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SUSTAINABLE INTENSIFICATION: A CALL FOR INNOVATION

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Summary: Sustainable intensification is a term that has been coined in order to describe the need for global agriculture to produce more food on a limited amount of available land, with lower inputs and less environmental impact. In reality it is about balancing a number of different trade-offs, at different scales between increasing productivity on managed land and the impacts on the wider environment. A number of different studies have gone some way to quantifying these trade-offs in terms of carbon and greenhouse gas emissions. Impacts on biodiversity have proven much more difficult to quantify. Innovation in agriculture is vital in order to deliver sustainable intensification. There is some evidence to suggest that crop yield improvements have plateaued. Genetic technologies can be harnessed to improve the yield potential of crops but this potential can only be realised if the cropping environment is managed optimally. Innovative tools and technologies are required in order to better understand the spatial and temporal variability of soil for improved management and better targeting of nutrient applications. A continued investment in ground-breaking research which increases knowledge, provides new insights and opens up new technological opportunities is essential but is not sufficient. There is an urgent need to build back the motivation, capacity and skills to translate science into practice through the varied processes of knowledge exchange.

INTRODUCTION

Sustainable Intensification: What is it? Why and How?

Sustainable intensification has been proposed as an integrating concept to meet the challenges of food security most effectively (Godfray *et al.,* 2010). It is defined as: "Simultaneously raising productivity, increasing resource use efficiency and reducing negative environmental impacts of agriculture". It is driven by the need to: reduce greenhouse gas (GHG) emissions and adapt to climate change; increase production efficiency; and increase competitiveness whilst sparing land for: carbon capture and storage; bioenergy production; biodiversity conservation; and the maintenance of ecosystems services.

The fact that the global population is expected to rise to at least 9 billion by 2050 is now well-documented, and it is estimated that the world population has passed the 7 billion mark only in the last few months. It has been rather less well publicised that the population in Britain is expected to rise to nearly 70 million by 2028, an increase of nearly 14% from 2008. A further factor to consider in responding to the demands of population increase is growing urbanisation in less developed countries. Globally, for the first time, in 2008, 50% of the global population

was urban – it was only 14% in 1900. The UK reached this situation at the end of the 19th century and is the most urbanised country in Europe; according to FAO statistics 90% of the population now live in conurbations of 100,000 people or more.

A significant amount of agricultural land on the globe could be used more productively and there is also degraded land where fertility could be restored. However, if deforestation and cultivation of permanent grassland are to be avoided, there is not much additional land that has not previously been cultivated which can be exploited to produce food for the growing population. Much currently uncultivated land is not suitable for food production or is important for other things, such as urban development or providing other critical environmental functions for the planet as reservoirs of biodiversity or carbon sinks. Exacerbating this further is the fact that plant growth is constrained on much of the Earth's land surface, due to limitations in solar radiation, temperature or water availability (Baldocchi *et al.,* 2004). When presented in this way the least-constrained food producing areas, such as northern Europe take on an even more important role in the future than current relative productivity levels would indicate.

The Policy Landscape

For the first time in many years, policy-makers, including defra are placing emphasis on increasing primary food production and the productivity of agricultural land. Defra have recently launched the Green Food project which aims to address the following core objectives:

- improve growth & competitiveness in the farming and food industry
- increase food production in the UK, and consider our role in global food security
- protect and enhance our natural environment

At the same time the draft proposals for the reform of the Common Agricultural Policy have been released. These proposals suggest that 30% of direct payments to farmers will now be contingent on a set of "greening" requirements being met. The measures include ecological focus areas (EFAs), crop rotation and requirements for grassland. It will be interesting, over the coming months and years, as policies and legislation become more refined, to see how the national and international policy-makers reconcile the inevitable trade-offs between production, land-use and environmental protection.

TRADE-OFFS BETWEEN LAND-USE AND ENVIRONMENTAL IMPACT

Measuring trade-offs

Delivering sustainable intensification depends on being able to quantify and optimise the management of trade-offs, at different scales, between man-managed agricultural systems and impacts on the wider environment. Looking first at the global scale, there is a clear trade-off between growing more food through agricultural expansion and net emissions of carbon dioxide from clearing forests or grasslands for crops. The outcome of this trade-off depends on the production capacity of the land that has been cleared and the carbon cost of the clearance and development of that land for agriculture. According to recent calculations (Foley *et al.,* 2011), the ratio between the current agricultural yields and historical carbon debt is poorest in tropical regions and comes close to zero in some temperate regions of the world. The ratio of low yields to high carbon losses illustrates the difficult trade-offs of many tropical areas and

highlights the environmental dangers of relying on tropical cropland expansion to meet future food demands; whereas increasing productivity through agricultural intensification in those areas that are inherently more productive may represent a positive trade-off. Indeed, a study which modelled the world as it is now, including the intensification of modern agriculture, and two alternative scenarios, in which crop production levels were kept at those of two decades ago, shows that the increase in greenhouse gas emissions from more productive agriculture are more than offset by the benefits of conserving land and the associated carbon sequestration (Burney *et al*., 2010).

The most significant cost, in carbon terms, associated with increasing production, is the cost of the production and impact of the use of nitrogen fertilisers and the above studies illustrate that the outcome of the trade-off between the costs associated with increasing productivity by applying additional N and the benefits associated with land conservation vary in different parts of the world, but that increasing agricultural intensification in those areas that are most productive is likely to have a positive outcome. If however the increased yields are lost to pest and disease attack, the outcome of the trade-off is immediately reversed. Recent work (Berry *et al.,* 2010) has quantified and demonstrated the positive effect of disease control on GHG emissions associated with wheat production in the UK. However a further trade-off must be reached between the benefits of reducing or eliminating waste from the system and the environmental impacts of the crop protection itself.

This study did not consider the cost to farmland biodiversity of increased agricultural intensification. However, many studies in grassland have shown that there is a clear trade-off between higher productivity and plant species diversity. The Rothamsted Park Grass experiment is an excellent long-term example of this effect and data from the experiment shows that under optimum pH, fertiliser additions that have tripled biomass yields have cut the number of plant species by half (Silvertown *et al.*, 2006). The cost benefit ratio associated with this trade off still remains to be estimated and the real issue here is that it has proven very difficult to quantify the economic (as distinct from the cultural) value of biodiversity whether it be at a field scale, at farm scale or globally.

THE NEED FOR INNOVATION

Increasing yields

There is a reasonable consensus that global sustainability and our ability to feed the growing population, has been greatly enhanced by increasing productivity on the land that has been already been cleared and cultivated. Further increases in productivity can be achieved by both increasing the genetic yield potential of crops or by ensuring that the yield potential is achieved by improving the management and agronomy of the crop and preventing losses through effective crop protection. Both activities are required and one cannot deliver the required outcomes without the other. Over the past fifty years, the improvement in genetic yield potential has been considerable. The effect of this can be clearly demonstrated by looking at the yields on another of the Rothamsted long-term experiments, Broadbalk. Further increases in yields will be required in order to achieve the predicted demand for food without the need to cultivate more land (the sustainable intensification agenda). However, the trends over the last two decades have not been encouraging. Defra statistics indicate that over the past 20 years yields of the major crops in the UK have plateaued. The rapid gains in yields that were seen in

the 1960s and 1970s have not continued and this has posed a number of important questions. Has the maximum yield potential for some of these crops been reached; or is it more to do with our ability to achieve that potential through crop management; or is it simply an issue of economics where lower crop prices and higher input costs have reduced the incentives for growers to strive for higher yields? There is also strong evidence from a current HGCA project on continuous cropping of oilseed rape that a contributory factor to yield decline in some crops has been the tendency for shorter rotations and repeated cropping of the most lucrative crops.

Whatever the cause of the yield plateau, preventing waste by minimising losses to pests and diseases through effective and efficient crop protection, is an essential element in ensuring the sustainability of agricultural intensification.

Challenges to chemical crop protection

Crop protection represents an arms race between developments in technology and the pests and pathogens as they evolve to overcome them. Today the challenges faced by those involved in the development of the technology are greater than ever, as increasingly stringent regulation limits capacity and imposes ever more complex technological hurdles and costs on the process. A new crop protection product may take more that 10-15 years to develop and cost up to £3 million, and even more in some cases. Pesticides are the most highly regulated chemicals in the world and currently we are losing products more quickly than they are being developed.

Innovation – genomics technologies

Genomics technologies will undoubtedly enable the development of strategies to develop more durable resistance to pests and disease through the efficient identification and selection of gene combinations. Real advances in the arms-race can be anticipated by the efficient development of durable resistance and creating particularly beneficial gene combinations using the techniques of direct gene transfer (genetic modification) or advancing methods of targeted mutation (sometimes referred to as “gene-editing”). There are many good examples now of where the molecular identity and capacity to isolate and manipulate resistance genes are well understood and technically feasible. This includes rust disease and soil-borne virus diseases of cereals.

Innovation – integrated management

Integrated management strategies will still be required to avoid rapid selection of virulent pathogen variants from the population which render the resistance ineffective. A clear recent example of the latter was the rapid loss of resistance to yellow rust in the wheat cultivar Oakley due to the increase in frequency of virulent pathotypes in the population. The genetic potential of the crop plant for grain yield can only be achieved if the constraints to production are removed and the cropping environment, including availability of resources, is optimised. All of the investment in innovation in crop genetics and plant breeding will have been of limited value if the cropping environment is not managed in such a way as to enable the genetic potential to be realised.

One of the reasons most frequently cited for the yield plateau is that despite continuing genetic improvement of crop varieties, the deterioration of the soil environment has meant that those improvements have not been realised. Undoubtedly soil remains the part of our environment

that is least understood. Leonardo da Vinci wrote, “We know more about the movement of celestial bodies than about the soil underfoot.” This statement is just as true today, 500 years after it was written. Soil is a hugely complex three-dimensional matrix that controls water availability, nutrient availability, nutrient leaching and N_2O release. It is variable in space and time and one of the biggest challenges that is faced in managing the soil environment is the paucity of available tools to measure and understand this variability. Soil is also a biologically active ecosystem and due to difficulties in isolating and culturing the many millions of microorganisms that inhabit soil, their various roles and even the importance of microbial diversity to the health and functioning of soil remain obscure. However, sequencing of the entire soil metagenome is now achievable and the international “Terragenome Project” is making great progress towards this. The next great challenge for this exciting area of research will be the translation of that science into tools that can be practically used for agriculture.

CONCLUSIONS

More than 20 years of complacency about food availability and supply in most countries (developed and less developed) has resulted in a significant erosion of the capacity to respond to the impending imbalance in supply and demand. With the exception of China and Brazil, investment in capital infrastructure, and human capital, through education, scientific research and innovation has steadily reduced over the last two decades. In the UK, the Agricultural Research Service which comprised twenty-nine Institutes at the end of the 1970s is now reduced to just six. The former National Agricultural Advisory Service which became the Agricultural Development and Advisory Service (ADAS) was privatised. Only three of the “Russell Group” universities confer degrees in agriculture or agricultural science.

Perhaps it is not surprising that at a time of financial stress and the need for austerity, increased funding for agricultural research and its application is not forthcoming. However, there is a clear need to coordinate and focus the available funding, both public and private, so that the benefits of investment in research can be most effectively realised. A continued investment in ground-breaking research which increases knowledge, provides new insights and opens up new technological opportunities is essential but is not sufficient. There is an urgent need to build back the motivation, capacity and skills to translate science into practice through the varied processes of knowledge exchange. This will require partnerships between the public and commercial sectors, a commitment to training and elevation of skills among both practitioners and delivery agents for innovation and technical advice and, in particular, a recognition of the commercial and market pressures that farm businesses face so that technical solutions can be considered part of business improvement.

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NEW EU PESTICIDE LEGISLATION – THE VIEW OF A MANUFACTURER

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Summary: The new pesticide legislation discussed in this paper are the Plant Protection Products Regulation 1107/2009 and the Sustainable Use Directive (SUD) 2009/128/EC. These pieces of European legislation are essential elements of the Thematic Strategy on Pesticides. Other European legislation briefly covered are the Maximum Residue Level Regulation 396/2005 and the Water Framework Directive 2000/60/EC. Regulation 1107/2009 replaced Directive 91/414/EEC on 14 June 2011. A main new element of the Regulation is that it provides the possibility to reject active substances on the basis of their intrinsic properties (including endocrine disruption). This concept is commonly known as 'hazard-based cut-off criteria'. The SUD is expected to be transposed into a UK regulation in early 2012. The UK is well positioned regarding many of the aspects of this legislation, however, some new controls will be introduced. Some practical implications of the legislation and the possible effect on the food supply chain are discussed.

INTRODUCTION

On 14 June 2011, EU Regulation 1107/2009 entered into force. This legislation replaces Directive 91/414/EEC, the previous main piece of legislation dealing with the registration of plant protection products (PPP) which came into force in 1993. The review process under this legislation for existing active substances saw the loss of over 600 active substances. For many people involved in the practicalities of the supply of food, the view is that PPP are already highly regulated and we are already experiencing some serious problems controlling weeds, diseases and pest problems, especially in minor crops. The fact that the introduction of new legislation could reduce the 'toolbox' of available PPP is of concern to the agricultural industry.

The new legislation published in December 2009, which forms the European Union Thematic Strategy on Pesticides is composed of four elements;

1. Plant Protection Products Regulation 1107/2009
2. Sustainable Use Directive (SUD) 2009/128/EC
3. Machinery Directive 2009/127/EC – which sets out standards for new equipment.
4. Statistics Regulation 1185/2009 – The key elements of this regulation are the provision of annual sales data and the provision of data every five years on usage on crops and the pesticides used.

Other relatively recent pieces of legislation also considered in this paper are the Maximum Residue Level (MRL) Regulation 396/2005 and the Water Framework Directive (WFD) 2000/60/EC.

REGULATION 1107/2009

The key thing to note is this is a regulation, not a directive, and as such is directly applicable in all Member States (MS). Most of the elements of the regulation did not need to be transposed into UK national legislation. The regulation was directly applicable on 14 June 2011.

The preceding legislation was the Plant Protection Products Directive (also known as the Authorisations Directive), 91/414/EEC which came into force in 1993 and was transposed into UK law as the Plant Protection Product Regulations in 1995. Under 91/414/EEC, registration of active substances was conducted at EU level, resulting in Annex I listing of the active substances. This was subsequently followed by registration (in the case of new active substances) or re-registration (in the case of existing active substances) of PPP at Member State level. One important aspect of this legislation was that evaluations and decisions were essentially risk-based.

Hazard-based cut-off criteria

Regulation 1107/2009 has introduced some significant changes. One of the main issues for the industry is the introduction of 'hazard-based cut-off criteria', whereby active substances will be eliminated from the evaluation process purely as a result of their classification (Table 1). No risk-based evaluations will be conducted for active substances that fail these criteria, irrespective of exposure or risk and whether safe uses can be identified (except in the case of some limited exclusions, discussed later in this paper).

Table 1. Criteria for the approval of active substances

Human Health	Environmental
Carcinogen C1A & C1B	PBT (Persistent, Bioaccumulative & Toxic)
Mutagen M1A & M1B	POP (Persistent Organic Pollutant)
Toxic for Reproduction R1A & R1B	vPvB (very Persistent, very Bioaccumulative)
Endocrine disruptor	Endocrine disruptor

It is important to note that some questions remain over the interpretation of some of the criteria e.g. Endocrine disruption and PBT, and the cut-off criteria will only take effect on renewal of each active substance (most taking place between 2016 and 2019).

Endocrine Disruptors

One of the criteria that will be used as a 'cut-off' will be endocrine disruption (Table 1.). Currently, this is the element that is causing the most uncertainty in the agrochemical industry, as there is no agreed testing and classification of active substances that are potential endocrine disruptors.

The endocrine system is a network of organs in the body that produce hormones which regulate reproduction, growth and development including puberty and moods. The endocrine system is naturally dynamic and responsive to various stimuli as part of its normal functioning and therefore any interaction with this system does not necessarily lead to harmful effects. Endocrine disruption is not an independent adverse effect, nor a new type of toxic property, nor a previously undetected hazard. It should be noted that despite the emotive association of endocrine activity and human reproduction, endocrine disruptors are not intrinsically bad for human health or dangerous to the environment. The inclusion of endocrine disruptors in 1107/2009 is a politically driven issue which is scientifically supported by some academic research and publications.

To put the issue of endocrine disruption more into context; many foods are associated with having endocrine active properties, some of which are used in holistic medicine to reduce menopausal symptoms. Soy beans, chick peas, and lentils are examples of commonly consumed foods that are considered to contain endocrine disruptors. Birth control pills are specifically designed to disrupt naturally occurring endocrine systems and as a result, normal female hormone cycles. They are used daily by millions of women, all over the world and are widely found in water at levels much higher than any agrochemical active substances. A number of active substances can under certain circumstances exhibit endocrine activity, but every regulatory approval decision is based on scientifically sound evidence on whether a substance is safe to humans and the environment.

The European commission has been tasked to draft proposals for the classification of endocrine disruption by 14 December 2013 and they have commissioned a "State of the Art Review" which is due to be completed by early 2012. Various other interested parties have also made proposals for endocrine disruption classification and evaluation, including the European Crop Protection Association, the European Food Safety Authority and a joint UK & Germany regulatory authority proposal. It is hoped that the Commission will take all these views into account when making the final proposal. Adoption of a final proposal is expected by the middle of 2014. If the new proposals have not been adopted by the time the first renewals of active substances, under 1107/2009, have taken place then there is a temporary definition in the legislation which can be used in the transitional period.

Various bodies, including regulatory authorities, industry and independent bodies have conducted analyses of the potential impact on active substance and product availability, and subsequently the decreasing crop yields if any become unavailable. One group of active substances which are being watched carefully are the azole fungicides, which are used in many crops for the control of various diseases. Whilst their classification as endocrine disruptors has been widely anticipated it should be stressed that this is not currently the case and every effort is being made by the agrochemical industry to ensure that authorisations for these important tools in the fight against plant diseases are maintained.

Possible exemptions to hazard-based cut-off criteria

1. Negligible exposure

There is provision in Annex II Point 3 of Regulation 1107/2009 which allows the approval of an active substance that fails the hazard-based cut-off criteria if "*the exposure to humans… under realistic proposed condition of use, is* ***negligible****…."*

It defines negligible exposure as residue concentrations in food or feed below the default concentration of 0.01 mg/kg. However, negligible exposure is not fully defined in the non-dietary area and work is ongoing to try to define under what circumstances this exemption could be used in relation to non-dietary exposure.

2. Derogation if a serious danger to plant health

Article 4.7 of Regulation1107/2009 provides the possibility for the approval of an active substance that fails the cut-off criteria if it is necessary to control a 'serious danger to plant health', that no (chemical or non-chemical) alternatives are available, that mitigation measures can be identified to limit exposure of humans and the environment, and that appropriate MRLs exist.

The main limitations to such an approval are that it has to be approved by all other Member States and the Commission, and is then only valid for a maximum of five years. An industry proposal has been made that any decision on whether an active substance is necessary to control a 'serious danger to plant health' should be made case-by-case on the basis of the evidence provided by the applicant and reviewed by the Member State. Industry recommends that particular attention should be given to maintaining a chemical diversity (retaining the approval of various modes of action against an individual pest) to ensure a sustainable agriculture in Europe and to minimise the development of resistance. The impact of withdrawing existing active substances on minor crops and/or uses must be taken into account when evaluating whether alternatives are suitable or not. Industry also recommends that non-chemical methods be evaluated using the same guidance, criteria (including efficacy) and safety standards as chemical methods e.g. the health costs of replacing the use of a herbicide with manual weeding.

Comparative Assessment & Substitution

Under Article 24 of Regulation 1107/2009, active substances that meet certain specified health and environmental criteria (specified in Annex II (6) of 1107/2009) will be identified by the Commission as 'Candidates for Substitution'. For these active substances, approvals will be only granted for seven years and any PPP containing that active substance will be required to undergo 'Comparative Assessment' at Member State level (Article 50). This new process aims to compare a PPP with other approved PPP and non-chemical methods of control or prevention, and substitute the more 'hazardous' with a 'safer' alternative. It is well recognised that this will not be an easy process as for example, one PPP may pose more of a risk to earthworms and the other more of a risk to birds – which is the 'safer'? Any potential for the replacement of a PPP by a non-chemical method not only requires an evaluation of the efficacy of the technique, but also a full assessment of the true cost in terms of safety and economics.

It is planned that this process will take place at Member State level, each time a product containing a Candidate for Substitution is registered. This means that any PPP containing more than one active substance will be reviewed many times. The Member State will be required to consider the availability of alternatives (including minor uses), resistance pressures, economics and practicality. It should be noted that the need to conduct these additional evaluations places an additional workload on Member State Regulatory Authorities.

Zonal Evaluation

Under Regulation 1107/2009, the EU is divided into 3 zones; Northern, Central & Southern. The concept is that once a PPP approval is granted in one Member State, other Member States in that zone are able to use the evaluation to grant an approval (a process commonly known as Mutual Recognition), as long as any national specific data requirements and risk assessments have been completed. This process is intended to speed-up decision making and to encourage a level playing field within a zone in terms of pesticide availability. In order to achieve this, it is important that any national specific data requirements and risk assessments are minimised. Both industry and the MS authorities are currently making submissions and evaluations according to this procedure and are hopeful that the use of the zonal process will reduce the number of evaluations conducted and time to authorisation.

Loss of Provisional Authorisations

Regulation 1107/2009 provides set timelines and a clear process for the evaluation of new active substances (Articles 7 to 13). In the event that these timelines are not met by the regulatory authorities and the evaluation has not been completed within 30 months, Article 30 contains a derogation allowing Member States to grant National Provisional Authorisations as long as an appropriate MRL has been set.

Under 91/414 the ability to grant a National Provisional Authorisation represented an important 'safety net' for the R&D industry, ensuring the rapid entry onto the market of PPP containing a new active substance, before approval of the active substance at European level. This process has been well used in the UK with many new active substances being available to the market with 12-18 months of submission and the loss of this option is important to industry. It should, however, be stressed that industry's preference is to ensure a process that supports and ensures rapid approval decision-making on new active substances, which in turn will allow PPP containing these new active substances to be fully authorised throughout Europe. A clear process for the granting of National Provisional Authorisations is not provided in the new Regulation.

Using the timelines in Regulation 1107/2009, the first National Provisional Authorisation may be expected 30-36 months after submission to the Rapporteur Member State. It should be noted that this is significantly longer than current timelines under Directive 91/414/EEC where the first National Provisional Authorisation may be granted 12-18 months after the submission of the active substance dossier to the Rapporteur Member State.

MRL REGULATION 396/2005

The MRL Regulation came into force on 1 September 2008 and requires MRLs to be set at EU level before any PPP approval can be granted in a Member State. The process requires a dossier to be submitted to a Rapporteur Member State, and evaluation takes place over a period of approximately 3-12 months. The resulting report is then evaluated by the European Food Safety Authority (EFSA) over a period of 3-6 months. For both evaluations, the time taken really depends on the complexity of the dossier and whether a new active substance is involved. Following their evaluation, EFSA make a recommendation to the Commission and a vote takes place at an appropriate Commission meeting (approximately 3 months later). The

approval for the use of the PPP cannot be granted until publication (at least 4 months after the vote).

The setting of the MRL is a potential 'rate-limiting step' for the introduction of new active substances and it is a major obstacle to the timely introduction of additional crops to PPP, especially for minor uses.

The crop protection industry's preference would be for a streamlined regulatory process that removes unnecessary administrative time delays and provides more economic incentives for industry to invest (such as additional data protection for the minor uses); minimising the need for specific rules and exemptions for minor uses.

SUSTAINABLE USE DIRECTIVE 2009/128/EC

Unlike Regulation 1107/2009, the SUD requires transposition into national legislation. The SUD was required to be transposed into MS legislation by 26 November 2011, but no MS attained this. In the UK, a consultation on the implications of the SUD was conducted in May 2010, with a summary of responses published in December 2010. The regulation is expected to be introduced in the UK in early 2012. The SUD covers the use of pesticides (including PPP and biocides) in the EU and will come into force in stages from 2012 to 2020. The UK is well positioned regarding many of the aspects of this legislation, however, some new controls will be required.

National Action Plans

Article 4 of the SUD requires Member States to develop National Action Plans (NAP) designed to reduce the risks relating to the use of pesticides and to reduce their use wherever possible. In the UK, the development of the NAP involved many stakeholders (a requirement of SUD) and have already been published (Anon., 2008). There are existing UK NAP on Biodiversity, Amenity, Availability, Water, Human Health and Amateur use. All these NAP will be continuously updated as experience and new ideas develop, and at least every five years.

Training

Article 5 of the SUD requires Member States to ensure that all professional users, distributers and advisers have *access* to appropriate training by 26 November 2013, rather than stating that all sprayer operators and advisors *must be trained and certified* by law. In the UK, there are already extensive training opportunities in place, including certification bodies. If the wording of the SUD is followed exactly in UK legislation, this would mean a reduction in current standards in the UK. Much of the agrochemical industry has urged the Government to ensure that the UK regulations reflect the intent of the SUD to reinforce controls on the use and distribution of pesticides, rather than weaken them. In particular, they want to see a continuation of the UK's current statutory requirement for certification of sprayer operators and an extension of this requirement to all advisers, as well as new statutory provisions for ongoing training and professional development.

Aquatic Environment and links to the Water Framework Directive (WFD) 2000/60/EC

Article 11 of the SUD requires Member States to ensure that appropriate measures are adopted in order to protect the aquatic environment and drinking water supplies. This Article links into the WFD and it is likely that measures taken to implement the directive could result in restrictions or prohibitions on the use of PPP on a local or national basis. The WFD has resulted in many EU Priority substances and UK Specific Pollutants being identified, for which action is required to be taken. Under the WFD, there is a possibility for a loss of PPP causing WFD non-compliance, but it is more likely that risk mitigation measures, such as the use of low drift nozzles and buffer zones and enhanced voluntary measures, will be adopted to address any localised issues.

Integrated Pest Management (IPM)

Article 14 of the SUD requires Member States to take all necessary measures to promote low pesticide-input pest management, giving priority to non-chemical methods. It should be noted that the concept of IPM, or more correctly Integrated Crop Management (ICM), has been and is fully supported by the agrochemical industry. Industry only ask that when considering the use of non-chemical methods of pest control the true cost, both in terms of safety and economics, needs to be fully considered alongside the crop protection product and any real or perceived biodiversity benefits.

DISCUSSION

The new legislation is now in place and industry and regulators have to find a way to make it work to the advantage of all stakeholders. Under this legislation, there is a potential for the loss of active substances and products. The challenge is to ensure that any losses of active substances and/or products are only due to safety reasons and are not just because one product appears to be less safe than another. Objective, evidence-based science should remain the backbone of EU regulation and the agrochemical industry encourages legislators to reconsider the narrow hazard-based approach of 1107/2009. Only by adhering to fully-informed, 360-degree risk assessment can we fulfil the combined imperatives of agricultural productivity, environmental protection and enhanced food safety. The toolbox of crop protection products (and modes of action) is reducing and it is important to remember that we still have to feed the world. World-wide, there is increasing awareness of the requirement for food security and the maintenance of not only the staple food supply, but also the variety of crops.

In order to show the true value of crop protection to the UK food chain and living standards, the UK Crop Protection Association sponsored a report, which concludes that without pesticides to keep weeds, pests and diseases in check, crop yields would fall to half their current levels and food prices would rise by 40%, an increase to UK consumers of some £70 billion per year in food costs (Rickard, 2010).

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EU PESTICIDE LEGISLATION – AN UPDATE

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Summary: At the end of 2009, the EU published four pieces of legislation which make up the Thematic Strategy for Pesticides. In 2010 the Government consulted on key elements of this legislative package. Some of the laws necessary to implement the Strategy have been made, others will be introduced shortly.

INTRODUCTION

At the end of 2009, the EU published four pieces of legislation which make up the Thematic Strategy for Pesticides. They are:

- a regulation concerning the placing of plant protection products on the market (which replaces Directive 91/414/EEC);
- a directive on the sustainable use of pesticides;
- a regulation on pesticide statistics; and
- an amendment to the machinery Directive 2006/42/EC, enabling standards to be set for new pesticide application equipment being brought to the market.

The Strategy is designed to further reduce the impact of pesticides, particularly plant protection products, on human health and the environment. Its specific objectives are to:

- minimise the hazards and risks to health from the use of pesticides;
- improve controls on the use and distribution of pesticides;
- reduce the levels of harmful active substances, including through substituting the most dangerous with safer (including non-chemical) alternatives;
- encourage low input control by raising awareness, promoting good practice and consideration of possible application of financial instruments; and
- establish a transparent system for reporting and monitoring progress made in fulfilling the objectives of the Strategy, including the development of suitable indicators.

This paper focuses on the key elements of this package: the new authorisation regulation (Regulation (EC) No 1107/2009) and the Directive on the sustainable use of pesticides (Directive 2009/128/EC). These are very different in nature. In general terms, the Regulation is much more prescriptive, setting out rules and processes which offer no or relatively limited discretion in the rules governing the marketing of pesticides. The Directive, by contrast, offers Member States a far greater degree of flexibility as to how the objectives set out in this legislation should be met.

THE NEW REGULATION

Regulation (EC) No 1107/2009 came into force in June 2011. It aims to increase the level of protection given to human health, animal welfare and the environment, simplify procedures and offer a more even choice of products to farmers and growers. Key elements which strengthen the previous regime include establishing a system under which similar products could be compared and the more hazardous removed from the market (subject to certain conditions), and introducing cut-off criteria designed to exclude the most hazardous compounds.

The UK Government supports most of this legislation, but voted against it over concerns about the lack of proper impact assessment of new criteria to eliminate active substances because of their intrinsic hazard, rather than their risks in use. There was particular concern that the criterion to eliminate substances with potential for adverse effects on human hormone systems (endocrine disrupters) could have significant impacts on crop protection but without achieving any clear benefit for consumer health. This is, however, drafted in indicative terms, and thus it is not possible to determine with certainty what the impacts will be. The adopted text requires the Commission to come forward with proposals for definitive provisions on endocrine disrupters by December 2013. Although the Government has stressed the need to remove the current uncertainty, the Commission has made clear that they will take the full time allowed them to make proposals. Substances which breach one or more of the hazard criteria could be retained under a derogation that allows temporary approvals where no alternative controls are available.

The Regulation will also introduce new measures which aim to simplify the process by which pesticides are authorised. Under the current system, companies apply separately to each Member State, which conducts its own assessment to determine whether to authorise the product. In contrast, the Regulation divides the EU into three zones. Within each zone, a product can be authorised on the basis of one Member State's evaluation. Other Member States in the same zone can simply accept that evaluation and authorise the product on the same conditions, though some may wish to apply additional conditions where they are needed to reflect particular national circumstances. This is intended to speed up decision-making and ensure a level playing field within the zone in terms of pesticide availability. It aims to avoid unnecessary duplication of work and thus save registration costs for the industry. Member States are working to develop detailed procedures for handling applications under these arrangements.

THE NEW DIRECTIVE

Directive 2009/128/EC is the first substantive piece of EU legislation governing the use of pesticides and comes into force in a number of stages from 2011 to 2020. Member States currently operate a variety of controls on the use of pesticides and the Directive should bring a greater degree of harmonisation to these. Taking the EU as a whole, the Directive should help bring standards up to levels similar to those which apply in the UK.

The key feature of the Directive is a requirement for Member States to develop National Action Plans designed to reduce further the risks associated with the use of pesticides and promote the use of low input systems. The Directive lists a number of measures which should populate the plans, including: initial and on-going training of users, distributors and advisors;

controls on sales; regular testing of application equipment; protection of water courses, amenity and conservation areas; controls on handling, storage and disposal; and promotion of low-pesticide input approaches (in particular Integrated Pest Management (IPM)). Most of the measures already feature as part of the existing national range of statutory and voluntary controls.

Government policy is that EU legislation should be transposed, wherever possible, through the use of alternatives to regulation, ensuring that UK business are not put at a commercial disadvantage compared to their European counterparts, and that necessary implementing measures come into force on (rather than before) the transposition date specified in a directive. In December 2010, the Government published its summary of, and response to, the public consultation that was conducted earlier that year on how the Directive should be implemented. It concluded that the UK's regulatory regime for pesticides and other existing statutory and voluntary controls place it in a good position with respect to many of the outcomes required by the Directive. In a number of areas, only minor changes to current arrangements were identified as necessary to meet the new requirements.

The Government response to the public consultation indicates the sort of measures that may be included in the implementing legislation for the Directive.

- Requirements for the Government to develop and maintain a National Action Plan (NAP), taking account of the views of stakeholders, to reduce further the risks which can be associated with the use of pesticides and promote the use of alternative methods of control.
- Requirements on training and certification programmes - Certification programmes for professional users, advisors and distributors must be in place by 2013.
- Requirements on sales - From 2015, only those holding training certificates will be able to purchase professional products, and distributors will have to ensure sufficient staff are available to provide information at the point of sale from 2013.
- Requirements relating to the testing of application equipment - The machinery must be inspected in accordance with the timetables set out in the Directive (all equipment to be tested once by November 2016, on a five-yearly basis until November 2020 and three-yearly thereafter). These requirements will not apply to knapsacks, handhelds and equipment that is not used for spraying pesticide products or equipment that represents a very low scale of use, as listed in the NAP.
- Requirements related to aerial spraying - The existing legislative control regime will be adapted to ensure the continuation of properly regulated spray operations, through a consent-based approach.
- Requirements relating to the protection of water - Current statutory and voluntary controls and measures relating to the use of pesticides and protection of water afford a high degree of protection to water resources and cover specific measures detailed in the Directive. The Water Framework Directive is likely to require more action to be taken to comply with its requirements. In many cases, local approaches to local issues will be required. This should be achieved using existing legal powers and through development of the existing controls. The Government will consider whether additional legislative references are necessary to implement the Directive. The Government will primarily seek to work with the industry to enhance voluntary measures that improve knowledge transfer to users and develop mitigation measures that can be adopted in areas where pesticides are causing problems.

- Requirements on use in public spaces and conservation areas - The Government will primarily rely on existing control measures and additional voluntary guidance, but will consider whether additional legislative references are necessary to implement the Directive.
- Requirements on handling and storage - Existing controls address the requirements of the Directive. The Government will primarily rely on existing control measures and additional voluntary guidance, but will consider whether additional legislative references are necessary to implement the Directive.

On promotion of integrated approaches, there is good evidence of existing practice of IPM principles, particularly in the agriculture and horticulture sectors. IPM is already promoted through industry schemes. Most training and Continuing Professional Development already include elements on IPM. However, to strengthen the current position, there will be a review of IPM coverage in training to ensure it consistently reflects the general principles outlined in the Directive. In addition, amenity stakeholders will be encouraged to promote best practice and uptake of IPM tools in that sector.

The Directive required Member States to bring into force the necessary laws, regulations and administrative provisions by 26 November 2011. Initially, it was planned to conduct a second consultation on the implementing legislation in the UK. However, Government policy on implementing European Directives provides that consultation should be carried out only where there is scope to influence the policy outcome. In this case, the policy approach has already been decided in relation to the vast majority of issues. A consultation on draft regulations therefore serves little purpose and would be an unnecessary burden on stakeholders, particularly as the Government will be asking them to contribute to drawing up the NAP. Given that further consultation would be of limited value, the Government considers that time constraints are also a factor to be taken into account, given that the transposing Regulations were not be made by the deadline for transposition. A decision was taken that there would be no further formal consultation. Work on transposing the Directive into UK law is underway and will be carried through as rapidly as possible.

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DIFFUSE POLLUTION FROM RURAL LAND USE: SCOTLAND'S APPROACH TO MITIGATION IN 14 PRIORITY CATCHMENTS

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Summary: The Water Framework Directive sets ambitious targets for Scotland's water environment. The most significant pollution pressure preventing these targets from being reached is diffuse pollution from rural land use. A focused management approach is now being rolled out across 14 priority catchments in Scotland to help identify, understand and mitigate diffuse pollution. This approach involves detailed catchment studies (desk and field based), awareness raising campaigns and finally 1:1 farmer visits.

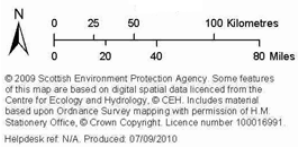
The field based surveys have provided good physical evidence of the extent of diffuse pollution pressures within each of the catchments. Non-compliance with statutory diffuse pollution legislation has been found to be high with over 5000 non-compliances being noted across the 14 catchments. The challenge now is for SEPA to work with local stakeholders, land managers and community groups to heighten diffuse pollution awareness and implement practical, land-based solutions that will ultimately drive water quality improvements, without compromising profitability.

INTRODUCTION

One of the most pressing issues preventing good water quality in Scotland is diffuse pollution from rural land use (SEPA, 2007a and 2007b). Heavily influenced by climatic conditions, diffuse pollution is most evident during and following rainfall events when nutrients, soil, chemicals and bacteria are mobilised from the land to local surface and groundwaters. Sources of diffuse pollution can include riverbanks poached and eroded by livestock, leached fertilisers/pesticides following application and surface runoff from cultivated fields. Typically minor at a field scale, these sources of pollution become significant when combined across a catchment, with notable impacts on the chemistry and ecology of the receiving waters.

The first national, coordinated approach to mitigating rural diffuse pollution in Scotland is now well underway, under the guidance of the Diffuse Pollution Management Advisory Group (DPMAG). This partnership group was established to coordinate diffuse pollution mitigation efforts and to ensure representation from a variety of key rural stakeholders. The Rural Diffuse Pollution Plan for Scotland (DPMAG, 2010) encompasses a two-tiered strategy consisting of a national approach to awareness raising, training and inspection and a targeted priority catchment approach in those areas worst affected. This paper provides an update on the priority catchment approach only.

Over one hundred catchments have been identified as being impacted by rural diffuse pollution across Scotland and 14 priority catchments containing the most important waters for conservation and protection; have been identified for action between 2009 and 2011. These are the Eye Water, River Tay, River South Esk, River Dee, Buchan Coastal, Ugie Water, River Deveron, River Ayr, River Doon, River Irvine, River Garnock, and the North Ayrshire, Galloway and Stewartry coastal catchments.



CATCHMENT_NUMBER	CATCHMENT_NAME
30	River Deveron
31	Buchan Coastal
32	River Ugie
37	River Dee (Grampian)
41	River South Esk (Tayside)
46	River Tay
67	Eye Water
77	Stewartry Coastal
80	Galloway Coastal
87	River Doon
88	North Ayrshire Coastal
89	River Ayr
90	River Irvine
91	River Garnock

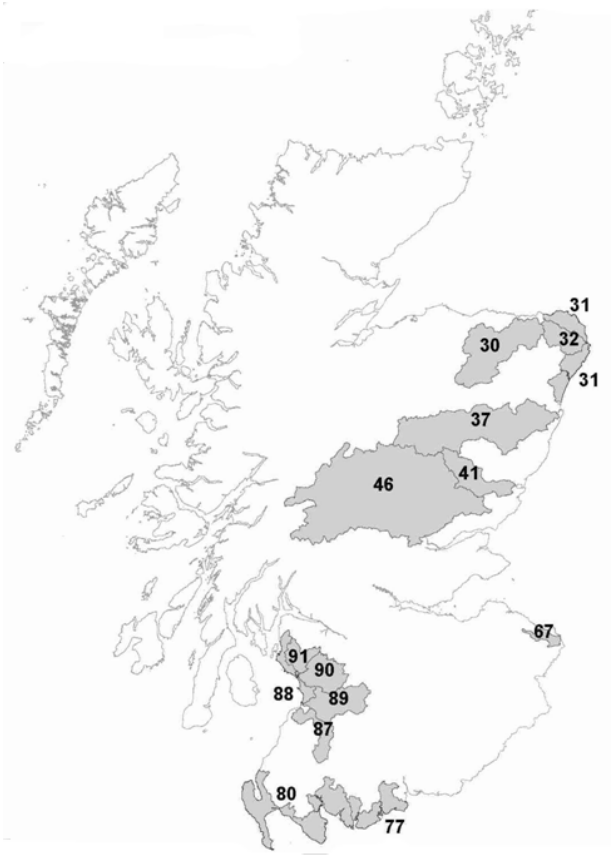


Figure 1. Map showing the location of the first 14 priority catchments

A key component of the strategy is to increase and promote awareness of diffuse pollution and the Diffuse Pollution General Binding Rules (DP GBRs). Introduced in April 2008 the DP GBRs cover a range of activities including cultivation, pesticide application and the storage and application of fertilisers. Based on accepted standards of good practice, these rules introduced a statutory baseline for all land managers.

THE PRIORITY CATCHMENT APPROACH

A three phased approach consisting of catchment characterisation and evidence gathering, awareness raising and finally 1:1 engagement and inspection, has been adopted in each of the first 14 priority catchments. This phased approach aims to identify, understand and mitigate rural diffuse pollution by working in partnership across the impacted catchments.

Catchment Characterisation and Evidence Gathering

Monitoring data collected by SEPA and its predecessors demonstrates water quality impacts in surface and/or groundwater across the 14 catchments. In the South Esk for example,

groundwater is classed as poor in the lower catchment due to levels of nitrate and the occasional presence of a fungicide epoxiconazole. Several surface water bodies are also downgraded as a result of elevated phosphorus levels and there are also concerns over the level of silt entering the river system (SEPA, 2011). The River South Esk is a designated Special Area of Conservation on the basis of its populations of Atlantic salmon and freshwater pearl mussels. Surveys carried out by Scottish Natural Heritage in 2003 and 2009 assessed the population of freshwater pearl mussels to be in 'unfavourable condition'. The species are known to be particularly sensitive to elevated nutrient concentrations and silt inputs.

As part of the characterisation process, all scientific data available internally and externally, has been collated and interpreted for each catchment. This looks in detail at the science underpinning the status of the catchment, providing information on the sources, transport pathways and impacts for the key pollutants of concern. Basic catchment characteristics such as geology, soils, land use, rainfall and climate data are also brought together with information on flood risk, abstraction and morphology. Characterisation reports are being published for all priority catchments, along with a more user friendly summary report.

In order to gain a clearer understanding of local land management practices and diffuse pollution pressures, SEPA staff walked the main rivers and burns within the 14 priority catchments. Principally, catchment walk surveys assessed compliance with the DP GBRs, but information relating to point source discharges, morphological pressures and invasive non-native species was also captured, to help provide a more comprehensive picture of the water environment. Land use in the riparian zone, the presence/absence of fencing and examples of good practice were also recorded. A photographic log of the issues was captured, along with GPS readings to allow the data to be mapped.

Awareness Raising

The DP GBRs have been in place for several years; however, knowledge of the legislation is believed to be relatively poor among many of those working in the rural sector. A variety of awareness raising campaigns and communication methods will be used across the catchments to highlight local diffuse pollution issues as well as the rules that must be complied with. Events and workshops will also provide practical advice on mitigation measures and funding mechanisms.

Critical to the success of the awareness raising campaigns will be the level and effectiveness of engagement at both a stakeholder and land manager level. Without local buy-in and a true partnership approach, water quality targets are unlikely to be met. As a regulatory body, this represents a significant challenge for SEPA.

1:1 Engagement and Inspection

Following a period of awareness raising, SEPA will initiate a programme of 1:1 engagement and inspection. Resource intensive 1:1 inspections will be targeted to areas most at risk within a priority catchment and where the most value can be gained.

Key components of environmental legislation (to which SEPA is the responsible authority) will be assessed during the inspection, including a more detailed assessment of the DP GBRs. The findings will highlight the diffuse pollution risks associated with the holding, along with any good practice measures currently being adopted. These findings will be reported back to the

land manager in person, where feasible, to allow discussions to take place over potential mitigation measures and to agree timescales for action. Follow up visits will be undertaken as necessary to secure compliance.

PROGRESS TO DATE

At the time of writing, 1:1 engagement had commenced in the River Ayr, River Doon, Ugie and Eye water. This section will therefore focus on the characterisation and awareness raising aspects of the priority catchment approach.

Catchment Characterisation

During 2010 and 2011 5632 kilometres of river was surveyed by SEPA staff in the 14 catchments and over 5000 non-compliances with legislation was recorded. Table 1 below provides a summary of the total number of DP GBR non-compliances per catchment and a breakdown of the most common ones encountered. For the majority of catchments, an average of 1 non-compliance per kilometre was recorded with the most prevalent relating to significant poaching and erosion of riverbanks by livestock (GBR 19) and the cultivation of land within 2 metres of the water environment (GBR 20). It should be noted that not all DP GBRs or all aspects of the DP GBRs could be assessed by way of the catchment walk surveys, particularly those in relation to pesticide application, sheep dipping and nutrient management.

Table 1. Summary of DP GBR non-compliances across 14 Priority Catchments

Priority Catchment	Total DP GBR non-compliances	Details of main non-compliances			River length (km)	DP GBR non-compliances per/km
		Fertiliser (GBR 18)	Livestock (GBR 19)	Cultivation (GBR 20)		
Eye	**286**	1	263	18	130	2.20
South Esk	**504**	10	254	234	400	1.26
Tay	**798**	1	489	298	950	0.84
Dee	**287**	4	261	20	457	0.63
Buchan Coastal	**249**	3	238	4	324	0.77
Ugie	**233**	3	189	38	278	0.84
Deveron	**346**	11	270	63	846	0.41
Ayr	**450**	32	383	35	350	1.29
Doon	**85**	3	70	11	140	0.61
N Ayrshire coastal	**66**	1	59	5	65	1.02
Irvine	**425**	13	399	9	525	0.81
Garnock	**289**	4	263	18	252	1.15
Galloway	**587**	2	498	52	629	0.93
Stewartry	**404**	1	382	16	286	1.41
TOTAL	**5009**	89	4018	821	5632	

This information is of particular value when viewed at a catchment scale, providing information on the geographic footprint and nature of non-compliances within a particular sub-catchment. Figure 2 shows the most prevalent DP GBR non-compliances across the River South Esk. In the Quharity sub-catchment, the incidence of GBR non-compliance was noted to be exceptionally high with an average of 7 per kilometre, the majority of which related to poaching and erosion caused by livestock. The South Esk itself was also found to have a high number of issues as a result of livestock watering points on the main river channel.

As anticipated, few DP GBR non-compliances were noted in the upper South Esk and its tributaries, which include the White Water, Burn of Heughs, Glenmoye, White Burn and Prosen Water. By contrast, the lower tributaries such as the Pow, Lemno and Melgund Burns demonstrate between 3 and 5 non-compliances per kilometre of river surveyed. These are highly productive arable catchments, with more than 50% of the land being devoted to arable and horticultural use. These three South Esk sub-catchments are also at less than good status as a result of diffuse pollution and other pressures such as morphology and abstraction.

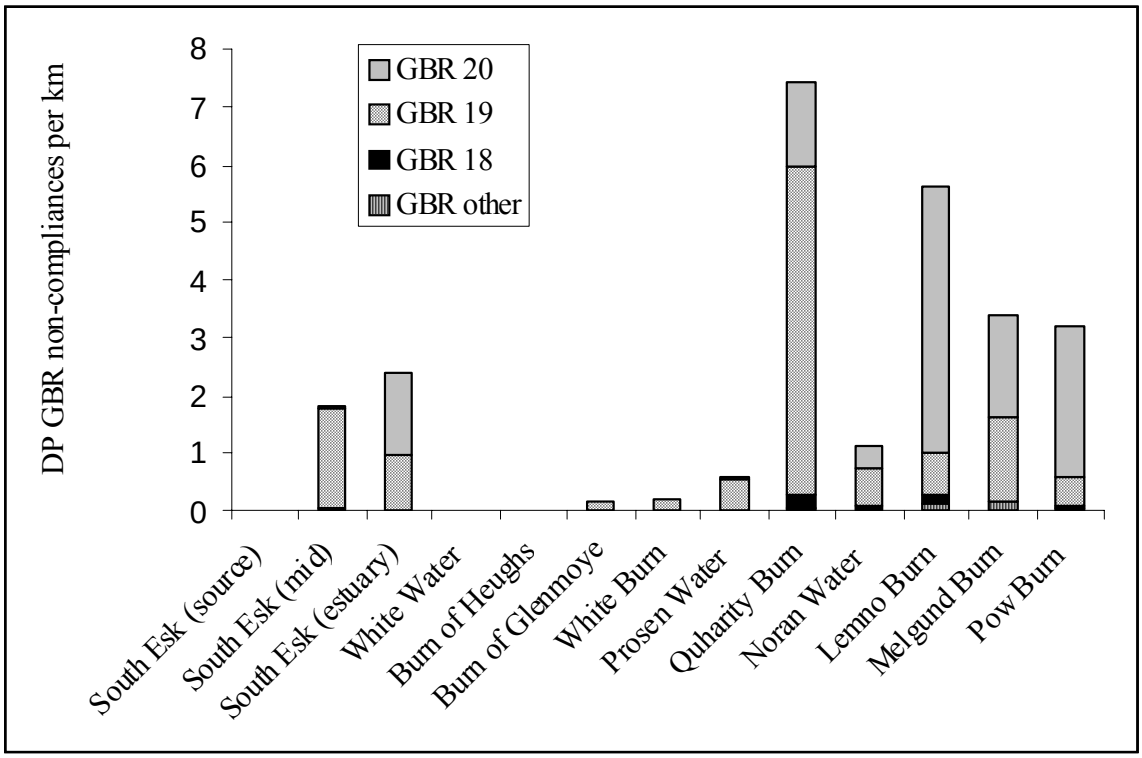


Figure 2. Summary of DP GBR non-compliances per River South Esk sub-catchment

A significant amount of data was also collected on other sources of pollution pressures including septic tanks, sewer overflows and contaminated runoff from stables, quarries and opencast mining activities.

Awareness Raising

From an early stage in the priority catchment process, SEPA has attempted to engage with a variety of stakeholders and land managers to raise awareness of the DP GBRs and to highlight the PC approach. Various methods of communication have been used by SEPA and partners

and include letters, press releases, radio and TV interviews, text alerts, postcards and e-news bulletins. Priority catchment web pages have also been developed by SEPA which house catchment walk findings, characterisation and summary reports and links to guidance material, including the DP GBR leaflets. More importantly, over 60 catchment specific meetings and workshops hosted by SEPA and partner organisations have been held across the 14 priority catchments, with good feedback being received on these events.

Stakeholder groups have also been set up in many of the catchments to steer the work carried out by SEPA. These groups also help to pull together a variety of interested parties and organisations, to share information, establish training needs and to coordinate awareness raising events. In some catchments, principally in the South West of Scotland, land manager groups have been established during trial 1:1 inspections. This has proved to be exceptionally useful and has influenced how SEPA will report back to land managers in this and other catchments.

DISCUSSION/CONCLUSION

The catchment walk surveys have provided good pictorial evidence to highlight diffuse pollution sources and pathways across each of the priority catchments. That, combined with a detailed scientific appraisal, provides a sound evidence base to inform and educate land managers and local stakeholders of the need to address rural diffuse pollution. The challenge for SEPA and partners will be to influence and change land management practices across Scotland to reduce diffuse pollution sources and where necessary, implement mitigation measures to protect Scotland's water environment.

ACKNOWLEDGEMENTS

We would like to acknowledge the help of all SEPA staff involved in this work, as well as the ongoing and invaluable support from local stakeholders and partnership groups.

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SEPA PESTICIDE MONITORING: A NEW APPROACH

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Summary: SEPA has developed a pesticide monitoring strategy designed to gain a better understanding of environmental concentrations of pesticides and the potential impacts in Scotland's waters. A range of monitoring techniques is being employed to identify and target areas of greatest concern. While the episodic nature of pesticide inputs presents a challenge to representative monitoring, using a variety of approaches and targeting strategies is providing valuable information on pesticide occurrence and behaviour in some of Scotland's most sensitive catchments.

INTRODUCTION

Historically, SEPA has generally not engaged in comprehensive pesticide monitoring in catchments, with sampling usually confined to end-of-catchment locations. While this approach is useful for some purposes (e.g. assessing pesticide loads reaching the marine environment), it cannot provide any detail of sources and movement of pesticides within catchments.

In Europe, the introduction of the Water Framework Directive (WFD), in 2000 heralded a paradigm shift in water management for Member States, with a move away from water quality assessment of individual waterbodies based solely on chemical parameters towards whole ecosystem assessment of river basins, measured by ecological parameters. Importantly, the Directive includes a specific requirement to control diffuse pollution. At present, diffuse pollution is the biggest threat to Scotland achieving good water quality; an estimated 4,600km of rivers, 300km² of lochs, and 80km² of coastal waters are adversely affected by rural land use. Scotland must aim to meet improvement targets by 2015, and SEPA has identified over 100 catchments in Scotland which are currently failing (or are predicted to fail) environmental quality standards, and require a catchment-wide approach to reduce diffuse pollution risks. Fourteen catchments have been selected for immediate action, known as diffuse pollution priority catchments, and work is currently underway in these catchments to target and reduce diffuse inputs, and return failing waterbodies to good ecological status.

In a number of the priority catchments, plant protection products (referred to generally as pesticides in the rest of this paper) have been identified as a potential cause of water quality downgrades, in the main through detrimental effects observed in in-stream ecology. To improve understanding of pesticide inputs into these catchments SEPA has developed and initiated a comprehensive monitoring strategy for pesticides. This paper uses case studies to provide an overview of and rationale for SEPA's current programme of pesticide monitoring.

SEPA PESTICIDE MONITORING

Pesticide monitoring is expensive and time-consuming, and if not targeted correctly can deliver poor returns. As a result, there is limited historical data on environmental levels of pesticides in Scottish waters. SEPA is currently utilising a number of approaches to provide a fuller picture of pesticide occurrence in the priority catchments. Sampling approaches need to be tailored to the specific questions to be answered in each catchment, and in each case there has to be careful consideration of where, when, and how to monitor most effectively.

Where to look?

A downside to the most commonly used "end-of-catchment" style of monitoring is that pollutant concentrations can be substantially diluted by the time a river reaches its mouth. In the case of pesticides, this can mean they are present, but not capable of being detected by the analytical methods used. However, even trace quantities of these toxic compounds have the potential to adversely affect ecology. Targeting sampling further up the catchment, and in tributaries rather than the main stem river, provides a more targeted approach, focusing on those areas where pesticides are applied. This provides better information on whether pesticide levels detected in water bodies further up the catchment are causing any adverse environmental impacts. In the priority catchments, SEPA is taking a 3-phase approach to ensure monitoring is targeted effectively;

1. *Identifying areas of concern*: Biological screening (using SPEAR analysis of invertebrate data) identifies water bodies with impacted ecology. This is used in combination with desk-based assessments of physical risk factors such as slope and land use to select potential locations for further investigation.
2. *Chemical screening*: A program of regular spot sampling is carried out over 6 months across the catchment at the locations identified in the initial phase. Comparing the results of this screening flags any 'hotspot' locations, and is used to prioritise sites for continued investigation.
3. *Targeted monitoring*: More intensive monitoring at sites selected through the screening process will provide data to support the assessment of environmental impact and identify any improvement. The monitoring will be focused on hotspots identified in the stage two screening and targeted to appropriate usage periods.

Case study: River South Esk priority catchment
The South Esk catchment lies in the Angus region of Eastern Scotland, flowing from its source in the Eastern Cairngorms to the North Sea at Montrose. Land use in the lower half of the catchment is dominated by arable agriculture, and ecological data shows evidence of pesticide impact in this area, particularly in the tributaries. Pesticides have also been detected in groundwaters in the lower catchment. This information was used to select four tributaries for 6 months of screening monitoring, and a summary of the results are shown in Figure 1. This comparison of locations clearly identifies tributaries A and C as potential candidates for further investigation, having more detections than B and D. Site specific assessments are now underway in preparation for the implementation of more intensive monitoring, including consideration of which pesticides have been detected, their usage patterns and physico-chemical properties. This will help to inform potential environmental pathways and will support the selection of appropriate monitoring techniques.

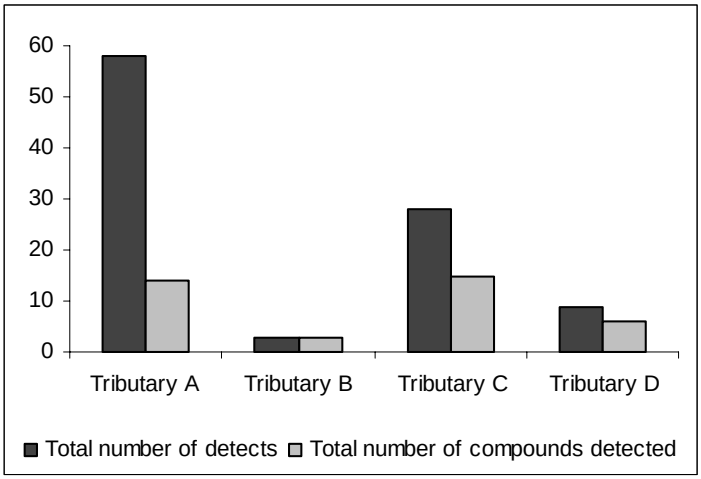

Figure 1. Summary data South Esk priority catchment chemical screening, comparing numbers of pesticide detections at the locations sampled.

When to sample?

Pesticides are applied to either prevent or treat disease and/or pests, and tend to be applied at certain times of the agricultural year. Targeting monitoring during these times of high use offers the best chance of determining if environmentally significant concentrations are reaching watercourses. A good understanding of land use in the catchment is vital to predict with any confidence when peak concentrations of certain compounds are likely to occur. A classic example of this is metaldehyde, the active ingredient in slug pellets. Metaldehyde is typically applied in late autumn/early winter, and while SEPA have only included metaldehyde routinely for the past few years, results show a clustering of detections over this period. Year-round sampling is more resource intensive, but can provide information on exposure pathways and whether constant low level exposure can lead to impacts on stream ecology.

How to sample?

Pesticide releases into the environment are generally episodic in nature, and therefore collecting data that can provide a representative picture of pesticide behaviour within catchments is difficult. Usage patterns vary with land use and seasonality. In addition, the range of plant protection products that are used from year to year can change as new products are approved for use and existing products are removed from the market. There are also a number of different potential routes by which compounds can reach watercourses and these need to be considered when designing catchment specific monitoring. SEPA employs a variety of techniques in its pesticide monitoring program to improve our understanding of pesticide concentrations in these catchments. Each method has its strengths, and the selection of the most appropriate approach is driven by the questions to be answered.

Spot sampling

Regular collection of discreet water samples is the simplest and most widely used method of monitoring, and provides valuable spatial and temporal information on pesticide concentrations within the catchments monitored. This is particularly useful when identifying “hot spot” locations, as demonstrated by the results of spot sampling in the Ythan catchment presented in Figure 2. This is the method currently being used in the initial screening of the priority catchments.

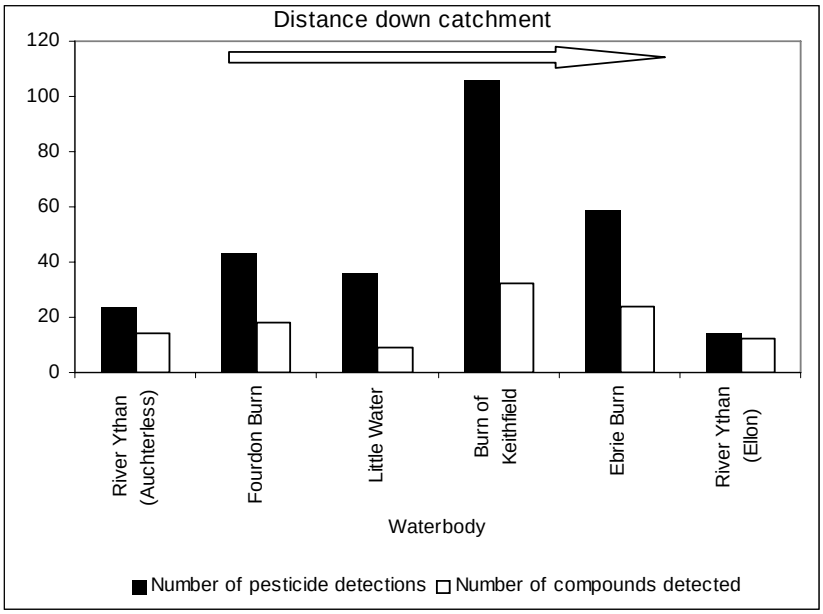

Figure 2. Summary data from pesticide screening in the River Ythan and its tributaries, comparing numbers of pesticide detections at the locations sampled.

Event sampling

Automated samplers can be deployed to carry out a variety of sampling programs. They can be set to trigger in response to rainfall, collecting samples at regular intervals to capture any flush of pollutants in the river resulting from overland runoff or leaching. This provides high resolution data on peak concentrations mobilised via runoff. Evidence from event sampling in the Lunan catchment shows that, while some compounds move with the sediment, this is not always the case, as demonstrated by the graphs presented in Figures 3 and 4. Auto-samplers can also be used to collect composite samples over periods of intense pesticide usage, or to carry out more frequent spot samples over targeted periods.

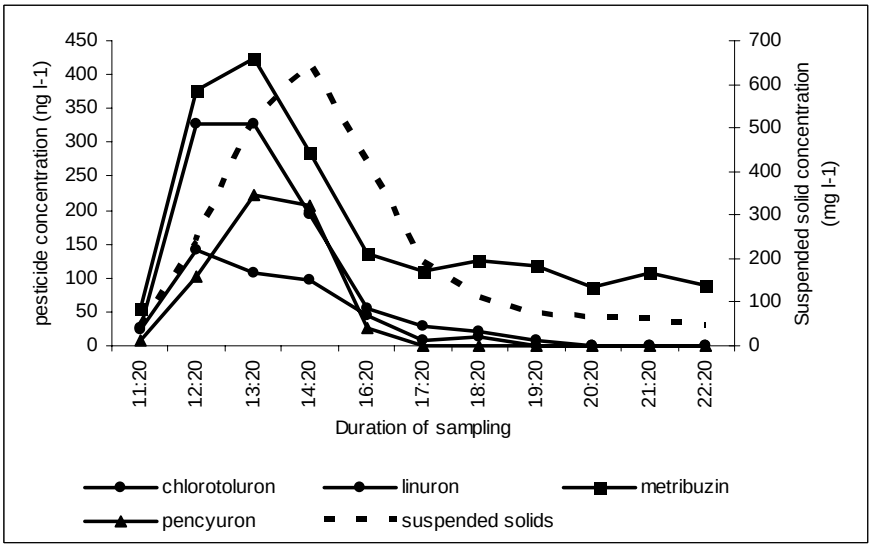

Figure 3. Event monitoring in the Lunan Water catchment: concentrations of pesticides measured in samples collected hourly during rainfall event, Oct 2010 (Location 1).

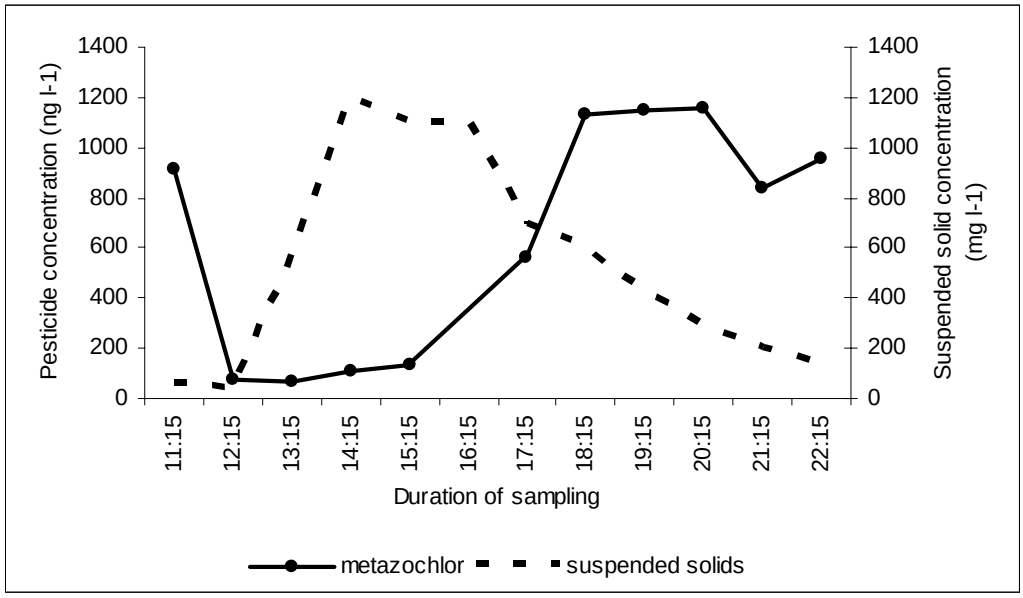

Figure 4. Event monitoring in the Lunan Water catchment: concentrations of pesticides measured in samples collected hourly during rainfall event, Oct 2010 (Location 2).

Passive samplers

Passive samplers are in-situ devices which allow uptake of chemicals directly from the water column. They therefore offer an alternative approach to providing time-integrated data, capturing peak concentrations which may otherwise be missed using typical spot sampling techniques. There has been a significant amount of research and development of these tools over recent years and SEPA is currently considering how these tools may be incorporated into current monitoring approaches. The samplers are typically deployed for several weeks at a time, during which time substances accumulate on their surface. The substances can then be extracted from the sampler and analysed using existing methodologies. The data may then be used qualitatively, as part of the screening approach to identify "hotspots", or quantitatively to provide an average concentration over the sampling period.

What to look for?

This is a serious challenge for any pesticide monitoring programme. Over 200 pesticides are currently approved for agricultural use in the UK, and more are being developed all the time; covering all possible compounds in analysis would be prohibitively expensive and time-consuming. SEPA has therefore developed a number of analytical methods designed to detect the most commonly used compounds (including a range of herbicides, insecticides, fungicides and molluscides) which are constantly reviewed and re-prioritised to reflect emerging issues. In addition to currently used compounds, historic monitoring shows that some withdrawn compounds have the ability to be persistent and may be detected in the water environment many years after they have been withdrawn from use. It is therefore important to continue to monitor for these types of compounds and consider their potential for longer term impacts.

The recent usage patterns of isoproturon and chlorotoluron provide a good example of how quickly trends in pesticide usage can change. Both are herbicides, used mainly to control

grasses and other weeds amongst cereal crops. Isoproturon was the most commonly used of the two before being withdrawn from use in 2009, after which time chlorotoluron use has increased. The results of this change in usage can be seen clearly in Figure 5, which shows results of routine monthly sampling in the River Ugie between 2007 and 2011. As the concentration of isoproturon detected in the river has reduced, chlorotoluron concentrations have increased.

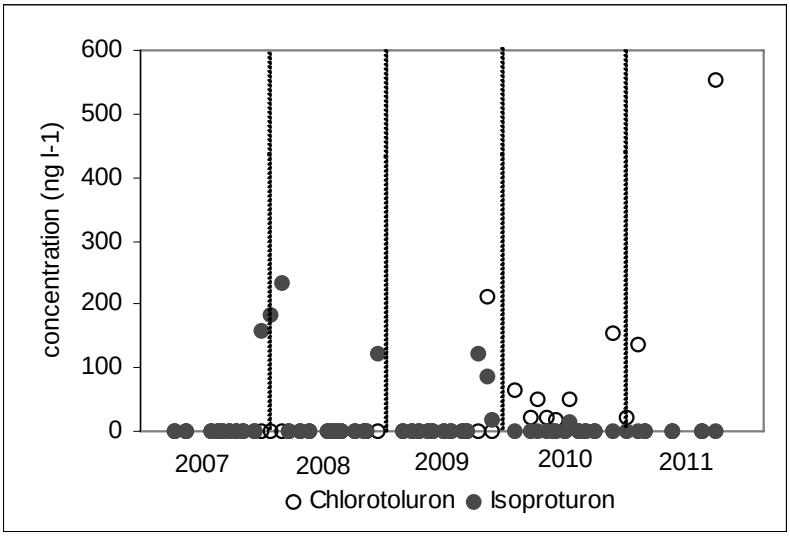

Figure 5. Concentrations of isoproturon and chlorotoluron measured in the River Ugie, 2007-2011.

CONCLUSIONS

Contamination of surface and groundwater by pesticides has been identified as a cause for concern in some of Scotland's most sensitive catchments, and a potential source of water quality and ecology downgrades. With limited historic data available, SEPA has developed and implemented a comprehensive programme of pesticide monitoring to identify areas of impact and provide information on potential sources and pathways of these compounds within catchments. A range of techniques and approaches are being used, with information on land use and usage patterns feeding into the process, along with chemical and ecological monitoring. The results generated will help to target and reduce diffuse pesticide inputs in impacted catchments, with an ultimate goal of improving water quality and returning failing water bodies to good ecological status.

ACKNOWLEDGEMENTS

The help of the many SEPA staff involved in this work and the ongoing support from local stakeholders and partnership groups in gratefully acknowledged.

PESTICIDES IN THE RIVER UGIE – DEVELOPING A CATCHMENT MANAGEMENT APPROACH TO PROTECT A DRINKING WATER SOURCE

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Summary: The catchment of the River Ugie in north-eastern Scotland is an area of active farming and the source of drinking water for supply to the town of Peterhead and surrounding areas. On occasions, the raw waters contain levels of plant protection products that are in conflict with standards to protect drinking water. Scottish Water is working collaboratively in the catchment to identify key sources and transport pathways. Catchment monitoring has been implemented as a method to identify sources and pathways to assist in the identification of potential catchment interventions to protect the source of drinking water. A rolling programme of raw water (river water) monitoring at 10 locations across the catchment commenced in May 2011.

INTRODUCTION

The River Ugie, in the north-east of Scotland, is the source of drinking water for the town of Peterhead and the surrounding area. The abstraction point for the drinking water supply is low in the catchment, above the tidal limit. The catchment of the Ugie is actively farmed and comprises arable, livestock and mixed farms. During the course of each year, the quality of the water from the Ugie is affected by a range of plant protection products (referred to as pesticides hereafter), sometimes to the extent that drinking water quality standards are not adhered to. This paper outlines the emerging catchment management approach that Scottish Water is taking, in partnership with others, to address the problem of pesticides in drinking water. The emerging catchment approach is intended to be complementary to conventional water treatment processes. In particular, this paper considers the role of catchment monitoring in identifying sources of pesticides and pathways of transmission into the River Ugie, as an essential pre-cursor to selecting effective catchment measures.

Partnership working is central to the approach being taken within the River Ugie. Scottish Water has been collaborating with the Scottish Environment Protection Agency (SEPA) on the approach and all results from the monitoring are shared with SEPA and other stakeholders within the catchment.

MONITORING THE CATCHMENT

Monitoring of the river water near the abstraction point has been on-going since 2002. This monitoring has shown that the river water, at times, carries pesticides associated with agricultural activities. However, it has not previously been established where the load arises. Given that the catchment is actively farmed, the number of potential sources and arisings of different pesticides may be considerable.

The purpose of undertaking monitoring within the Ugie catchment itself was to identify the areas of land that appear to be contributing the greatest load of pesticides. A catchment monitoring strategy was devised and implemented at the end of May 2011. The catchment was sub-divided into 10 components and the sample frequency was set at 2 weeks. In September 2011 the sampling frequency was increased to weekly. Sampling locations were identified on the basis of ease of access; hence all locations are on bridges on public roads.

Monitoring in the catchment provides data for a baseline, against which future improvements may be measured. Additionally, it is intended that monitoring of the catchment will provide the evidence-base to justify the implementation of measures. (It must be noted that implementation of catchment measures to protect the quality of raw waters is beyond the scope of this paper.)

The River has two main tributaries, the North and South Ugie. Catchment sample points are located on both these main tributaries, as well as the smaller tributaries. Table 1 and Figure 1 below identify the sample locations.

Table 1: Sample point locations within the River Ugie catchment

Sample point No.	Sample point location	Waterbody ID	Watercourse
1	Bridge of Buthlaw (close to Flushing)	23217	Faichfield Burn
2	Bridge at Longside (Main St. close to Longside WwTW)	23225	Burn of Ludquharn
3	Bridge at Braehead (A950) close to Longside	23224	South Ugie Water – Stuartfield to Longside
4	Bridge at Strichen (A981)	23222	North Ugie Water – upper catchment
5	Baluss Bridge (A952) close to Mintlaw	23224	South Ugie Water – Stuartfield to Longside
6	Bridge at Mill of Bruxie (A950)	23228	Leeches Burn
7	Old Maud Bridge - close to Maud (B9106)	23230	South Ugie Water – New Deer to Stuartfield
8	Bridge at Mill of Gaval (close to Fetterangus)	23221	North Ugie Water – lower catchment
9	Bridge of Rora (1.6 mile to the north from Longside)	23221	North Ugie Water – lower catchment
10	Sample point close to the Artlaw Bridge	23215	River Ugie North/South

There were four major pesticides being detected in the River Ugie and in the final water from Forehill WTW during 2010. Table 2 below presents the suite of determinands selected for analysis, which contains, in bold, the pesticides that were found in final treated waters in 2010. At the time of sampling, local weather conditions are recorded. Simple flow monitoring is also undertaken at the time of sampling, as this allows an estimation of load to be calculated.

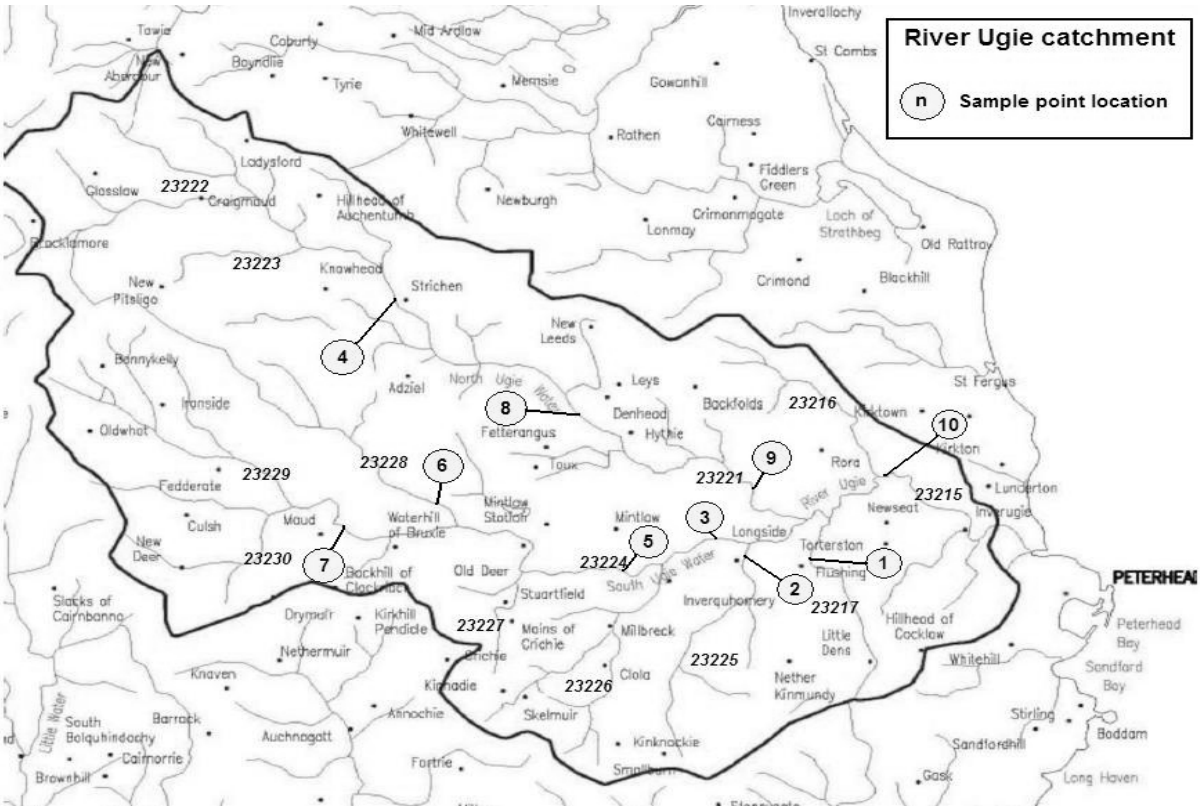

Figure 1: Location of catchment monitoring sites in Ugie catchment

Table 2: Analytical suites for pesticide analysis

Component	Units	LOD	Component	Units	LOD
2,4-D	µg/l	<0.003	**MCPA**	µg/l	<0.004
2,4-DB	µg/l	<0.004	MCPB	µg/l	<0.005
Atrazine	µg/l	<0.005	MCPP	µg/l	<0.003
Bentazone	µg/l	<0.004	**Metaldehyde**	µg/l	<0.006
Bromoxynil	µg/l	<0.005	**Metazachlor**	µg/l	<0.004
Carbendazim	µg/l	<0.005	Metsulfuron-methyl	µg/l	<0.006
Chlorotoluron	µg/l	<0.004	Monolinuron	µg/l	<0.006
Chloroxuron	µg/l	<0.004	Monuron	µg/l	<0.005
Dicamba	µg/l	<0.008	Propazine	µg/l	<0.006
Dichlorprop	µg/l	<0.004	Simazine	µg/l	<0.006
Diuron	µg/l	<0.003	Thifensulfuron-methyl	µg/l	<0.009
Ioxynil	µg/l	<0.004	Tribenuron-methyl	µg/l	<0.005
Isoproturon	µg/l	<0.004	Triclopyr	µg/l	<0.015
Linuron	µg/l	<0.002	Trietazine	µg/l	<0.003

SUPPORTING DETERMINANDS					
Colour	mg/l Pt/Co	<2	Turbidity	NTU	<0.3
Hydrogen ion	pH value	-			

RESULTS

Sampling commenced in May 2011. The baseline data are emerging into a comprehensive picture of the type, extent and duration of pesticide usage in the catchment. However, as there is only a limited time series at time of writing, only preliminary results are available.

Firstly, the catchment monitoring in 2011 is showing a wide spectrum of pesticides in use within the catchment. During the period from May to October 2011, seasonal changes in pesticide usage are apparent. For instance, herbicide usage is apparent over the summer months in both the North and South Ugie. By mid-September 2011, a much broader spectrum of pesticides was detected across the catchment.

The water treatment works at Forehill does not have any formal pesticide removal capability. Many results show that levels of pesticides in the raw water are very low, well within any drinking water standard. However, on occasions the concentrations found in the raw waters in the catchment were sufficiently high to contravene the drinking water quality standards (PCV is set at 0.1 µg/l for any individual pesticide and 0.5 µg/l for all pesticides combined at customers' taps). A full analysis of the results is beyond the scope of this paper, but will be conducted during 2012.

Figure 2 provides a summary of the most significant results from the catchment monitoring between May – November 2011. The PCV does not apply to the raw water itself, but as the works at Forehill has no formal pesticide treatment capability, results in the raw water are strongly correlated with failure at customers' taps. Dilution within the river as it flows further downstream provides some additional benefit. The results show that 5 pesticides have presented concerns thus far in 2011, at a number of sample locations.

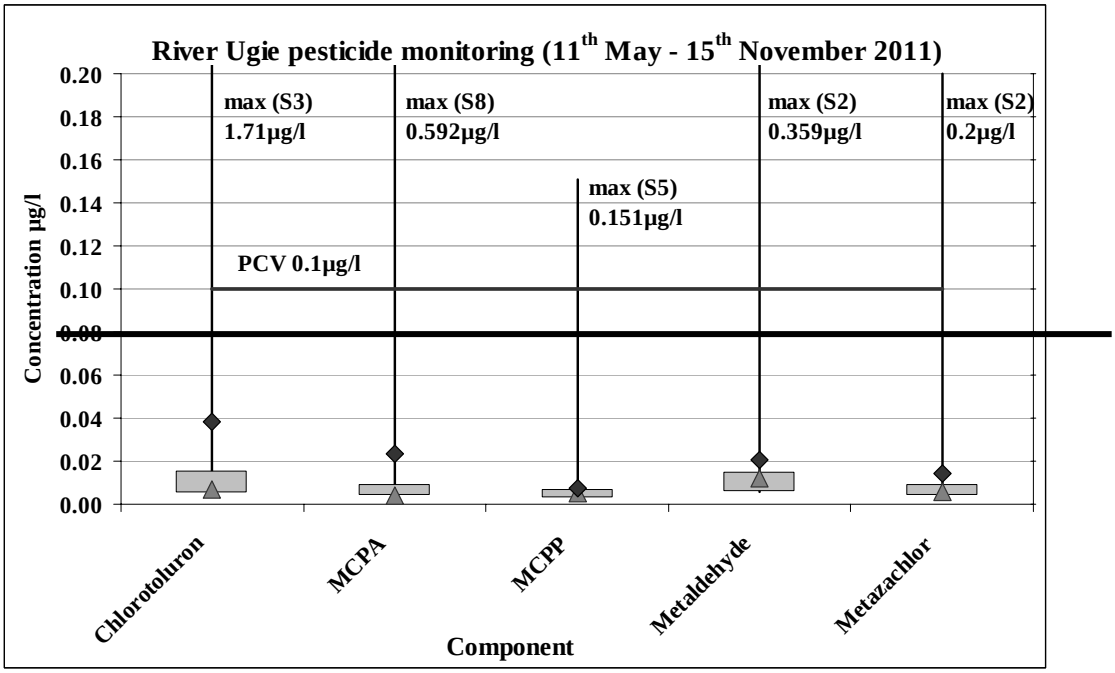

Figure 2: Summary of the most significant results from catchment monitoring on the River Ugie (11 May – 15th November 2011). Box and whisker plot show: Mean – black diamond; Maximum – upper "whisker"; Minimum – lower "whisker"; Median – grey triangle; Interquartile range – grey box

DISCUSSION

Protection of raw water quality and compliance with drinking water standards is the key motivation for Scottish Water to be undertaking catchment monitoring of pesticides. Implementation of catchment measures may provide alternatives to conventional treatment, where treatment costs are disproportionate or where pesticides are unaffected by conventional treatment methods (such as metaldehyde).

The benefits of catchment monitoring are that it will allow measures to be targeted on localities that either provide a significant loading of pesticides into the raw waters or where pathways for the transport of pesticides are significant. Catchment monitoring is the first step in developing an evidence-base to inform the identification of measures.

At the present time, all monitoring is being conducted by samplers taking spot samples on a weekly rolling programme. Analysis is conducted in Scottish Water's laboratories. Whilst this approach provides full quality assurance (UKAS accreditation) of the results and a robust data set, the process is time-consuming, comparatively expensive and availability of results is dependent upon the laboratory schedules. A key lesson to date concerning catchment monitoring is that in order to extend catchment monitoring in future, significant enhancements in efficiencies are required, without compromising the quality of the results. For scientists dependent upon catchment monitoring, this is a significant challenge for the future.

Another key lesson from the catchment monitoring is that the results themselves are of interest to stakeholders within the catchment. Consequently, Scottish Water has had to improve communication with stakeholders and ensure that results are shared rapidly and openly. This requires explaining the limits of spot sample data results and ensuring that stakeholders understand the context in which the data will be used. For instance, working with stakeholders to ensure that they understand that the data will help inform how Scottish Water can contribute to developing effective measures in the catchment.

ACKNOWLEDGEMENTS

The authors acknowledge support of Scottish Water Laboratory Services in taking the samples and collating results.

The authors acknowledge the River Ugie catchment stakeholders who are involved in the Sustainable Land Management project.

The authors acknowledge the work of the Diffuse Pollution Management Advisory Group (DPMAG) for setting the strategic direction on rural diffuse pollution management in Scotland.

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GLOSSARY OF TERMS AND DESCRIPTIONS

LOD	Limit of Detection
PCV	Permitted Concentration Value
µg/l	Micrograms per litre
2,4-D	(2,4-dichlorophenoxy)acetic acid
2,4-DB	4-(2,4-dichlorophenoxy)butyric acid
MCPA	2-(4-chloro-2-methylphenoxy)acetic acid
MCPB	4-(4-chloro-2-methylphenoxy)butanoic acid
MCPP	2-(4-chloro-2-methylphenoxy)propanoic acid

Component	Description
2,4-D	Systematic phenoxy herbicide used in the control of broadleaf weeds
2,4-DB	Systematic phenoxy herbicide used in the control of broadleaf weeds
Atrazine	Systematic herbicide used in the control of broadleaf weeds
Bentazone	Contact herbicide used to control broadleaf weeds in winter and spring cereals
Bromoxynil	Herbicide used for post-emergent control of annual broadleaf weeds
Carbendazim	Widely used broad-spectrum benzimidazole fungicide
Chlorotoluron	Soil-acting and contact herbicide used to control broadleaf weeds in winter and spring cereals
Chloroxuron	Phenylurea herbicide used for the control of annual grasses and broadleaf weeds
Dicamba	Systematic herbicide used in the control of broadleaf weeds
Dichlorprop	Systematic phenoxy herbicide used in the control of broadleaf weeds
Diuron	Pre-emergence residual herbicide for total control of weeds and mosses in non-crop areas and woody crops
Ioxynil	Contact herbicide used to control broadleaf weeds
Isoproturon	Soil-acting and contact herbicide used to control broadleaf weeds in winter and spring cereals
Linuron	Herbicide used to control broadleaf weeds and annual grass
MCPA	Selective, widely-used phenoxy herbicide
MCPB	Selective, widely-used phenoxy herbicide
MCPP (Mecoprop)	Selective, widely-used phenoxy herbicide
Metaldehyde	Mulluscicide used to kill slugs, snails and other gastropods
Metazachlor	Herbicide used for pre-emergence and early post-emergence control of winter and annual grassed and broad-leaved weeds
Metsulfuron-methyl	Herbicide used to control broadleaf weeds and annual grass
Monolinuron	Herbicide used to control broadleaf weeds and annual grass
Monuron	Phenylurea herbicide used mainly for the weed control of non-crop areas
Propazine	Pre-emergence selective triazine herbicide
Simazine	Pre-emergence selective herbicide used for control of broadleaf weeds and annual grass on a variety of deep-rooted crops
Thifensulfuron-methyl	Pre-emergence selective herbicide used for control of broadleaf weeds
Tribenuron-methyl	Pre-emergence selective herbicide used for control of broadleaf weeds
Triclopyr	Systematic herbicide used in the control of broadleaf weeds
Trietazine	Herbicide used to control broadleaf weeds

THE SUPPRESSION OF COMMON COUCH GRASS (*ELYTRIGIA REPENS*) BY BUCKWHEAT

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Summary: A container-based experiment was carried out to investigate the suppression of common couch grass (*Elytrigia repens*) by buckwheat sown at a range of sowing densities, timings and varieties, against a range of planting lengths of couch rhizomes. Delaying buckwheat sowing until three weeks after the couch rhizomes were planted resulted in 39.6% more couch fresh weight than when buckwheat was planted at the same time as rhizomes. In practical terms delaying drilling of a buckwheat cover crop for up to three weeks after cultivating may result in reduced levels of weed suppression and a higher weed burden. The buckwheat density should be high (80-160 kg/ha) to aid weed suppression and there were differences between the suppressive effects of the two buckwheat varieties (cv. Pyra and Billy), as Pyra was more vigorous in growth habit.

INTRODUCTION

Common couch is a major problem in organic farming systems, due to its competitive nature and ability to spread by both seed and rhizomes. Where numerous cultivations are carried out the rhizomes are chopped into smaller fragments and often dragged to the soil surface where new shoots grow readily. In 2007 an EU survey investigating the status of weed management in organic farming (Glemnitz *et al*., 2007) was conducted and common couch was listed as one of the most problematic weeds in all EU member state countries.

Buckwheat is a broad-leaved crop, in the *polygonaceae* family and is commonly grown for grain or as a cover crop. It has the ability to suppress weeds (Bjorkman & Shail, 2010) via competition and allelopathic properties (Batish *et al*., 2002, Iqbal *et al*., 2003, Golisz *et al*., 2007). It is very fast growing, which enables it to out-compete the weeds if an early dense plant cover is established. However, it is not frost tolerant, so is only spring sown in the UK. The aim of this investigation was to determine the levels of suppression of common couch (*E. repens*) from a range of sowing densities, sowing timings and varieties of buckwheat.

MATERIALS AND METHODS

Plastic containers measuring 23.5 cm x 30.5 cm x 15 cm deep were filled with a standard soil based medium container compost (Levington C2) and watered to field capacity before any seed or couch rhizomes were sown. All containers were placed outside on a concrete standing area at ADAS Boxworth. The experiment was a fully randomised design with four replicate blocks, with treatments listed in Table 1.

Couch rhizomes were sown at two different lengths, either 20 cm lengths x four pieces, or 10 cm lengths x eight pieces per container. All rhizomes were weighed before sowing. When four pieces of rhizomes were used all were from the growing tip end of the rhizome piece. When eight pieces were sown four were from the growing tip end and the other four were from just behind that region on the rhizome. The total number of nodes per rhizome was counted and recorded at the time of weighing. Two varieties of buckwheat (cv. Billy (1) and Pyra (2)) were included at 2 different sowing densities equivalent to a 'normal' and double field rate (80 kg/ha and 160 kg/ha). Four timings of sowing the buckwheat were included in the experiment with a one-week interval between them. The first timing (T1) was on the same day that the couch rhizomes were planted, 31 March 2009, (T2) 7 April 2009, (T3) 14 April 2009 and (T4) 21 April 2009.

Table 1. The treatment list (repeated for the four different sowing dates).

Treatment	Buckwheat field rates (kg/ha)	Buckwheat density/ container	Couch rhizomes/ Length container)
Couch alone	n/a	n/a	20cm x 4
Couch alone	n/a	n/a	10cm x 8
Couch + BW cv. 1	80	0.6g (30 seeds)	20cm x 4
Couch + BW cv. 1	80	0.6g (30 seeds)	10cm x 8
Couch + BW cv. 2	80	0.6g (30 seeds)	20cm x 4
Couch + BW cv. 2	80	0.6g (30 seeds)	10cm x 8
Couch + BW cv. 1	160	1.2g (60 seeds)	20cm x 4
Couch + BW cv. 1	160	1.2g (60 seeds)	10cm x 8
Couch + BW cv. 2	160	1.2g (60 seeds)	20cm x 4
Couch + BW cv. 2	160	1.2g (60 seeds)	10cm x 8

Buckwheat vigour was assessed on 17 June 2009, using a visual score 0-9 scale, where 9= healthy plants and 0= dead plants. Plant counts (number per container) and fresh weight (g per container) assessments of the couch and buckwheat were carried out on 18 June 2009. The fresh weight assessment required all plant material to be cut at the base of the stem and weighed as separate species. The containers were then left outside and regularly watered and any re-growth of the couch plants was assessed on 30 July 2009.

RESULTS

The buckwheat variety Pyra was more vigorous in this particular experiment compared to variety Billy. Pyra scored a mean of 8 and Billy scored a mean of 4 based on a scale of 0-9.

The full data set were analysed against couch fresh weight using analysis of variance in Genstat (Table 2). The presence of buckwheat always reduced the fresh weight of the couch compared to the couch sown alone. The highest levels of suppression from the buckwheat were always in the early sowing timings T0 and T1, when buckwheat was sown with the couch rhizomes (T0) or one week later (T1). By the time the couch had been in the soil for three weeks before the buckwheat was sown the level of couch suppression was reduced in all treatments.

Table 2. The mean couch shoot fresh weight (g), from a range of treatments including buckwheat variety, sowing density, sowing timing and couch rhizome length.

	Mean couch fresh weight (g) (sowing date)			
Treatments *Plant, variety, density, length*	At sowing (T0)	Post 1 week (T1)	Post 2 weeks (T2)	Post 3 weeks (T3)
Couch 20cm	146.9	n/a	n/a	n/a
Couch 10cm	168.3	n/a	n/a	n/a
BW (cv.1) low, couch 20cm	83.9	109.3	116.3	135.7
BW (cv.1) low, couch 10cm	125.5	129.7	111.0	135.7
BW (cv.2) low, couch 20cm	95.5	126.3	128.1	129.2
BW (cv.2) low, couch 10cm	109.4	98.2	86.4	139.5
BW (cv.1) high, couch 20cm	82.2	74.7	105.5	127.7
BW (cv.1) high, couch 10cm	68.9	88.2	76.0	123.1
BW (cv.2) high, couch 20cm	92.4	96.7	112.0	136.8
BW (cv.2) high, couch 10cm	64.8	87.3	103.5	144.3
s.e.d		*17.82*		
df		*96*		

(*BW= buckwheat*)

The individual treatments do show some significant differences. For example there was a significant difference (p=<0.001) between sowing dates, as shown below (Fig. 1). Delaying buckwheat sowing until three weeks after the couch rhizomes were planted resulted in 39.6% more couch fresh weight than when buckwheat was planted at the same time as rhizomes.

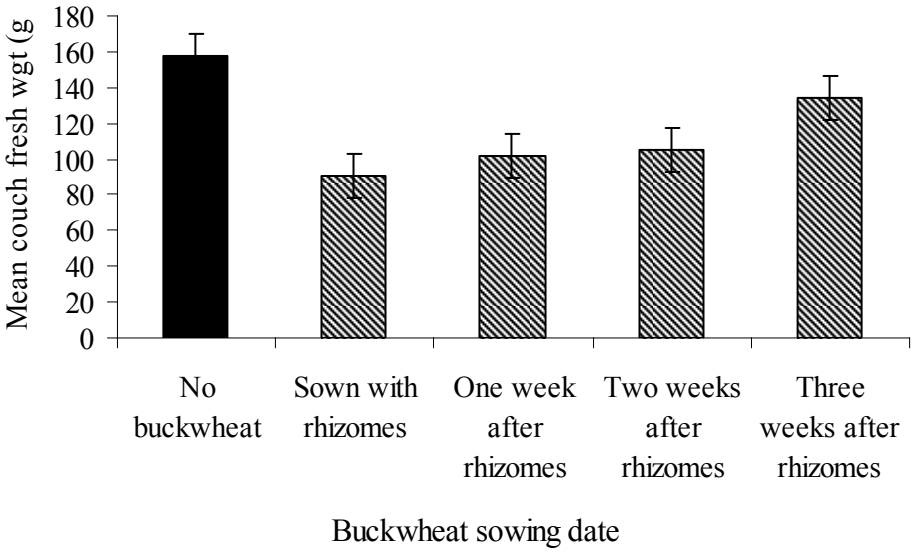

Figure 1. The mean couch shoot fresh weight (g) against four timings of buckwheat sowing dates, meaned across couch density, buckwheat density and variety. (*Bars represent SEM*)

There was a significant difference (p= <0.001) between the two sowing densities of buckwheat, meaned across all treatments, with a mean of 116g couch shoots/container at the low density and 99g couch shoots/container at the higher sowing density. At the high density of buckwheat the couch fresh weight was a mean of 77g/container when the buckwheat was sown on the same day as the rhizomes (T0) and this increased to 133g/container when the buckwheat was sown three weeks later (T3). There were no significant differences (p=0.170) between couch rhizome planting density (lengths) and sowing dates, as they ranged from a couch fresh weight of 88.5g and 92.1g for 20cm lengths and 10cm lengths respectively at T0, to 132.4g and 135.7g at T3. By taking buckwheat variety out as a factor and meaning the two varieties there were also no significant interaction (p= 0.145) between treatments of buckwheat density, couch density and sowing date (Table 3).

Table 3. The mean couch shoot fresh weight (g), from a range of treatments including buckwheat sowing density, timing and couch rhizome length, meaned across two buckwheat varieties.

	Mean couch fresh weight (g) (sowing date)			
Treatments	At sowing (T0)	Post 1 week (T1)	Post 2 weeks (T2)	Post 3 weeks (T3)
Couch 20cm	146.9	n/a	n/a	n/a
Couch 10cm	168.3	n/a	n/a	n/a
BW low, couch 20cm	89.7	117.8	122.2	132.5
BW low, couch 10cm	117.5	114.0	98.7	137.6
BW high, couch 20cm	84.5	85.7	108.8	132.3
BW high, couch 10cm	66.9	87.8	89.8	133.7
	s.e.d		15.43	
	df		96	

(*BW= buckwheat*)

The full data set were also analysed against the number of couch shoots per container, using analysis of variance in Genstat (Table 4). There were always more couch shoots per container from the rhizome pieces that had been cut into the shorter lengths of 10cm compared to 20cm lengths in all treatments and timings, except where buckwheat was sown two weeks after the rhizomes (T2). From the analysis, couch rhizome density as a factor alone was significant (p=<0.001) in terms of couch shoot numbers. The number of nodes per rhizome piece was counted at planting and there was a mean of 22 nodes/container for the 20 cm length pieces, compared to a mean of 26 nodes/container for the 10 cm length pieces.

Drawing conclusions from couch shoot counts alone would have been misleading in this experiment and having fresh weights of the couch has provided a clearer understanding of the treatment effects. For example the differences between the number of couch shoots at T0 (rhizome sowing) compared to T3 (three weeks later) are often very minimal or even less in a few treatments (Table 4). However, the fresh weights of the couch always show an increase between T0 and T3 (Table 3).

Table 4. The mean number of couch shoots per container, from a range of treatments including buckwheat variety, sowing density and timing and couch rhizome length.

	Mean no. couch shoots/container (sowing date)			
Treatments	At sowing (T0)	Post 1 week (T1)	Post 2 weeks (T2)	Post 3 weeks (T3)
Plant, variety, density, length				
Couch 20cm	16.72	n/a	n/a	n/a
Couch 10cm	22.25	n/a	n/a	n/a
BW (cv.1) low density, couch 20cm	17.50	16.25	22.50	14.25
BW (cv.1) low density, couch 10cm	19.00	22.50	20.50	21.50
BW (cv.2) low density, couch 20cm	15.25	17.75	18.00	20.25
BW (cv.2) low density, couch 10cm	20.00	21.75	19.50	22.00
BW (cv.1) high density, couch 20cm	15.00	18.50	20.25	22.00
BW (cv.1) high density, couch 10cm	20.25	22.75	23.50	22.50
BW (cv.2) high density, couch 20cm	11.75	15.50	20.75	17.25
BW (cv.2) high density, couch 10cm	23.38	19.50	18.75	20.50
s.e.d		*3.763*		
df		*97*		

(*BW= buckwheat*)

DISCUSSION

This experiment included a range of sowing dates of buckwheat to mimic field cultivation and drilling on the same day, or drilling one, two or three weeks after 'cultivating' the land. It can be concluded from the results that the highest levels of couch suppression from the buckwheat always occurred when the buckwheat was sown at the same time as the rhizomes or within a week of planting the rhizomes. When buckwheat planting was delayed up to three weeks after the couch rhizomes were planted there was a 39.6% increase in the couch shoot fresh weight, due to the fact that the couch had no competition for space or resources in this period of time allowing it to grow quickly. This was obviously in controlled conditions where moisture was not limiting, there was no competition from other weeds and the rhizomes had all been planted at a shallow depth. In a field situation the rhizomes would have been at a range of different depths in the soil profile resulting in germination over a more protracted time period. If the weather conditions had been dry at cultivation and drilling then the couch rhizomes may have been slower to germinate and emerge. It is highly likely, particularly in an organic situation, that there would also have been a high level of competition from other weed species potentially resulting in a lower level of couch emergence. However in practical terms these results are indicating that drilling a cover crop, in this case buckwheat, very soon after cultivating increases the levels of couch suppression, through plant competition.

The experiment included a number of different rhizome lengths to mimic cultivations that would have occurred before drilling, as certain machinery would chop up the rhizomes into shorter lengths. The results of this experiment showed that for a given weight of rhizomes

those cut into shorter pieces of rhizome (10cm lengths) had more nodes/container and slightly more couch shoots/container than rhizome pieces cut to 20cm lengths. In practice multiple cultivations would cut the rhizomes into shorter lengths and this may be detrimental to couch control. However this is currently being investigated further in a field experiment as this relationship has been based on a container experiment in controlled conditions.

It can be concluded that in this experiment the buckwheat variety Pyra was more vigorous at controlling/suppressing couch compared to variety Billy. There are a limited number of buckwheat varieties available in the UK and further work could investigate varietal differences versus weed suppression. These results show that the density of buckwheat sowing does have an effect on the level of couch control and that high sowing rates should always be used. However, limited density levels have been compared in these trials and further work should incorporate sowing density and weed suppression levels.

Based on this work and other information the most appropriate way to deploy these findings would be to leave the stubble uncultivated until the buckwheat is ready to be sown and carry out both cultivating and drilling on the same day. This would prevent the couch emerging before the buckwheat has a chance of germinating and establishing a good cover crop.

ACKNOWLEDGEMENTS

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ASSESSMENT OF AGRICULTURAL SOIL PHOSPHORUS MANAGEMENT IN SCOTLAND'S DIFFUSE POLLUTION PRIORITY CATCHMENTS

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Summary: Phosphorus loading from agricultural sources is listed as a specific diffuse pollution pressure in the 14 priority catchments established by SEPA in 2010 under the River Basin Management Plan. This paper reviews current efforts to delineate best management for agricultural usage of phosphorus (P) and a data set of Soil Test Phosphorus (STP) results taken from SAC advisory soil database between mid 1996 and 2010. A preliminary assessment has suggested that no more than 20% of Scotland soils can be shown to be above the targeted moderate status. Accurately proportioning the loading of P to surface waters attributable to soil mediated processes is important if we are to determine the suitability of this targeted approach to P management. If soil mediated sources are shown to be primary, then attributing this to the probable small proportion of Scotland's agriculturally managed soils that are above the moderate status will be challenging.

INTRODUCTION

In 2010 the Scottish Environment Protection Agency (SEPA) launched the Diffuse Pollution Priority Catchments Programme to provide a focus for activities to help meet the water quality improvements required under Scotland's River Basin Management Plan and under the Water Framework Directive at the European Level. SEPA is initially targeting its diffuse pollution mitigation activities between 2011 and 2015 at 14 Diffuse Pollution Priority Catchments (Table 1) and has started detailed studies of these high priority river catchments to identify pollutant sources and possible mitigation actions. These studies will form the basis of detailed plans for co-ordinating the work of SEPA, its partners and other organisations in working with farmers to ensure the appropriate diffuse pollution mitigation actions are taken. This catchment-targeting approach (with an additional set of Diffuse Pollution Priority Catchments being targeted between 2015 and 2021 and a further set between 2021 and 2027) will focus appropriate diffuse pollution mitigation measures into each area.

Table 1. List of priority Catchments.

North Ayrshire Coast	River Irvine	River Ugie
River Ayr	River South Esk	Galloway Coastal
Eye Water	Buchan Coastal	River Deveron
River Doon	River Garnock	Stewartry Coastal
River Tay	River Dee (Grampian)	

A key message under the programme is that diffuse pollution from agriculture is a significant issue for groundwater, rivers, lochs, transitional and coastal waters. (SEPA, 2009)

The catchments are geographically spread across Scotland and represent the full range of agricultural production. Each catchment has been identified as having specific main pressures that has warranted its inclusion in the programme but one common thread across all the catchments is the identified risk from soil and non-soil mediated phosphorous (P) loading from agricultural activities.

The amounts of P applied annually to crops in these catchments along with P resource stored in the soil are now to be included in a national monitoring strategy. This paper provides an overview and recent changes that have occurred in P nutrient recommendations and what initial steps have been taken toward understanding soil mediated phosphorus loading to surface waters.

NUTRIENT RECOMMENDATIONS

In 2009 - 2010 SAC updated a range of its Technical Notes on fertiliser recommendation for all the main crops in Scotland (Table 2). The work was funded by the Scottish Government as part of its public good pollution prevention activities (funded under the Veterinary & Advisory Services programme) with the aim of helping balance productivity with the need to consider risk to water quality from agricultural diffuse pollution.

Table 2. List of SAC Technical Notes Updated in 2009 – 10.

TN621: Fertiliser recommendations for vegetables, minority arable crops and bulbs
TN622: Optimising the application of bulky organic fertilisers
TN623: Fertiliser recommendations for soft fruit and rhubarb crops
TN625: Nitrogen recommendations for cereals, oilseed rape and potatoes
TN632: Fertiliser recommendations for grassland
TN633: Phosphorus, potassium, sulphur and magnesium recommendations for cereals, oilseed rape and potatoes

A common change across all the Technical Notes is the approach to P recommendations. A stronger emphasis has been placed on basing recommendations on data obtained from soil analysis combined with information on crop yields. For soils with a moderate status for STP (4.5 to 13.4 mg/l based on SAC techniques) the recommendations for all crop types are targeted to replace plant offtake and avoid any further build up of soil P levels. For soils with high STP status (≥13.5 mg/l based on SAC techniques) the recommendations call for a fixed reduction in calculated rates for P to ensure that available soil resources are drawn down.

As part of its programme to target diffuse pollution and provide a compliance tool for Nitrate Vulnerable Zone (NVZ) areas the Scottish Government has also commissioned the development of PLANET Scotland (Planning Land Applications of Nutrients for Efficiency and the environmenT) which is a computer based nutrient management programme (http://www.planet4farmers.co.uk). PLANET Scotland provides field-level nutrient

recommendations for potassium, phosphorus, nitrogen, and sulphur based on the revised SAC Technical Notes. A key feature of PLANET is that at least one year of previous cropping history must be supplied to obtain a recommendation emphasising a budgeting approach. The programme also records the application of bulky organic fertilisers and directly accounts for their contribution toward meeting crop P requirements based on either custom or standard values for nutrient content of the bulky organic fertiliser. More details are available at http://www.sac.ac.uk/consulting/slurrymanagement/nmep5/.

SCOTLAND'S SOIL PHOSPHORUS RESERVES

Standard agronomic advice in Scotland recommends the build-up and maintenance of soil P at moderate STP level. This is achieved by applying P at rates exceeding crop requirements when STP levels are shown to be low.

The total amount of P already stored in Scotland's soils is of interest both from a soil protection perspective and as part of efforts to address diffuse pollution issues (Dobbie et al., 2011). Advisory soil data is the only widely available data that can provide a time series of change in soil P status against changing land use and nutrient additions.

Data has been extracted from mid 1996 to 2010 from the SAC soil analysis advisory database. This represents soil samples from over 19,000 individual farms that have been submitted from the 23 SAC agricultural office areas within Scotland. The data included extractable soil P (STP), soil pH and derived organic matter.

The full data set comprises >130,000 topsoil samples. The results of preliminary analysis of the data set are summarised below from the report to SEPA "Soil Phosphorus levels in diffuse pollution priority catchments", contract reference 30107 (Sinclair *et al.,* unpublished).

The annual distribution of sample numbers is shown in Figure 1, and the distribution of samples across Scotland is shown in Figure 2 for the each agricultural sub-region as defined by the Scottish Government (http://www.scotland.gov.uk/Topics/Statistics/19972/21087). The distribution by P status across the entire dataset is summarised in Figure 3 and Tables 3 and 4.

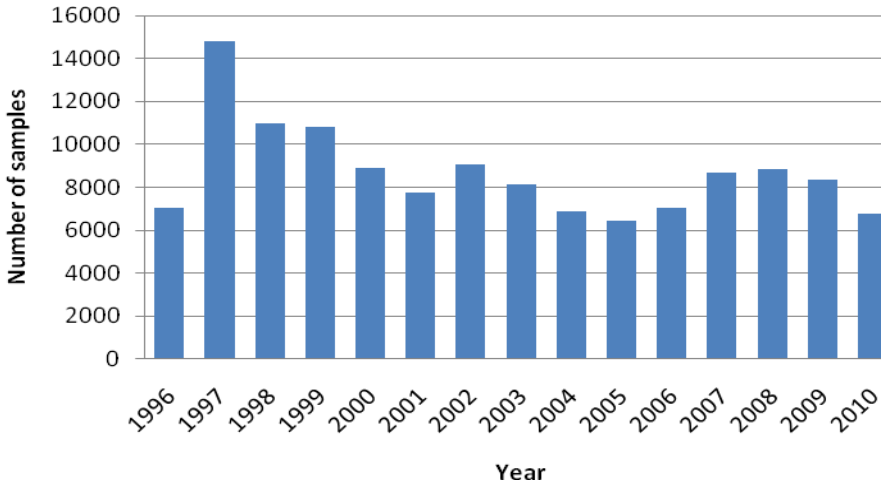

Figure 1. Total numbers of samples by year (1996-2010), 1996 is a part year **(**Source: Sinclair *et al*., unpublished).

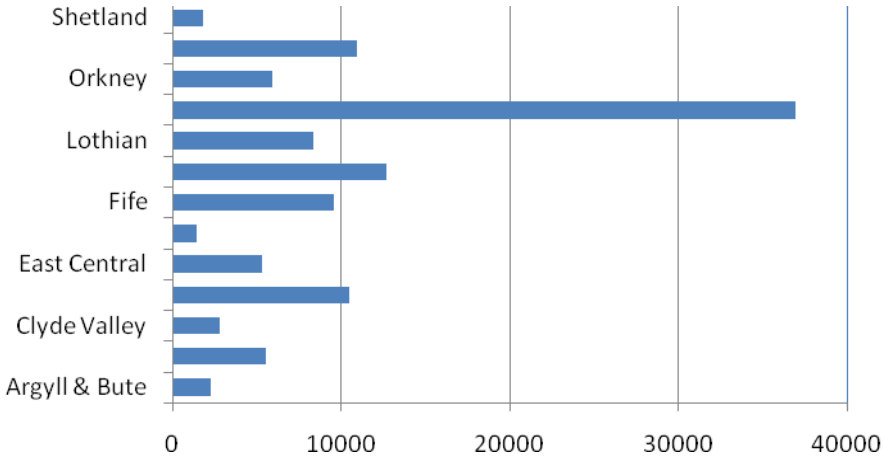

Figure 2. Total number of samples per sub-region (Source: Sinclair *et al.*, unpublished)

A preliminary estimate of the density of soil sampling across the various sub-regions, where the sample density would represent the averaged number of samples collected annually for each sub-region divided by the area of crops and grass (excluding rough grazing), shows that much of the predominately grassland areas in the Central and South Western region of Scotland are poorly represented in the data base. A basic assessment of the entire data shows that the majority of the fields tested are at or below the recommended moderate status for STP. There is no evidence in this database to suggest that there is a systemic over use of P arising from agricultural application of fertilisers as compared to current best practice.

Approximately 15% of those soils analysed required less than maintenance P applications. On an annual basis this has fluctuated with the highest being in 2008 at approximately 20% and the lowest at 10% in 2010.

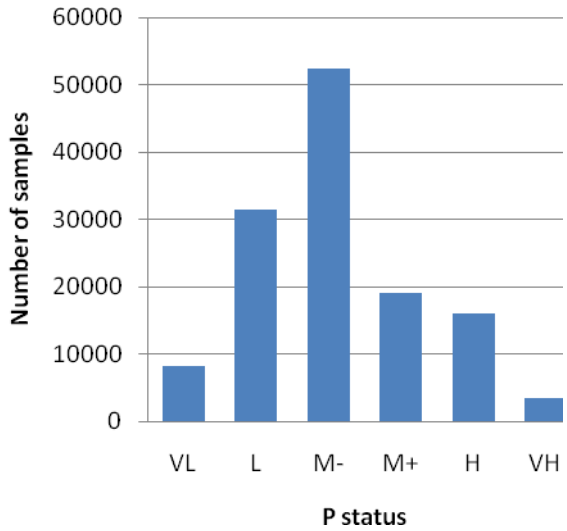

Figure 3. Distribution of soil P status within the complete dataset of ~130,000 samples. VL, very low; L, low; M, moderate; H, high; VH, very high (Source: Sinclair *et al.*, unpublished)

Table 3. Breakdown of P Status into percentage of the complete dataset.

Range(mg P/l)	< 1.7	1.7-4.4	4.4-9.4	9.4-13.4	13.4-30	>30
Percentage of total samples	6.2	24.1	40.2	14.6	12.3	2.6

(Source: Sinclair *et al*., unpublished)

Individually these fields are of interest since they represent a localised risk to surface water quality. It is unclear if these high P soils are from static set of fields within individual farms or are dynamic and arising as a result of how P is managed for crop rotations. Caution needs to be applied before extrapolating these results up to a national base since at best these results are representative of less than 20% of the land under agricultural management over a 4 year sampling cycle and only represent 4% on an annual basis.

DISCUSSION

As the Priority Catchment programme moves into its next stage, documented water quality improvement will be required within the 14 identified catchments if Scotland is to meet it is obligations under the Water Framework Directive. Of concern is the level and impact of soil mediated phosphorous loading. The release of nutrient advisory notes that incorporates diffuse pollution concerns along with the development of PLANET Scotland has been a first step in creating a baseline to establish best practice in agricultural usage of P. A preliminary assessment of the SAC advisory data has shown that it is possible that no more than 20% of Scotland's soils can be shown to be above the targeted moderate status. Accurately proportioning the loading of P to surface waters attributable to soil mediated processes is important if we are to determine the suitability of this targeted approach to P management.

Table 4. Distribution of samples by soil P status as a proportion (%) of total number of samples for individual years.

Sample year	VL	L	M-	M+	H	VH
1996	6.5	21.9	39.9	15.0	13.9	2.9
1997	5.7	23.3	38.9	15.8	13.1	3.2
1998	6.2	22.5	40.5	15.1	13.0	2.7
1999	4.9	21.7	42.7	15.3	12.8	2.6
2000	5.9	25.1	40.1	14.4	11.5	2.9
2001	8.4	24.2	40.5	13.6	11.1	2.2
2002	7.2	24.4	39.7	13.5	12.8	2.5
2003	6.7	25.2	40.4	13.7	11.5	2.4
2004	7.3	26.0	40.7	13.8	9.6	2.5
2005	5.9	25.6	43.0	12.7	10.3	2.4
2006	5.6	24.7	38.8	14.8	12.9	3.1
2007	3.9	20.9	40.3	16.4	16.0	2.5
2008	4.1	21.5	38.0	17.2	16.1	3.1
2009	8.3	28.4	40.0	12.5	9.0	1.8
2010	8.4	30.0	39.6	11.7	8.0	2.2

(Source: Sinclair *et al*., unpublished)

If soil mediated sources are shown to be primary, then attributing this to the probable small proportion of Scotland's agriculturally managed soils that are above the moderate status will be challenging. Ongoing work is focusing on assessing STP levels in each of the Priority Catchments and developing additional risk factors based on management practices. In the Irvine catchment this will be extended to develop an understanding of the individual soil characteristics which will be used to develop a risk assessment process for phosphate leaching that extends beyond basic STP. Detailed information on the physical and chemical nature of many of these soils already exists and these will be compiled and contrasted to identify those soils within the catchment that represent the higher risk for soluble P leaching and are at a greater risk of saturation of the P-sorption capacity.

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ADHERENCE, BIOFILM AND MOTILITY CHARACTERISTICS OF PLANT ASSOCIATED *SALMONELLA ENTERICA*.

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Summary: Colonisation of plants by human pathogenic bacteria has previously been associated with the ability of the bacteria to form biofilms and demonstrate motility. Analysis of eight *Salmonella enterica* that have a heritage with plants showed significant variations in their ability to adhere to plant tissue, which did not correlate with either biofilm formation or motility. Biofilm formation was dependent on temperature and media, but there was variation in how the isolates responded to differences in these growth conditions. There was an inverse correlation between biofilm formation and motility, which was highly significant for bacteria in MOPS media at both temperatures.

INTRODUCTION

Food-borne pathogens such as *Escherichia coli* and *Salmonella enterica* are traditionally associated with their primary reservoirs; cattle, poultry and pigs. However, there are an increasing number of outbreaks arising from contaminated fresh produce, which for some bacteria can account for up to 30 % of all food-borne outbreaks (Greig & Ravel, 2009). These bacteria are not simply vectored through the food-chain by plants, but interact with them and can colonise them in a similar way to other members of the *Enterobacteriaceae*. One of the first steps in colonisation is adherence to the host tissue. Bacteria use a variety of mechanisms for adherence and can be found in association with some hosts as a biofilm, alternatively specific interactions can take place between bacterial surface factors, such as flagella, and host cells.

Biofilms comprise a matrix of bacteria and surface expressed proteinaceous and nucleic acid factors. The composition varies change under different environmental conditions, in particular lower temperatures (White-Ziegler *et al.,* 2008). In *S. enterica* curli fimbriae and cellulose are thought to play an important role, especially in the context of plant colonisation (Barak *et al.,* 2005; Lapidot & Yaron, 2009). Other studies have shown a role for flagella in initial colonisation and invasion into plant tissue (Cooley *et al.,* 2003; Berger *et al.,* 2008), although flagella of related bacteria have also been shown to bind directly to host tissue (Mahajan *et al.,* 2008).

The aim of the work was to determine whether biofilm formation and motility correlated with the ability of plant-associated *Salmonella enterica* isolates to adhere to plant roots. The group of bacteria comprise clinical isolates that have been associated with consumption of contaminated fresh produce, and a re-call isolate from a contaminated salad product.

MATERIALS AND METHODS

Bacteria and media

Plant-associated *S. enterica* isolates were obtained from the Scottish *Salmonella*, *Shigella* and *Clostridium difficile* Reference Laboratory. Eight isolates were used that had an association with plants: seven were clinical isolates associated with fresh produce outbreaks and one was a salad re-call isolate. The isolates were all non-typhoidal *S. enterica* and designated A to H for clarity. The bacteria were maintained in LB media at 37 ^{o}C with aeration and inoculated into different media at the appropriate temperature as described. Media used for biofilm, motility and adherence assays was either LB media or rich-defined MOPS media (RD MOPS) supplemented with glucose (Neidhardt *et al.* 1974). The media was solidified with 0.35 % (w/v) agar for motility assays. Selective XLT media (Oxoid, UK) was used to identify *S. enterica* colonies from the root adherence assays.

Biofilm formation

Biofilm assays were performed essentially as described in (Merritt *et al.* 2011). Bacteria were grown for 18 hours in LB media at 37 ^{o}C and sub-inoculated into fresh LB or RD MOPS that had been pre-warmed to 37 ^{o}C or 18 ^{o}C, to an optical density (OD_{600}) of 0.02. The suspensions were plated into a 96-well polystyrene microtitre plate at 100 □l per well and incubated at 37 ^{o}C for 24 hours, or at 18 ^{o}C for 48 hours. Crystal violet (1 % (w/v)) was solubilised with a mixture containing 80 % ethanol and 20 % acetone and the absorbance read at 590 nm. Each experiment contained five replicate samples and the experiments were repeated three times.

Motility

Motility was measured on LB or RD MOPS motility plates. Bacteria were plated onto LB agar at 37 ^{o}C and grown for 18 hours. Single colonies were picked and stabbed into the centre of motility agar plates that had been pre-warmed to 37 ^{o}C or 18 ^{o}C. The motility plates were incubated at 37 ^{o}C for 16 hours or at 18 ^{o}C for 42 hours and the diameter of the colony measured. Each experiment contained 3 replicate colonies and the experiments were repeated twice.

Tomato root adherence

Bacteria were grown for 18 hours in LB media at 37 ^{o}C and sub-inoculated into fresh RD MOPS to an optical density (OD_{600}) of 0.02 and grown for 24 hours at 18 ^{o}C. The bacteria were diluted to an OD_{600} of 0.02 in PBS for the infections. Tomato plants were grown in standard (non-intercept) compost from seed, for four weeks with a 16 / 8 hour light / dark cycle, respectively, at 70 % humidity. The roots were separated from the plants and washed in sterile distilled H_2O to remove compost particles. The root weights were recorded and the roots placed into a 50 ml tube containing 20 ml of bacterial suspension, for two hours at 18^{o}C, with gentle agitation (80 rpm). The roots were then washed with ~ 25 ml PBS and loosely adherent bacteria removed by vortex, for 15 seconds. Three such washes were carried out and the roots macerated in 2 ml of PBS with sterile sand. Ten-fold serial dilutions were plated onto XLT agar and incubated at 37 ^{o}C for 24 hours.

Antibody agglutination

A *Salmonella*-specific flagella (H) polyclonal antibody mixture (MAST diagnostics, UK) was mixed with a bacterial suspension on a microscope slide. Agglutination (cross-linking between flagella and H antibodies) was defined as the formation of visible clumps, which formed within two minutes. Flagellated *Escherichia coli* was used as a negative control.

RESULTS

The ability of the plant-associated *S. enterica* isolates to form biofilms was tested under two different temperatures; 37 ^{o}C and 20 ^{o}C, to mimic mammals and plant host temperatures, respectively. Two different media were used: defined (MOPS) and complex, undefined (LB) to determine any media effects and because the isolates were always grown in defined media prior to plant infection assays. In general, biofilm formation was greatest for bacteria grown in MOPS media, at 18 ^{o}C, although it varied significantly between the isolates with some isolates completely unable to form biofilms under the conditions tested. Two isolates (E & G) showed differential media responses, especially at 37 ^{o}C.

Motility was measured on swimming agar plates, also at 37 ^{o}C and 20 ^{o}C. Motility was strongly inhibited in MOPS media compared to LB, at both temperatures. All of the isolates except for isolate A were able to swim, at both temperatures. Whether isolate A produced functional flagella or not was assessed using anti-H *Salmonella* flagella antibodies. A strong agglutination reaction occurred, to a similar level as the other isolates, indicative of production of flagella. Microscopic visualisation of the bacteria showed that they were able to tumble, but not move, indicative of a flagella rotor mutant (Yim *et al.,* 2011).

Adherence to plant tissue was assessed on tomato plants because tomatoes have been implicated in several major food-borne outbreaks of *Salmonella*. Previous work has shown tomato to be susceptible to other plant and human pathogenic members of the *Enterobacteriaceae*. The ability of the bacteria to adhere to tomato roots varied greatly: isolate A was unable to bind, while isolate B showed the highest level of adherence.

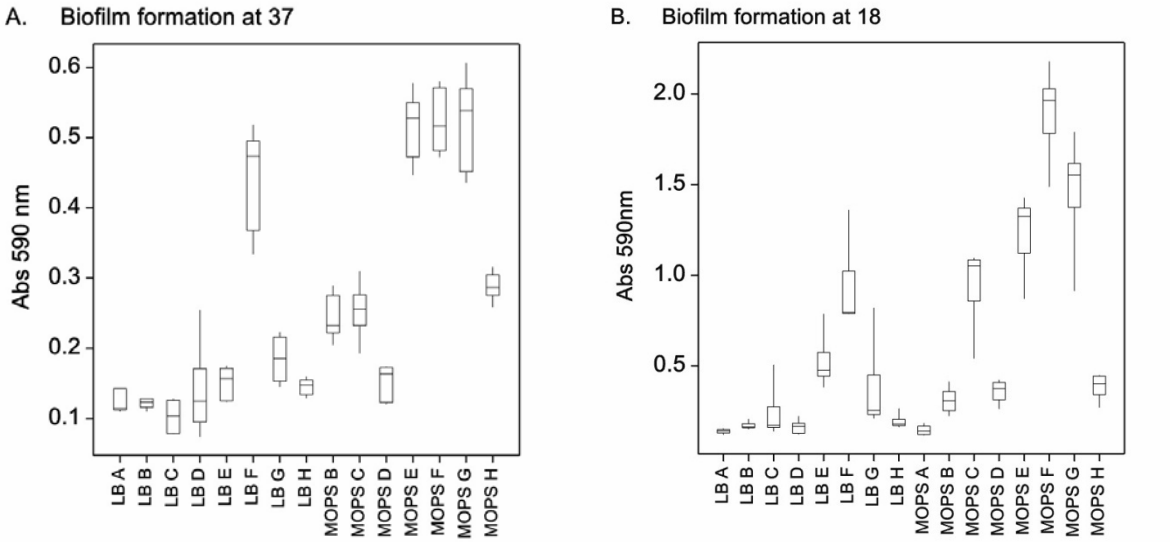

Figure 1. Biofilm formation of eight *S. enterica* isolates (A - H), in LB or MOPS media, at 37 ^{o}C or 18 ^{o}C. The absorbance (OD_{600}) measurements from 5 replicate samples are presented as a box plot. The data is representative of 3 repeated experiments.

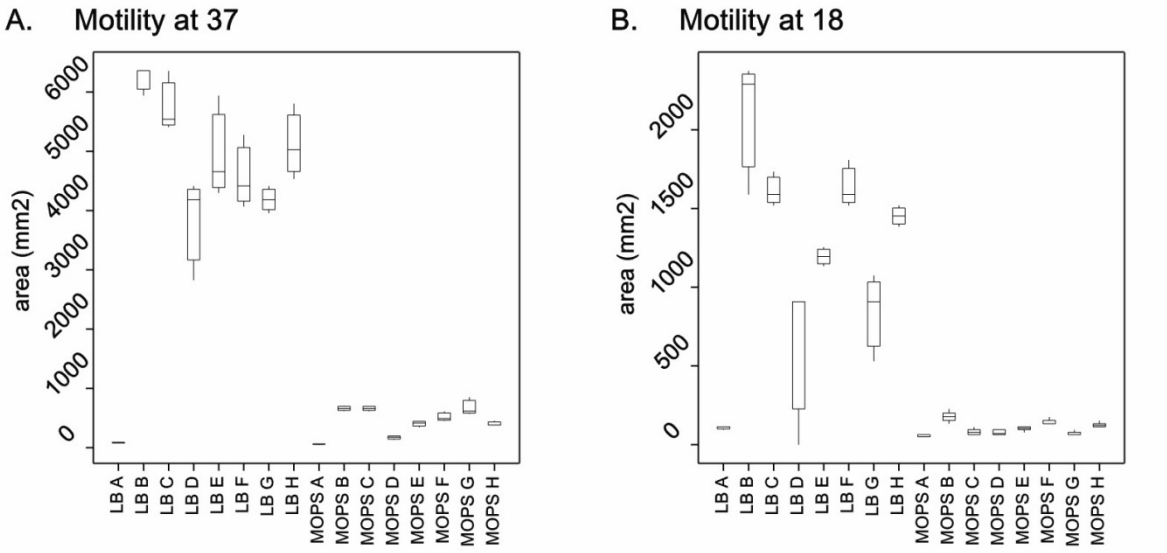

Figure 2. Motility measurements for eight *S. enterica* isolates (A - H), in LB or MOPS media, at 37 ^{o}C or 18 ^{o}C. The colony diameter (mm^2) from 3 sample replicates is presented as a boxplot. The data is representative of repeated experiments.

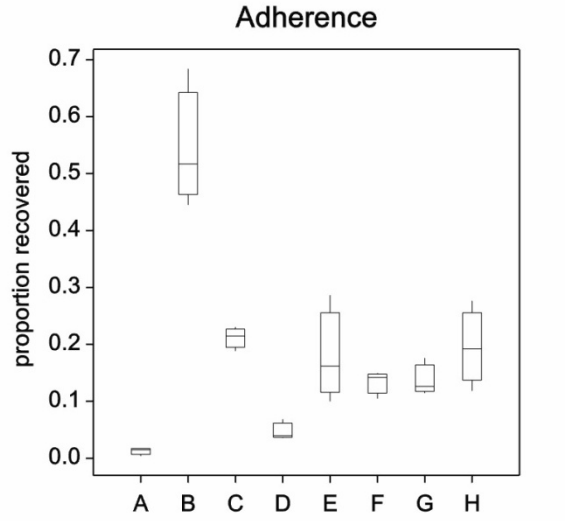

Figure 3. Adherence to tomato roots for eight *S. enterica* isolates (A - H), at 18 ^{o}C. The number of bacteria recovered from 3 sample replicates was converted to a proportion of the inoculum population and presented as a boxplot. The data is representative of repeated experiments.

DISCUSSION

Colonisation of plants by food-borne pathogens depends on a number of factors, both plant and bacterial, that determine the outcome. A key question surrounds the initial interactions that

occur, when the bacteria adhere to plant tissue, before colonisation becomes established, and whether *in vitro* phenotypes play a role *in planta*. We have shown that *in vitro*, an inverse correlation exists between motility and biofilm formation that is dependent on the media type. This was observed for both media types and at both temperatures. One exception occurred with *S. enterica* isolate F, which in contrast to all the other isolates at 37 ^{o}C in LB, formed significantly greater biofilms and was as motile as the other isolates in this media. It is possible that the biofilm components of this isolate also include flagella. *S. enterica* can express two different flagella types, which are controlled by a phase variable promoter, generating heterogeneous populations (Joys *et al.* 1974). One possibility is that isolate F has a greater proportion of one of the flagella types that is used for adherence in biofilms. Development of biofilms normally cycles between motile and sessile bacteria, and flagella are clearly fundamental to motility. However, flagella can also be used as an adherence factor under defined conditions.

Adherence to tomato roots was very variable between the isolates, with extremes of adherence shown by isolates A and B. Isolate A was recovered from tomato roots in very low levels. This isolate was found to be unable to swim, despite production of antigenic flagella, and also showed very low levels of biofilm ability. In contrast, isolate B adhered very strongly, resulting in recovery of the majority of the starting inoculum. However, this isolate demonstrated poor levels of biofilm formation, especially in the media used prior to infection of the tomato roots. The strongest biofilms in this media were shown by isolates E, F and G, but these isolates adhered to tomato roots at similar levels to other isolates (C and H).

Taken together, there are no correlations between either motility or biofilm formation and adherence to tomato roots, under the conditions tested. It is likely that multiple factors contribute to biofilm formation and plant adherence and, despite growth under similar environmental conditions, different factors are involved in attachment to the different surfaces (plastic 'vs' plant).

ACKNOWLEDGEMENTS

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DO WE NEED TO BE WORRIED ABOUT THE POTENTIAL THREAT OF INVASIVE SPECIES TO CROPS AS THE CLIMATE CHANGES?

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Summary: There are pests that have the potential to invade the UK and cause problems to a wide range of crops. These include invertebrates, fungi and plant species. With climate change ongoing, the conditions within UK crops will become favourable for the spread of pests currently within the UK, and establishment of several invasive species, which will provide significant issues for their management.

This paper will summarise the potential for several current and potential invasive pest species to spread and establish under current and future climates, and discuss the likely impacts they will have on specific UK crops.

INTRODUCTION

Climate change is now an accepted phenomenon and its impact on Scottish agriculture will become more noticeable over the next 50 years (Davies *et al*, 2007). Over the last half century temperatures have increased in every season in all parts of Scotland, and in some areas in Scotland, particularly northern and western areas, winter rainfall has increased on average by 60%. Climate change scenarios produced by the UK Climate Projections Programme (UKCP09) and the Intergovernmental Panel on Climate Change (IPCC) suggest Scotland is to get warmer winters and summers, with an increase in winter rainfall, but a decrease in summer rainfall, and with more extreme weather events (see Table 1). These changes in temperature and rainfall coupled with increases in carbon dioxide concentration will affect crops as well as the pests, weeds and diseases that live on or in them.

The climate will also become more favourable for species that have yet to establish in the UK, as increase in temperatures will directly affect pest physiological processes, such as the rate of growth and development. The growth period of most temperate species occurs during the warmer summer period of the year. A rise in summer temperatures may be expected to increase the speed of development, allowing for more generations to develop within a season in multivoltine species (Bale *et al*, 2002).

This paper summarises how climate change is likely to affect the pests we currently see on crops in Scotland, and identify potential 'new' threats that could take advantage of the changes in climate over the next half century or so.

Table 1. The likely changes in Scottish climate by 2050

Climatic variable	Likely change
Temperature	Warming of between 1-2°C, with greatest warming during the autumn except for the extreme north of the country. There will be more extremes of temperature in the summer and autumn, with fewer very cold days, especially in the winter.
Rainfall	Winter rainfall will increase by 15-20%. Summer rainfall will decrease by 15-30%.
Humidity	Relative humidity will decrease slightly.
Soil moisture	There will be a reduction in soil moisture in the summer and autumn of between 10-30% except in the Highlands. Winter soil moisture will increase up to 10% from current levels.
Thermal growing season	This will increase in all areas allowing earlier sowing of crops to occur along with earlier harvests and potential for novel crops to be grown.

METHODS

Climatic modelling can be used as a tool in pest risk assessment, and has a major role in determining the effects of global change on ecosystems (Baker *et al*., 2000). CLIMEX is a software package that contains two distinct functions: the 'Match Climates' function in CLIMEX uses an inductive approach, as it compares meteorological data from different areas directly (Sutherst *et al*, 2000). This function calculates a 'Match Index', which is a measure of the overall similarity of climatic variables at different locations. CLIMEX also contains a 'Compare Locations' function, which is based on a deductive approach as using species-specific climate response models (Sutherst, 2003). This function generates an 'Ecoclimatic Index' representing a measure of the overall climatic suitability of a location for a specific species (Baker *et al*, 2000; Sutherst *et al*, 2000). The ability to predict the risk of establishment plays an important role in the management of invasive species, particularly in determining priority species for control and regions most vulnerable. CLIMEX is now used for quarantine, biological control, pest management and conservation worldwide, and is applicable for a diverse range of species, pests and diseases. Within this paper, CLIMEX has been used to determine the suitability of the projected Scottish climate for 2050-2099 (based on the A1B medium emissions scenarios produced in UKCP09 and the IPCC) for the establishment and spread of a range of current and invasive crop pest species.

CLIMATE CHANGE AND CURRENT PESTS

The impact of climate change on pests of crops is driven by the response of invertebrates to temperature, moisture and carbon dioxide. Some pests such as cereal aphids will reproduce more rapidly at the elevated carbon dioxide levels forecast for 2050, and temperature increases will accelerate the rate of multiplication even further, allowing more generations per season (up from 18 to 23 for some aphid species). This inevitably has consequences for the crops that

aphids infest, particularly for crops such as seed potatoes where virus transmission by aphids is a potential threat.

We are already seeing 'new' pest problems arising in Scottish crops which are, in part, in response to climatic changes: cabbage stem flea beetle and rape winter stem weevil in winter oilseed rape and orange wheat blossom midge in cereals for example.

As the Scottish climate changes over the next 50 years, many of these pests will become serious problems (see Table 2).

Table 2. Likely increase or decrease in severity of specific crop pests by 2050

Common name	Crops affected	Increase/decrease in severity
Turnip sawfly	Oilseed rape, vegetable brassicas	Increase
Gout fly	Cereals	Increase
Wheat stem sawfly	Wheat	Increase
Wheat bulb fly	Wheat and spring barley	Decrease
Aphids	All crops	Increase
Cereal leaf beetle	Cereals	Increase
Wireworm	Cereals, potatoes	Increase
Cabbage root fly	Vegetable brassicas, oilseed rape	Increase
Pea moth	Peas	Decrease
Potato cyst nematode	Potatoes, tomatoes	Increase
Diamondback moth	Brassica vegetables	Increase
Carrot fly	Carrots, parsnips	Decrease
Cutworm	Field vegetables, potatoes	Increase
Slugs	All crops	Decrease, except in the northwest

Some pests that are sensitive to moisture will decrease to some extent. Slugs for example could decrease in severity due to lower rainfall in the summer months in most areas (Willis *et al*, 2006), however, any increase in summer irrigation will negate this benefit. Conversely, wheat bulb fly will become less of an issue in future years due to egg mortality increasing due of the projected increase in winter rainfall, as survival is low if annual rainfall exceeds 840mm (Thomas, 1948).

Many pests that are already present could increase in importance (see Table 1), with cabbage root fly for example becoming a significant pest of winter oilseed rape as in Germany, where it is now considered to be one of the most important pests of this crop.

INVASIVE PESTS

Several pest species invade Scotland annually as they are unable to overwinter under the current climate. One example is the Silver Y moth (*Autographa gamma*), which is widespread across Europe. In spring variable numbers migrate north reaching as far as Iceland Greenland, and Finland. In Scotland adults are present in significant numbers from May onwards with numbers

dwindling in late autumn as they are killed off by frosts. Numbers of moths caught in pheromone traps in East Lothian since 2001 are summarised in Table 3.

Table 3. Peak No. of Silver Y moth caught in pheromone traps in East Lothian 2001-2011

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Peak No.	58	101	200	92	119	219	145	435	221	728	114

Populations of silver Y moth vary from year to year (Table 3), with some years such as 2010 having a significant invasion, which subsequently caused problems on several crops. The projected increase in winter temperatures, and reduction in frosts has the potential to allow Silver Y moth to survive in Scotland and become a significant pest by the middle of the century, especially as it can attack a range of crops such as potatoes, brassicas, peas, carrot, lettuce, wheat and maize.

Several potato pests have the potential to be introduced (or re-introduced) into the UK. Breeding colonies of Colorado potato beetle (*Leptinotarsa decemlineata*) have occasionally been present in the UK, but the last was eradicated in 1977. Colorado potato beetle (CPB) is a notifiable quarantine pest, whose introduction is prohibited under the EC Single Market Protected Zone arrangements for Plant Health.

Using a CPB climate response model in CLIMEX (Kocmánková *et al*, 2010), the current climate is suitable for establishment of the beetle in south east England (Fig. 1a), and by 2050 (Fig. 1b) areas in East Lothian, Fife and Angus will be suitable for establishment, with most of the UK suitable by 2080 (Fig. 1c), with 3 generations of CPB possible in south east England, and 2 generations in Scotland.

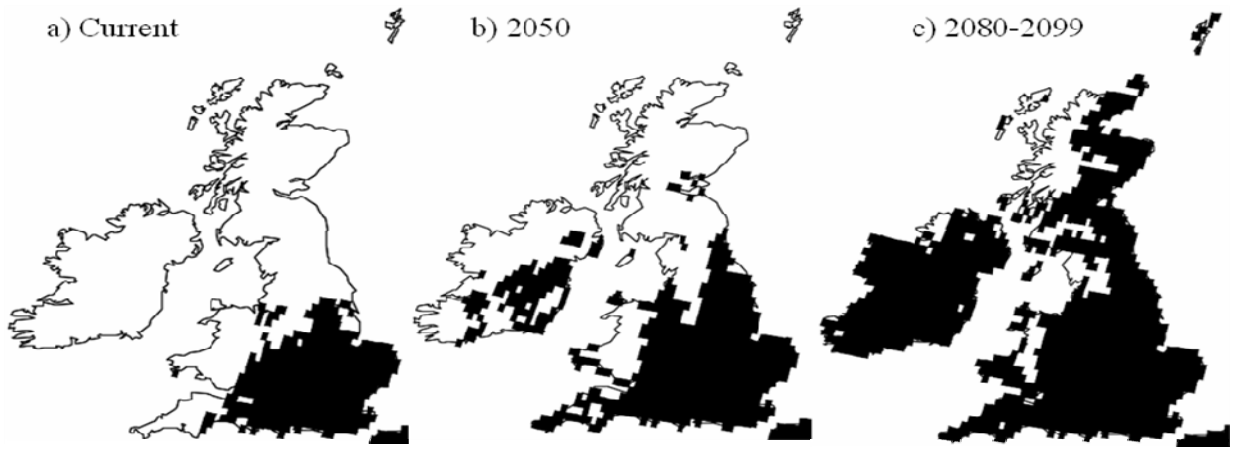

Figure 1. The potential distribution of CPB (shaded black) in the UK under the current climate (a), climate projected for 2050 (b) and climate for 2080-2099 (c).

There are several potato flea beetle species (*Epitrix* spp.) that are currently at risk of establishment within Europe (EPPO, 2010). Two species; *E. cucumeris* and *E. similaris* have been found in Portugal, with *E. similaris* also being found in Spain. The beetles feed on the leaves, but the larvae feeding on the tuber can cause holes up to 1 cm into the tuber flesh significantly affecting tuber quality. Matching the climate of Porto, Portugal where *Epitrix* spp. are present in potato crops with the current and forecast UK climates indicates that over the next 40-90 years the UK and Scottish climate will become suitable for the establishment of this pest (Fig. 2).

SUMMARY

As the climate slowly changes, and we see seasons vary from year to year, pest problems gradually become more common and noticeable. When wheat bulb fly egg counts exceed 35 million/ha (as in 2010), and Silver Y moth trap catches exceed 700 in a week (also in 2010), outbreaks of pest problems are likely to increase in frequency and significantly affect crops. Whilst several pests will be 'forced out' by the changes in climate making the country unsuitable for establishment, other pests are waiting for the opportunity to get into the country and take advantage of the climate becoming suitable for their establishment and survival.

Measures are in place at UK and EU levels to try to prevent many of these invasive species such as *Epitrix* spp., CPB, Zebra chip disease vectored by psyllids, western corn rootworm (already present), European corn borer (already present) and others spreading in the UK or getting into the country. However, growers, agronomists and researchers need to be vigilant to spot pest infestations at an early stage to allow management, and where possible, eradication, to take place.

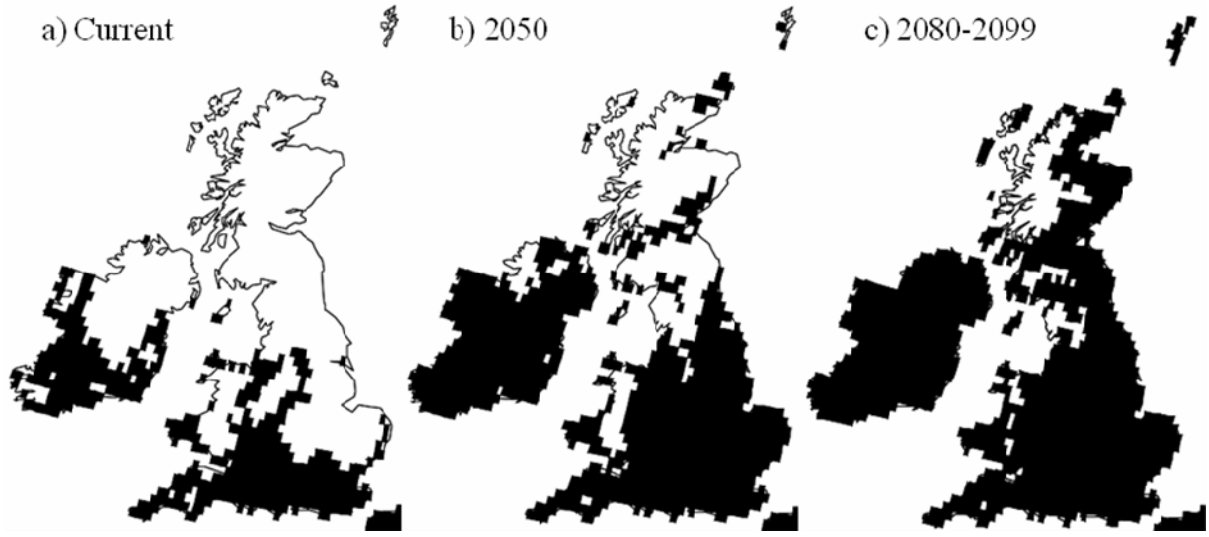

Figure 2. Climate matches for the UK with Porto, Portugal (shaded black) where *Epitrix* spp. are found in potato crops: current climate (a), climate projected for 2050 (b) and climate for 2080-2099 (c).

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FARMING FOR A BETTER CLIMATE; ENCORAGING PRACTICAL CLIMATE CHANGE MITIGATION MEASURES ON FARM

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Summary: It is widely accepted that our climate is changing as a result of greenhouse gas emissions attributable to human activities. It is estimated that Scottish agriculture and related land use activities account for around a fifth of Scotland's greenhouse gas emissions. SAC is working with the Scottish Government on their Farming for a Better Climate initiative to highlight steps farmers can take to reduce greenhouse gas emissions from routine farm activities whilst also benefitting the farm business. There are four climate change focus farms with input from a farmer discussion group and support from SAC specialists. This paper outlines some of the mitigation measures discussed by climate change focus farm discussion groups in the first half of the project.

INTRODUCTION

We know that it is highly likely that our climate is altering over a short timeframe; far reaching consequences have been predicted on a global scale as a result of climate change that could make some parts of the world uninhabitable (IPCC, 2007). Debate continues about the impact and extent of climate change we can expect, but there is wide agreement that unchecked emissions of greenhouse gases by human activities is altering climate systems and action needs to be taken (Smith, 2010b; Pachauri and Reisinger, 2007).

In terms of agricultural emissions, the key greenhouse gases are methane (CH_4) and nitrous oxide (N_2O); both more damaging in terms of global warming potential than carbon dioxide (CO_2) (IPCC, 2007). Although the global agricultural and rural land use sector is a significant source of greenhouse gases, it can also act as a sink; sequestering carbon dioxide from the atmosphere (AEA, 2008; Scottish Government, 2009a and 2009b; Smith, 2010b; Del Prato *et al*., 2010). A more detailed review of the science behind climate change and the role played by greenhouse gases is available in Smith, 2010a and Reay *et al*, 2010.

Under the Climate Change (Scotland) Act (2009), Scotland is committed to reducing greenhouse gas emissions by 42% by 2020, with this target rising to 80% emission reduction by 2050 (both targets based on 1990 emission levels) (Scottish Parliament, 2009). Scottish Government (2010 and 2011) estimate 20% of Scotland's greenhouse gas emissions could be attributable to the agricultural and rural land use sector.

Scottish Government (2009a and 2009b) has suggested a range of measures to reduce greenhouse gas emissions alongside steps to adapt to a changing climate. In order to help the agricultural sector in Scotland identify the required reductions in greenhouse gas emissions, the Scottish Government commissioned SAC to deliver its Farming for a Better Climate initiative.

The Farming for a Better Climate initiative aims to help farmers in Scotland recognise sources of greenhouse gas emissions, understand the financial impact this could have on their business, take steps to reduce these losses and consider how they could adapt to climate change risks in the future (SAC, 2009).

SAC are working in partnership with four farmers who volunteered to act as 'climate change focus farms'. Under the focus farm concept, a facilitator from SAC works together with the focus farmer to identify practices for further investigation on their farm and provide a forum for discussion of these ideas with neighboring farmers and invited specialist speakers. The focus farmers represent the beef and sheep, arable and dairy sectors with one of the four farms providing information directly to the public about the steps farmers are taking to reduce greenhouse gas losses via their farm shop and ice cream parlour.

The mitigation measures suggested under the initiative all have the potential to reduce greenhouse gas emissions and lead to direct financial benefits for the majority of farm business in Scotland (depending on which measures the farm is already implementing and the type of farming system). The measures, based on work carried out by Moran *et al* (2008), have been grouped into five key action areas (SAC, 2009). The five key action areas for consideration under the Farming for a Better Climate are:

- Energy and fuel use
- Development of on farm renewables
- Locking carbon into soils and vegetation (includes afforestation)
- Optimising fertiliser, slurry and manure use
- Managing livestock and storage of livestock wastes

SAC maintains and updates the Farming for a Better Climate website (www.farmingforabetterclimate.org). The website acts as a focal point for the initiative; it provides information on the climate change focus farms, promotes mitigation and adaptation measures in relation to the five key action areas and hosts a quarterly e-newsletter. Mitigation and adaption measures are illustrated in downloadable practical guides and farmer case studies. Findings from the focus farms are also reported through other media and demonstration events to reach a wider audience.

The aim of this paper is to highlight some of the practical mitigation measures discussed by the climate change focus farms at the half way point of the project.

METHODS

In 2009, volunteer farmers were sought to participate in the Farming for a Better Climate initiative. This was done by promotion in the press and at Farming for a Better Climate meetings across Scotland. Three farms were identified to represent dairy, upland beef and

sheep, and arable sectors. A fourth farm, a mixed dairy and arable unit with farm shop and ice cream parlour, was also invited to participate. It was felt this would be a good opportunity to promote some of the steps farmers are taking to reduce greenhouse gas emissions to the wider public.

Following the farmer's agreement to participate in the initiative, data were collected on the farm by the SAC farm facilitator, discussing current practices with the farmer and using existing farm records, for example IACS (Integrated Administration and Control System) forms, fertiliser records and fuel and electricity bills. Data were analysed to identify resource use on the individual farms and to inform the production of a carbon footprint for each of the focus farms using the SAC Carbon Footprinting tool. Data analyses will be repeated at the end of the initiative to assess change in terms of greenhouse gas emissions and attribute to individual actions.

Following an open meeting on the farms to launch the initiative, the climate change focus farms then hosted smaller discussion group meetings, inviting other farmers in their locality. Topics for meetings were decided in discussion with the focus farmer, depending on their individual farming systems, with a view to cover all five key action areas during the meeting programme. Guest speakers viewed as specialists in their area were invited to participate in the meetings; the focus farmer meetings provided a forum for discussion of the five key action areas with neighboring farmers. Specialist advisors also highlighted the benefits that could be achieved from taking a second look at routine practices within each of the five key action areas.

Findings from these meetings were communicated to the wider agricultural sector through articles on the website, newsletters, translated into practical guides and as press articles. In addition the initiative was also promoted at other events and agricultural shows.

RESULTS AND DISCUSSION

Focus farm discussion groups

Working with a respected farmer and SAC facilitator on the farm to provide a forum for discussion with neighbouring farmers and guest speakers is an effective way to "*facilitate intereaction, learning and innovation"* (Blackstock *et al*, 2009). Discussion between the focus farmer, invited specialist speakers and other farmers within the group often provided new ideas or points for discussion and facilitated information exchange from farmer to farmer. This approach is preferable over a one-way downwards cascade of scientific information from scientific researcher to farmer (Blackstock *et al*, 2009).

Specialist advisers and guest speakers showed how small changes in practice could or have benefitted the focus farms in terms of financial gain and emission reduction. Their expertise was also key to suggesting how the measures could be easily adopted by the meeting participants. Discussion amongst the group was encouraged. It was important that meetings suggested practical ideas with clear business benefits that farmers could take away and apply at home; meetings have to be seen as useful to attend and not just a talking shop (Grist, 2010). Topics investigated on the farms in the first half of the initiative included the following:

Energy and fuel use

With fuel and energy prices increasing it was deemed that better use of electricity and fuel were areas that all farmers could identify with and realise financial savings though improved practices on the farm. The energy audits provided a good guide for the focus farmers, highlighting potential areas where savings could be made. Metering, measuring and recording usage of electricity and fuel were deemed to be key in identifying and quantifying potential savings. Simple measures, for example putting up a whiteboard to record the amount of fuel used against individual tasks and installation of additional electricity meters, allowed energy use to be monitored.

Dairy farming uses a considerable amount of electricity to heat water and cool milk (Dunn *et al*, 2010). A number of efficiencies were identified ranging from no/low-cost steps such as checking thermostat time, temperature settings and tank insulation, to more costly activities such as replacing a plate cooler in the dairy. All measures were given an indicative payback period ranging from an instant return to around seven years.

Scope for renewables

Within the rural sector there is an interest in renewables, this stems from increasing energy costs and the level of funding through feed-in tariffs (FITs). Practical, technical and financial feasibility are essential considerations when assessing renewable energy opportunities. Scope for renewables was investigated on the focus farms; additional data is being collected to allow feasibility studies to be undertaken. Renewable technologies deemed technically suitable for the focus farms include wind, solar PV, micro-hydro and wood fuel.

Fertilisers and manures

Increasing fertiliser costs over the past few years has forced farmers to review their management of both organic and inorganic fertilizers. Optimising both organic and inorganic nutrient applications can lead to reduced emission, purchase and application costs, plus added benefits in terms of water quality and biodiversity. Analysing soil from individual fields on the focus farms provided information about their nutrient status; one farm recorded 8 out of 29 fields at the optimum pH level, the remaining 21 fields needing small changes in current practice. This demonstrates that there is still scope for 'good farmers' to revisit and adjust current farm management policies to optimise business benefits. PLANET Scotland (ADAS, 2010) is a useful tool to help farmers plan organic and inorganic fertiliser application and is being used by some of the focus farmers.

Optimising livestock productivity

Improving the efficiency of livestock on the farm can favorably alter the ratio of milk or meat produced to greenhouse gas emissions and improve farm profitability. A number of issues were discussed and considered by the dairy and beef focus farms such as herd health, condition scoring, age of calving, age of finishing and production of good quality forage. For example forage analysis was highlighted as being key to identifying forage quality, allowing rations to be better tailored to suit the animal's needs.

Carbon footprinting

The carbon footprint analysis on the four farms highlighted the amount of carbon dioxide equivalents (CO_2e) per kg or litre of product from the farm. Upon benchmarking with similar enterprises across Scotland, it was seen that the farms in the study already compared well, but there were still actions they could take that would reduce their carbon footprint and in turn,

could financially benefit their business. The enhancement of 'green' credentials leading to access to markets, 'legacy' in terms of improved sustainability of the farm business and improvement of biodiversity and habitats were also deemed to be additional benefits to be gained from managing carbon on the farm.

In summary, agricultural activities have the potential to contribute to reducing Scotland's greenhouse gas emissions. The first year of this initiative has illustrated that there is scope for most farms to take a second look at previously accepted practices and identify changes that will reduce greenhouse gas emissions and in turn, have the potential to improve the profitability of the farm business.

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NINE MONTHS OF WINTER FOLLOWED BY THREE MONTHS OF BAD WEATHER. WIND ENERGY AND CROP PRODUCTION IN THE OUTER HEBRIDES.

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Summary: The dry weight of mustard (*Brassica hirta*) grown with the aid of artificial illumination synchronised with the wind was compared with that of mustard grown using other lighting regimes on the Isle of Lewis, Scotland. Thirty six samples grown over three eight day trials produced mean dry weights of 7.78g for natural light, 8.23g for wind pattern illumination and 8.61g for an 18 hour pattern of artificial illumination. This demonstrates that the yield of mustard could be increased by the direct utilisation of wind power, without the need for electricity to be stored or supplemented.

INTRODUCTION

Historically, wind speeds in the Outer Hebrides of Scotland have been viewed as problematic for growing crops (Hance, 1952). Low light levels can also be a limiting factor at these latitudes (Both, 2000). Fundamentally, plants require a source of energy to grow and the wind is a source of energy, but its suitability as an aid for growing plants has not been fully explored. In order for plants to be able to utilise wind energy for photosynthesis the available wind power needs to be converted firstly into electricity and then into light. The technology to achieve this is well developed but critics of wind power cite the highly variable nature of wind as a major obstacle to its use. For example see Halkema (2008).

The variability of the wind can be overcome by using batteries that store the electricity produced during windy periods and allow its steady release over time. Batteries, however, add to the cost and complexity of systems. They also account for efficiency losses of 15- 25% (Xtronic, 2010) and can be of environmental concern during their manufacture and disposal (Kalantar & Mousavi, 2010). There would therefore be benefits if the energy from the wind could be directly applied to crops.

Wind is not the only force of nature that is variable. Solar radiation is also subject to change, with winter and summer, day and night, cloudy days and clear days. Plants have evolved to cope with such variation. This suggests the possibility that they could also accommodate some of the vagaries of wind energy.

The possibility of providing wind dependent illumination rather than strict diurnal lighting regimes would in many ways echo the reality of the non-optimal light that plants receive in the

natural world. Commercially viable crops are grown in naturally varying light conditions even if they seldom attain their full genetic potential (Albright, 2008).

The following experiment was carried out to test the hypothesis that horticultural production on the Isle of Lewis, Outer Hebrides, Scotland can be increased by the direct utilisation of wind generated electricity without having to store electricity in batteries.

MATERIALS AND METHOD

Three 0.75m^2 growing areas were prepared in a glass glazed greenhouse. The sides of each area were shielded from its neighbour with aluminium foil. Each area was illuminated subject to one of three lighting regimes. The experiment was repeated three times and the positions of the growing areas changed on each occasion. The three lighting regimes were

1. Natural light.
2. Natural light and 18 hours of supplemental light between 06:00 and 24:00.
3. Natural light and 18 hours of variable wind dependent supplemental light between 06:00 and 24:00.

The supplemental lighting was provided by commercial light emitting diode (LED) grow lights (ECO LED lights, Berkshire, England) rated at 120 watts per unit and positioned to provide 100 W/m^2. The component LEDs emitted red light at 630nm and 660nm and blue light at 460nm. A relay switched on the wind operated lights when a Rutland 910 wind turbine (Marlec, Corby, England) was exposed to winds above 2.6 meters per second (the turbine's cut in speed).

In each of the growing areas four 150 mm diameter plastic growing trays were supported above a water trough. Each tray had a central hole through which 850mm of 10mm pre-stretched 8 pleat polyester was passed to form an irrigation wick. The upper end of each wick was coiled beneath 3 pre-weighed layers of absorbent paper. Evenly distributed on the paper on each tray were 8.5g of white mustard seed (*B. hirta*). The seed had been weighed, soaked for 1 hour in water, placed in the trays and covered with polythene film and kept in the dark for 48 hours prior to the start of each experiment.

Each of the three experiments were run for 8 days and during this time a data logger recorded when the wind operated lights switched on and off. After 8 days the plants were removed and dried using a microwave oven (after Popp *et al.,* 1996) and their dry weights were measured. The use of a microwave oven rather than a thermal oven to dry the plants reduced the processing time to approximately half an hour per sample. The dry weights of the plants produced were then compared using a one-way randomised Analysis of Variance and a post-hoc Tukey HSD test. The experiments were conducted between 2nd February 2011 and 10th March 2011.

RESULTS

The mean exposure to the set diurnal pattern additional LED lighting was 121 hours, 1.5 minutes. The mean total exposure to the variable wind pattern additional LED lighting was 42

hours, 24 minutes. The mean duration of individual periods of variable wind pattern additional LED lighting was 8 minutes, 44 seconds. The dry weights of mustard from each of the lighting conditions are shown in Table 1.

Table 1. The mean dry weights of mustard (*B. hirta*) crops grown under different lighting regimes.

	Sample number	Mean	Standard deviation	95% Confidence Limits Lower	Upper
Natural light	12	7.78	.18	7.67	7.90
Set diurnal light	12	8.61	.26	8.45	8.78
Variable wind light	12	8.23	.18	8.11	8.34

A one-way randomised Analysis of Variance showed there was a significant difference between the dry weights of mustard produced ($F(2,33) = 47.07$, $p<0.001$). Post-hoc Tukey HSD tests showed that all three lighting regimes produced dry weights of mustard that were significantly different from each other ($p<0.005$ in all cases).

DISCUSSION

The dry weight of mustard (*B. hirta*) grown using a combination of natural light and artificial light dependent on the wind over 8 days was 5.69% greater than the dry weight of mustard grown solely using natural light. This supports the hypothesis that horticultural production on the Isle of Lewis, Outer Hebrides, Scotland can be increased by the direct utilisation of wind generated electricity without having to store electricity in batteries. This finding may be of significance regarding questions about the usefulness of wind power due to its variability and intermittency.

The dry weight of mustard grown using natural light and a set 18 hour pattern of artificial light was 10.7% greater than the dry weight of mustard grown using only natural light. Interestingly, the wind pattern lights were on for 35% of the time the set pattern lights were but yielded 53.26% of the gains produced using set pattern lights.
To increase the duration of wind powered illumination would require an increase in generating capacity as well as a means of storing the additional electricity which is then subject to associated efficiency losses. The implications of such a development would have to be weighed carefully against the potential gains. For the observed results, growing twice the

amount of plants under direct wind powered light would be more productive than the three fold increase in generating capacity required to produce the required steady 18 hour diurnal pattern.

The significance of supplemental lighting will vary throughout the year as levels of natural light alter with the seasons. Supplemental lighting can of course be used to increase the concentration of light as well as its duration and both can be of significance in northern latitudes (Runkle, 2007). It is very interesting to note that the darkest months of the year are the windiest in the Outer Hebrides (Met Office, 2011).

The decision to grow crops in Northern Britain is not always made because it is the best possible place to grow them, but rather because they still produce worthwhile yields. The potential to harvest energy at these same locations and feed it directly into crop production may influence this decision making process.

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RESULTS OF A FIVE YEAR STUDY OF CROP ROTATION ON SMALL MAMMAL POPULATIONS

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Summary: Small mammal populations were monitored overwinter on a 4 year rotation of potatoes, barley, swedes and fallow. Results indicated that wood mice numbers were considerably greater in the swede plots than those of barley, potatoes and fallow soil. The reason for the 10 fold difference increase in mice numbers in swedes over the winter months was that this crop afforded cover during the winter month which was not available from the other harvested crops. The implications of this finding for maintaining small mammal populations in the agricultural landscape and aiding the countryside's biodiversity is discussed since mice can play an important role as prey to a number of predators.

INTRODUCTION

Crop rotations have altered or disappeared from British agriculture since the Second World War and this has been associated with declines in some, but not all wildlife (Robinson & Sutherland, 2002). Farming practices have become increasingly more intensive with a decrease in habitat diversity e.g. a 50% reduction in hedgerows since the 1940s. The increased interest in stemming and reversing the reduction in biodiversity in agricultural land has lead to a number of measures and practices e.g. not spraying field margins with herbicides and insecticides (De Snoo, 1999). Some of these measures have the added advantage of controlling agricultural pest e.g. the introduction of beetle banks (Collins *et al*., 2002) and the U.K. government spent £497m on agri-environment subsidies in 2009.

Most recent research into biodiversity in agricultural land has concentrated on plants, insects or birds with little attention made to the impact of crop rotation or the introduction of conservation measures on small mammals. The present paper set out to investigate the impact of a four year rotation on small mammals.

MATERIALS AND METHODS

The study site was in a field at the Hutton Institute, Invergowrie, Dundee and the crop rotation area covered an area of just over 2 hectares. The four crops in the rotation were; barley (*Hordeum vulgare*), potato (*Solanum tuberosum*), swede (*Brassica napus*) and fallow. The area of each plot was approximately 100m x 50m. Due to a number of factors not least the home range of the animals involved and for practical considerations there was no replication of the treatment plots within each year but temporal replication did occur over the five year period 2006/2007, 2007/2008, 2008/2009, 2009/2010 and 2010/2011. Not all crops were sampled

every month in the period September to January each year, the start and end dates depending on when students could start and when project reports had to be handed in. Usually the potato and barley crops were harvested before the study took place while the swede crop was not harvested until after the study period was completed in January.

Data used in this analysis were based on twenty Longworth traps used to sample for small mammals on each of the plots over the period October-January. The traps were always set out in the same regular pattern with spacing between traps of 10m in an east/west direction and 20m in a north/south. Since, over the period of the study, 99% of animals caught were the wood mouse, *Apodomus sylvaticus* only that data were used in the analysis. Other animals caught in the Longworth traps included the field vole, *Microtus agresis* and a weasel, *Mustela nivalis*. Other data on the weather, sex and weights of the mice caught were collected during the study periods but are not presented in this paper.

RESULTS

The results of the investigation are shown in Table 1. Initially results in some years look inconsistent with trends seen in other years e.g. in September the Fallow plots in 2007/2008 and 2009/2010 had no mice recovered while in 2010/2011 12.8% of traps caught mice.

Explanations for some of these inconsistencies are as follows, in 2010/2011 the fallow plots did not receive the required weed killer and consequently they were overrun with weeds and these gave extensive, dense ground cover, while in the same year the reverse was true for the swede crop which did not grow well and produced little ground cover.

The potatoes crops in 2006/2007, 2007/2008 and 2010/2011 were late in having their haulms being burnt down and in the months of September 2007/2008, 2010/2011 and October 2006/2007 the potato shaws were still covering the drills. In 2007/2008 the barley crop was not harvested until 30th September and hence this unharvested crop also gave ground cover.

If the data from these aberrant crop results are removed (highlighted in bold) then the following trends become evident. The fallow crops had, for the months of September - January, an overall capture rate of 2.7% while comparable rates for potatoes, barley and swedes were 4.9%, 4.6% and 43.3% respectively. The meteorological data, and information on the sex and weights of the mice are not given but they did not alter significantly or impact on mice numbers in a consistent manner similar to that reported for crops during the study period.

Table 1. The percentage of traps catching the wood mouse *Apodemus sylvaticus* from the four crops over a five year period

Crop	Month	Year				
		2006/2007	2007/2008	2008/2009	2009/2010	2010/2011
Fallow	September		0		0	**13.8**
	October	0.6	2.5	1.3	5.0	
	November	0	7.5	0	4.0	
	December	2.5	6.3	0	8.0	
	January	7.5	2.5	0	1.0	**13.8**
Potato	September		**41.3**		6.0	**14.7**
	October	**35.6**	11.3	1.3	n.s.	
	November	11.3	2.5	2.5	n.s.	
	December	3.8	12.5	6.3	3.0	
	January	7.5	0	3.8	1.0	0
Barley	September		**21.3**		1.3	1.3
	October	7.5	0	16.3	1.3	
	November	3.8	5.0	0	12.5	
	December	1.0	6.3	0	8.8	
	January	2.5	12.5	5.0	1.7	0
Swede	September		50.0		35.0	**1.0**
	October	68.8	56.3	26.3	43.3	
	November	45.0	51.3	55.0	35.0	
	December	53.8	46.3	20.0	67.5	
	January	18.8	43.0	21.3	43.3	**2.5**

DISCUSSIONS

The raw data from the investigation into the impact of different crops on woodmice numbers initially gave inconsistent data which we were able to explain by the year to year differences in the growth and management of the crops. Once these data had been omitted then it is clear that the swede crop had significantly greater numbers of mice over-wintering in it compared with the numbers in the fallow, potato or barley stubble. The reason for this could have been that the swede supplied a better food supply but observations obtained from the other crops suggested that the driver behind the greater numbers of mice in the swede crop was the amount of cover afforded by the swede crop. This became evident when the swede crop in 2010/2011 failed and mice numbers were uncharacteristically low and the reverse occurred in the fallow area. The fallow areas in the preceding years had no vegetation but had in 2010/2011 become overgrown

with weeds which gave complete ground cover resulting in considerable greater mice numbers. This explanation was also supported by the elevated numbers of mice found in the potato crop before it was harvested in 2006/20087, 2007/2008 and 2010/2011, and the barley crop before it was harvested in 2007/2008.

Macdonald *et al*., (2000) investigated the impact of different crops in England and found rape fields has significantly fewer mice than winter barley or winter wheat. However they did not include a crop which remained in the ground over-winter in their rotation so there are no comparable data to that reported here. A reduction in mice numbers after harvest was reported by Tew & Macdonald, 1993 who found more than half of radio tracked mice were predated in the week following harvest by animals such as tawny owls (*Strix aluco*) and weasels (*Mustela nivalis*). Wood mice are a constituent of the diet of many avian and mammal predators and therefore play an important role in the food chain of these animals. The role of a ground covering crop such as swedes through the winter would therefore seem to possibly play an important role in enhancing the biodiversity of wildlife in the British countryside and should be encouraged.

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INTEGRATION OF HOST RESISTANCE AND FUNGICIDES

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Summary: Integrating host resistance and fungicide treatment reduces selection for fungicide insensitive pathogen strains, thus prolonging the effective life of modes of action. This benefit occurs because host resistance: (i) decreases the difference in relative epidemic growth rates of 'wild type' and insensitive strains at any given level of total fungicide dose, and (ii) allows effective control to be obtained with a lower total fungicide dose (fewer treatments per crop or lower dose per treatment), thus further reducing selection pressure (in haploid and/or clonal target species). The first of these mechanisms probably also applies in the opposite direction, i.e. fungicide treatment can slow selection for virulence. It follows that, over-dependence on either host resistance or fungicides is likely to increase the rate of loss of efficacy of host resistance genes or fungicide modes of action. The aim should therefore be to identify and implement an optimum balance between the two methods of control.

INTRODUCTION

Northern Britain is well placed to make a contribution to the predicted requirement for increased global crop productivity (Beddington, 2010). Low air temperatures, which prolong the yield-forming phase of crop development, combined with long daylight hours, provide a high potential for solar radiation capture to drive yield accumulation. Unfortunately, the crop canopy required to capture all that solar radiation, is necessarily packed with nitrogen and carbon, and is therefore an attractive ecological niche for pathogenic fungi whose survival depends on gaining access to those two crucial resources.

As foliar pathogens are mobile and have a large geographic range, the crops of Northern Britain form one corner of a vast European and global nutrient source for pathogens. The area of arable crops has rather little genetic variation at a species level. From in excess of a quarter of a million flowering plant species in the world, just four (*Triticum aestivum*, *Hordeum vulgare*, *Solanum tuberosum* and *Brassica napus*) are grown over the majority of the arable area of North Western Europe - thus maximising the potential for specialised pathogens to infect, grow, reproduce and disperse to repeat the life-cycle. Hence, there is a huge pathogen population from which new strains can emerge which are able to overcome control measures deployed against them.

By 1960, the 'boom and bust' cycles associated with the introduction of major ('race-specific', 'qualitative') resistance genes into crop cultivars resulted in a view expressed by Buxton (1960), that: *'Fungi have been described as a "mutable and treacherous tribe", but that even*

this is something of an overstatement is abundantly evident from the frequent and spectacular outbreaks of fungus diseases on previously resistant plants'. The idea dates back to Brierley (1931) who described fungi as *'the most highly mutable of all known organisms'*. However, work over subsequent decades, reviewed by Caten (1996) showed that: *'...in terms of their variability and genetic mechanisms, the fungi are no different from other eukaryotes… Their apparent variability and mutability arises from their rapid multiplication and dispersal, and from the large selection pressures that we place upon them when we deploy new resistance genes and fungicide*s'. In other words, don't blame the pathogen! Humans have created the evolutionary pressure on pathogens, and we need urgently to find better ways to manage the resulting selection.

The impact on yield of failure to maintain effective control has serious social, economic and environmental consequences. The last is not widely recognised. We demonstrated (Berry *et al*., 2008; 2010) that effective disease control is critical to minimise greenhouse gas emissions per tonne of production in wheat. Other workers used Berry *et al*.'s method to analysis these issues for barley and oilseed rape (Mahmuti *et al*., 2009), and arrived at the same conclusion. More recently, we have shown significant and consistent positive effects of disease control on water use efficiency (data unpublished).

This paper considers whether disease control is likely to be sustainable in the long and short term, and then focuses on maintaining effective control of foliar pathogens by integrating host resistance and fungicides. The evidence cited is predominantly from work on small-grain cereals, although the general principles apply to other crops.

IS DISEASE MANAGEMENT SUSTAINABLE IN THE LONG TERM?

If we consider 'long-term' to mean the capacity to maintain food security over many human generations, then the current rate of loss of efficacy is only sustainable if genetic (host resistance) and chemical (fungicide mode of action) resources are virtually limitless or renewable.

Genetic resources

The extent to which resistance genes can be considered as 'renewable' depends on their rate of occurrence, whether they become fixed in host populations by natural selection and the ease (or otherwise) with which the new genes can be found and introgressed into adapted germplasm. There are huge uncertainties in quantifying each of these steps, but plant breeding has substantially increased the ability to move resistance genes between related species and incorporate them in varieties sown across large areas. Compared with natural ecosystems, this has substantially increased the size of pathogen populations from which new virulent strains can emerge. It is unlikely that there has been a compensatory increase in the rate of occurrence of new genes.

However, there are three positive points. Firstly, resistance breakdown in this type of 'gene for gene' interaction between host and pathogen is not usually due to loss of effectiveness of the underlying physical or chemical resistance mechanisms, but rather the loss of effectiveness of the signalling mechanism between pathogen and host which triggers them. Hence, the field

remains open for developing alternative mechanisms of eliciting the defence response - although such mechanisms are unlikely to be so well targeted in space and time as a natural incompatible interaction. Secondly, quantitative resistance (also described by the loose synonyms 'partial', 'minor gene', 'horizontal', race non-specific) against the important necrotrophic and hemi-biotrophic pathogens tends to be more durable than is generally the case for major resistance genes against the biotrophs; although cases of deterioration in the performance of partial resistance have been reported (Cowger *et al*., 2000). Thirdly, the 'reservoir' of sources of resistance in genetically accessible progenitor species and wild relatives is probably sufficient to last for several decades.

Chemical resources

Organic chemistry is almost infinitely variable. However, target species (pathogenic fungi) have a finite number of major metabolic pathways which differ sufficiently from those in non-target species (mammals, birds, invertebrates, etc). This limits the number of points at which biosynthesis might be inhibited in a way which results in high toxicity to the target and low toxicity to non-targets. The number of useful pathways and sites of action is unquantifiable (because it depends partly on the extent to which smaller differences might be targeted with greater precision) but it should not be considered as infinite.

Hence, like mineral and petrochemical resources, genetic and chemical resources may not be exhausted, but are likely to become increasingly complex and costly to mine.

IS DISEASE MANAGEMENT SUSTAINABLE IN THE SHORT AND MEDIUM TERM?

Since the widespread introduction of synthetic fungicides in the 1970's there has always been at least one or two effective modes of action and/or major host resistance genes against each of the foliar diseases of economic importance on arable crops. For this to continue, the rate of introduction of new resistance genes or modes of action must be maintained above the rate of loss of effective genes and modes of action. Considering each in turn:

Rate of introduction – modes of action

The rate of discovery and introduction of new modes of action peaked in the 1960s and '70s. Since then the rate of introduction of new active compounds has been maintained partly by 'patent busting' areas of known activity discovered by a competitor (Russell, 2005). Hence, the introductions of new areas of chemistry, such as the azoles, strobilurins and succinate dehydrogenase inhibitors (SDHIs), were followed by a range of new molecules from these areas, from several crop protection companies. This approach makes commercial sense, but is risky given that there is often cross-resistance within a mode of action - the occurrence of QoI insensitivity caused by the G143A mutation prejudiced the performance of a string of products (Lucas, 2003).

Two areas of innovation may help. The first arises because natural ecosystems are a battleground of competing species which often use chemical weapons to gain competitive advantage. The agrochemical industry has increasingly used this as a diverse source of novel

organic chemistry (e.g. strobilurins). The second is that by understanding how fungi work it might be possible to design fungicides to disrupt specific metabolic processes and so provide disease control. Such approaches have proved difficult, but studies into fungal genomics/metabolomics provide opportunities for this approach (Russell, 2005) and modelling of binding sites is beginning to guide the organic chemists towards more active molecules.

However, these potential technological improvements are weighed against increasing commercial and regulatory pressures. The cost of developing a new fungicide continues to increase, along with the difficulty of achieving regulatory approval in Europe. Although development costs and risks are spread across a global market, these pressures on the agrochemical industry make it unlikely that there will be an increase in the rate of introduction of new modes of action.

PROGNOSIS

Maintenance of control so far has depended on two pieces of good fortune. Firstly, low cross resistance between some azoles (probably due to differences in binding modes between active substances) has meant that some more recent products have continued to be effective despite the performance of older molecules being seriously eroded. Secondly, the speed of evolution of insensitivity and virulence differs within and between species. For example, the basidiomycetes, which include the important rust pathogens, have proved very able to evolve virulence to overcome host resistance genes, but poor at evolving insensitivity against fungicides. Hence, cereal cultivars which are now rust susceptible continue to be grown successfully because fungicidal control remains effective. Conversely, *Mycosphaerella graminicola* (causal organism of septoria tritici leaf blotch) has been very adept at evolving insensitivity, but has been largely unable to erode the forms of host partial resistance deployed against it.

New systemic, broad spectrum fungicides have been introduced approximately every other decade; the azoles were introduced in the 1970s, the QoIs in the 1990s and the new generation of SDHIs are coming on-stream now. The new generation SDHIs have arrived just in time, as *in vitro* sensitivity studies and field data from HGCA fungicide performance trials suggest that the performance of the remaining effective azoles against *Mycosphaerella graminicola* continues a slow decline. Until now SDHIs (as the active substance boscalid, BASF) have been used on a relatively small proportion of the arable area. As new products are used on an increasing proportion of the arable area, the selection pressure for insensitivity will increase substantially. Resistance risk assessment remains an uncertain science, but FRAC rate the resistance risk as moderate to high. Evidence from SDHI insensitivity in pathogens of horticultural crops, and from laboratory studies, suggests some diversity between the different chemical classes within the SDHIs (Avenot & Michailides, 2010). But cross resistance between SDHIs is likely to be stronger than that between the azoles; so there is a serious risk of loss of efficacy, affecting most or all SDHIs, occurring in one or two steps. The availability of the remaining multi-site acting fungicides is under threat from pesticides legislation or the Water Framework Directive.

Although one remaining effective control method against each of the major diseases would be sufficient to avoid serious losses, this perilous situation must be avoided. As the number of remaining effective methods of control declines, the selection pressure increases because the

remaining methods would need to be applied more frequently or more extensively - resulting in a negative feedback loop which increases the probability of loss of control. The aim must therefore be to prolong the effective life of current and future methods of control, to maintain diversity of control.

INTEGRATION TO PROLONG EFFECTIVE LIFE

Although IPM has long been advocated, there has been relatively little mechanistic analysis of integrated control of plant pathogens, to understand its consequences and help guide deployment. Here we consider the epidemiological and population genetics interactions between two main elements of integrated control in arable crops.

Partial host resistance and fungicides both slow the relative growth rate of epidemics by similar effects on pathogen life cycle components; principally reducing infection efficiency and sporulation. Hence, host resistance and fungicides are complementary and can be considered as being substantially interchangeable. Integrated control exploits this to mitigate, and adapt to, pathogen evolution.

The adaptation benefit results from avoiding excessive dependence on one method of control - fungicides maintain control when a new virulence causes loss of host resistance, and host resistance can provide control when insensitivity affects fungicide efficacy. Mitigation of pathogen evolution is more complex and less well understood, but probably of greater long-term importance. There are many similarities between the evolutionary processes driving selection for virulence and insensitivity. One fundamental similarity is that the competitive advantage which drives selection for a new virulent or insensitive* pathogen strain is proportional to the difference in the relative growth rates of the 'wild-type' and new strain, in the presence of the relevant host resistance or fungicide. Hence, we can consider 'mixtures' of host resistance and fungicides, as having similar evolutionary consequences for pathogen populations as mixtures of fungicides.

*As this paper covers both host resistance and fungicide resistance, the terms 'insensitive' and 'insensitivity' are used instead of 'fungicide resistant' or 'resistance', to avoid confusion.

EFFECTS OF HOST RESISTANCE ON FUNGICIDE INSENSITIVITY

Using modelling methods developed to study the effects of fungicide mixtures (Hobbelen *et al*., 2011a & b) we have shown that integrating (i.e. 'mixing') partial host resistance and fungicide treatment reduces selection for a fungicide insensitive strain, thus prolonging the effective life of a mode of action. This benefit occurs because partial host resistance: (i) decreases the difference in relative growth rates of the two strains at any given level of total fungicide dose, and (ii) allows effective control to be obtained with a lower total fungicide dose (fewer treatments per crop or lower dose per treatment), thus further reducing selection pressure (van den Bosch *et al*., 2011).

It is important to note that the second of these beneficial effects applies to the majority of fungal plant pathogens (which are either haploid or reproduce predominantly clonally) but may <u>not</u> apply to invertebrate pests and weeds. The following example illustrates why this is the

case. Many insect pests are diploid, sexually reproducing organisms, so the possible genotypes in the population are SS, SR and RR, where S denotes a susceptible allele and R denotes a resistance one. In many cases the sensitivity of heterozygous (SR) individuals is intermediate to that of the homozygotes (RR and SS). Hence, if a low dose of insecticide is applied, a large fraction of heterozygotes may survive the treatment, whereas applying a high dose will cause higher mortality of heterozygous individuals. In the early stages of resistance selection, when resistance is still rare, almost all resistance alleles will be present in heterozygous condition, because virtually all individuals carrying resistance alleles will mate with susceptible homozygotes (SS). The result of such mating is a combination of SS and SR individuals. In such a scenario applying a high dose is likely to decrease selection and lengthen effective life by killing heterozygous individuals, thus removing R alleles from the population (in the case of absence of selfing and/or inbreeding). In contrast, if a particular pest or pathogen species is haploid or does not reproduce sexually, then dilution of resistance through mating won't occur. As a result, high doses are likely to increase selection and shorten effective life (see van den Bosch *et al*., 2011 for full explanation).

The effects of qualitative host resistance on insensitivity evolution are not known, but it is likely to affect insensitivity emergence (as defined in van den Bosch et al., 2011) by reducing population size.

EFFECTS OF FUNGICIDE TREATMENT ON VIRULENCE

There are strong mechanistic reasons, and some evidence from modelling studies (Giovanni Lo Iacono, analysis unpublished), to suggest that integrating (i.e. 'mixing') host resistance and fungicide treatment reduces selection for virulent pathogen strains, thus prolonging the effective life of resistance genes. This occurs because fungicides decrease the difference in relative epidemic growth rates of virulent and avirulent strains.

The mutually beneficial effect from integration described above implies that over-dependence on either host resistance or fungicides is likely to increase the rate of loss of efficacy of host resistance genes or fungicide modes of action. Hence, the aim must be to identify and implement an optimum balance between the two methods of control.

REMOVING CONSTRAINTS ON INTEGRATION

The two main categories of quantitative and qualitative resistance genes can be sub-divided into those which have been introduced into elite cultivars from related species (alien introgression) or from diverse, but less well adapted, germplasm of the crop species. In both cases there is often linkage to undesirable alleles, which has to be minimised in subsequent breeding.

The drive to achieve more durable disease resistance against the cereal rusts is focussing attention on sources of quantitative resistance (usually detected as quantitative trait loci (QTL)) which individually often have only a limited effect. Hence, two or more genes/QTL are often 'pyramided' in a cultivar for each disease, in order to achieve commercially acceptable control. As a result of pyramiding, and the range of pathogen species to be controlled, cultivars contain large numbers of resistance genes, some of which remain effective and others (usually major

genes) which have been 'defeated' by pathogens evolving virulence. The last sometimes results in segments of alien chromosome remaining in breeding material, despite their original function having been lost.

Breeding for disease resistance may have deleterious effects on yield (Brown, 2002), caused: (i) indirectly (heavy selection for resistant phenotypes in the early generations of breeding programmes reduces the number of lines from which to select for yield in later generations), (ii) via linkage between resistance genes and deleterious alleles, (iii) by deleterious effects from segments of alien chromosome, (iv) by a pleiotopic increase in susceptibility to pathogens of a different trophic group (Kliebenstein & Rowe, 2008), or (v) directly, by a physiological cost of the resistance gene to the plant. Such yield effects are difficult to measure and there is conflicting evidence in the literature, but recent evidence of hypersensitive resistance responses in barley causing stomatal dysfunction (Prats *et al*., 2009) is a particular cause for concern. Even if the yield cost of each individual resistance gene is small, the cumulative effect of accumulating many genes in cultivars could be a significant constraint on yield progress. This imposes a commercial limitation on breeding cultivars with better disease resistance (Fenwick & Berry, 2008). High yielding, disease resistant cultivars have generally proved elusive, so the choice facing the grower is often between growing high yielding susceptible cultivars or lower yielding resistant cultivars.

In contrast to host resistance, treatment with fungicides is highly cost effective in the presence of disease and does not have a deleterious effect on yield in the absence of visible disease. As a result, it is economically advantageous to grow high-yielding susceptible cultivars, despite the higher fungicide input costs, rather than lower-yielding resistant cultivars.

We conclude that implementing effective integrated control, to slow the rate of loss of effective fungicide modes of action, depends on progress with identifying and introgressing durable, quantitative host resistance with low yield costs.

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SCOTTISH OPPORTUNITIES FOR INCREASING AGRICULTURAL PRODUCTION AND FOOD SECURITY IN EMERGING MARKETS THROUGH SUSTAINABLE INTENSIFICATION

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Summary: The paper examines the opportunities, challenges and problems in increasing food and energy crop production in emerging markets through "Sustainable Intensification Systems" (SIS) with Scottish overseas inward investment and technology transfer. This examination is through the use of three major case studies in Ukraine, Cuba and Northern Sudan. The paper examines the implementation of new technologies, Targeted Inputs for a Better Rural Environment (TIBRE) and Integrated Farm Management Systems (IFM) approaches in remediation of agricultural land to aid food security and feed local urban populations. The twin aims of profitable production and environmental stewardship, together with local involvement, environmental protection and social partnership alliances in successful outcomes for all stakeholders, are highlighted.

INTRODUCTION

In the last five years the importance of food security has risen dramatically. From a position of relative complacency in a time of relative plenty for the affluent, national governments have cut back on agricultural research and extension funding. The publicity over food "mountains" in the EU led to a system of set-aside land of 15% in 1987, a situation which seems untenable in 2012. There were increasing assertions that modern agriculture was leading to significant environmental damage and health problems. Life expectancy continued to rise and the percentage of income spent on food dropped dramatically as food prices fell significantly in real terms. A number of food "scares" tipped the public perception against "intensive" farming and led to an increase in organic farming, although it remains a luxury requiring far higher land area per unit of output compared with other agricultural systems. With modern technology Sustainable Intensification Systems require less land resources than less intensive or organic systems, thus relieving the pressure on land for production of environmental services and goods.

In recent years the global consumption of coarse and feed grains has risen to unprecedented levels due to three multiplying factors:

1. The growth in population from 2 billion to 7 billion over the course of one lifetime (FAO data)
2. The increased demand for protein in the diet, especially from those countries whose economies are developing rapidly eg China, India and Eastern Europe.

3. The switch from beef and sheep meat which utilize forage crops towards greater use of pork and chicken which utilise grain crops.

This has led to dramatic rises in prices of commodity crops putting pressure on the poorer sections of society and food importing counties. Land available for food production has reduced, China alone lost 8mha of agricultural land in the last decade. Weather volatility, not climate change *per se*, has also had a significant effect on world stocks and led to both Ukraine and Russia ceasing wheat exports in 2010 in order to feed their own populations following severe droughts. Climate change has always been a constant and needs to be addressed through mitigating technology, although currently the political focus is on decarbonisation of economies, as the link between CO_2 and global temperatures is proving to be increasingly tenuous (Gerondeau, 2010). Nevertheless energy conservation is crucial in modern agricultural systems through "Sustainable Intensification Systems" (SIS).

The effect of higher food prices in countries with a high proportion of daily income spent on food is highly significant. In poorer nations this can rise to over 50% of income. (UK Foresight 2011). Price increases are catastrophic and concentrate the mind via the stomach. Egypt is the world's largest importer of wheat to feed its young population of 80 million. The wheat export bans from Russia and Ukraine meant Egypt had to look elsewhere for supply. In a mirror of the Solidarity Movement in Poland 20 years ago, which heralded the fall of Communism, it was food price rises which were a major factor in sparking the demonstrations in the Middle East. With 63% of the global undernourished in Asia-Pacific countries food poverty is a pressing problem for this theatre, which is having an increasing effect on global supply and demand. Asia is now consuming 45% of global wheat supplies. Instability and fear of shortage, coupled with climatic events are now forcing commodity prices ever higher.

Over the last 50 years the population has increased 111%, yet crop production has increased by 162% whilst the land used has increased by only 27% and yields per hectare have increased by 135% (Higgins, 2011). As a side effect this increase in yield per hectare has spared natural habitats from being cropped. Not only will new technology be required to increase production further but there will also need to be significant policy and social changes coupled with new regulatory regimes. The next 50 years are critical in terms of rapid population rises and the need for increased production. Technologies exist which can assist with increasing production but many political or regulatory barriers can have negative impacts.

Genetically modified (GM) crops have been grown for 15 years now and both public and private research is vibrant outwith the EU. A recent review showed that in developed countries GM crops had increased yield in 59% of research studies (whilst improving quality or reducing inputs) but studies in developing countries showed the same yield trend in 82% of studies, whilst there was a positive economic benefit in 72% of studies (Glick, 2011). The early biotech features have been shown by recent independent research to have released 9.6m ha of land from production through higher yields (Glick, 2011). In India, cotton yields have increased 70% from 2001 to 2008 and half of this increase is attributable to Bt cotton which protects against the bollworm. At the same time insecticide use on Indian cotton has decreased by 56%. Similar reductions in insecticide use have been achieved in Chinese GM cotton crops (Higgins, 2011). This has positive benefits for the environment. A similar positive example in a food crop is the use of RoundUp ready soyabean in Brazil which has resulted in an average 1.5 fewer herbicide sprays and with conservation tillage saved 82 million litres of diesel and 9.9 billion litres of water over the last ten years of GM soya use (Glick, 2011).

The switch of land to fuel crops has also had a major effect in the opposite direction by removing land from food production, particularly with government programmes in the US and Brazil. We need to question whether both growing fuel crops on food land and arable organic agriculture using more land per unit of output are sustainable against a background of food security and food poverty. It has been calculated that it would take 50% of US arable land to produce only 10% of their annual fuel requirement (UK Foresight 2011). In many cases the energy equation is questionable with some energy crops being driven by political programmes rather than the laws of thermodynamics; some resemble no more than large storage batteries with close to neutral energy flows. In 2010 a key milestone was reached with over 50% of the world's population now living in urban areas. This move allows higher incomes, greater social freedom and improved social conditions, especially for women. A further pressure is that 1.8 billion people are predicted to be short of water by 2025, and water use will be doubled by 2050 whilst grain production will need to rise by an extra billion tonnes by 2050 (UK Foresight, 2011). The shift in world demographics with contrasting trajectories for India and China will also have a major impact on water and food needs. Not all the effects of rising CO_2 levels are negative. Rising CO_2 levels may benefit us by increasing crop productivity, improved water use efficiency and more efficient carbon fixation by plants narrow the efficiency gap between C_3 and C_4 crops (Kirkham, 2011).

It would not be too much of an exaggeration to say that farmer productivity has provided not only the ability to afford luxuries but also holds the key to global stability. Whilst it is unlikely that we will experience a 'New Green Revolution' we do need a 'Green Evolution'. We can increase food production by increasing land usage or increasing production per hectare, or a combination of both. This paper concentrates on three differing projects to bring old agricultural land back into production using the best new technology to achieve sustainable profitable production and act as models for progress.

INVESTMENT IN AGRICULTURE

Due to the economic downturn there has been a move towards investments in real assets and opportunities as a means of diversifying investment portfolios. Agricultural investment is underpinned by strong long-term macroeconomic fundamentals Investment in agricultural companies gives a mix of current income and capital appreciation, uncorrelated with returns from equity markets and provides a strong hedge against inflation. All models need to meet the triple needs of economic, technical and environmental requirements to be sustainable. In emerging markets there are significant opportunities to bring land back into production by remediation and to raise yields on areas already being farmed. This represents a leap-frog forward in technology in many cases and avoids costly developmental processes. For example, in Western Ukraine, progress was from horse-drawn ploughs to Global Positioning Satellite (GPS) controlled tractors in two years. There are three main barriers to successful development:- lack of a credible legal system, poor access to efficient capital and lack of a system of land rights. A range of models are possible from contentious and destabilising "Land Grabs" through to fully integrated programmes, involving all stakeholders with a high degree of social partnership. The latter is likely to be a more successful model.

Access to capital can be via a number of routes and business models from sovereign wealth and Government funds through to private investment. In many cases Government development banks are too bureaucratic and slow. Often funds only become available when success has

already been achieved and initial risk capital left to the private sector. More pump priming with easier access to capital would be an important step forward. Government extension services are often lethargic and ill equipped to aid these developments. Risk is critical in any market and political, infrastructure, climatic risk and soil quality need to be taken into account. Any significant agricultural investment produces clear ripple effects in terms of social, workforce and infrastructure development, increasing employment, local economy and taxation and improving working conditions. Initially foreign skills are key, but development and good job prospects for locals must be part of any sustainable strategy.

TIBRE, LEAF AND SCOTTISH QUALITY CROPS

Three development/knowledge transfer programmes are used by Scottish farmers and are now embraced as core philosophies in the case studies discussed. TIBRE is a manual of new technologies in the form of a menu split into areas such as genetic improvement, crop nutrition and protection, information technology and machinery. Each is evaluated so that it shows an environmental benefit and is, at worst, cost neutral to the farming business. Often they will make a financial improvement after a period of training and implementation. They can be implemented alone or together with other options to produce desired synergistic outcomes. A GPS precision agriculture controlled pneumatic fertiliser spreader, applying quality controlled fertiliser according to real time crop density monitoring, gives an improvement in fertiliser efficiency (SNH 1997). This saves the farmer money whilst increasing crop yields and quality and protecting the environment. LEAF is a systems-based approach utilising Integrated Farm Management (IFM) with a significant knowledge transfer and public education function through the use of a network of commercial demonstration farms and public engagement events such as 'Open Farm Sunday'. This annual event welcomes people onto LEAF farms to help inform the public about how their food is produced. SQC (Scottish Quality Crops) is an industry-led certification process for cereals produced in Scotland. In order for the cereals to be sold under the scheme (covering over 90% of Scottish cereals) farms agree to abide by a detailed production and storage protocol, which is independently audited. It involves all stakeholders, including farmers, advisers, merchants and end users, and was, when introduced, the first such scheme in the EU.

DEVELOPMENT IN EMERGING MARKETS: THREE CASE STUDIES

Opportunities for Scottish expertise in global markets are highlighted using three development case studies by Scottish companies or utilising Scottish technical expertise. The case studies are in Ukraine, Northern Sudan and Cuba, and highlight the opportunities available and the differing business and investment models across differing agro-meteorological zones and political landscapes.

Whilst emphasis is on Ukraine, key issues and challenges in emerging markets are common threads in principle in most theatres of agricultural activity. In the first case study used, a Scottish firm of agricultural entrepreneurs, Continental Farmers Group PLC (CFG), identified in 2005 an opportunity with land left fallow since the dissolution of Russian collective farms 20 years ago in Western Ukraine. The break-up of large Soviet collective farms, post–independence, led to a system of land ownership with small individual villager holdings of 0.5-1.0ha, without sufficient capital/incentive to cultivate the land. Soils in Ukraine are world class chernozem soils and fertile for a wide range of crops. Climate in Western Ukraine is an

attraction with higher rainfall (800mm per annum) than further East in more traditional arable areas. The geographical position close to EU markets is also a factor as is historical/cultural links with Poland, where CFG's successful, proven arable model has been in place for 17 years.

An agricultural land sale moratorium is in place in Ukraine to avoid land grabs. This means painstaking, lengthy negotiations with individuals and village councils to aggregate a holding of sufficient size to allow economies of scale for modern production. The "economies of scale" are often cited as important factors in development but the "economies of skill" are also crucial to success. The initial phase in many developments is one of distrust in inward investment, based on history. Degradation of fertile land by profit grabbing entrepreneurs is also a fear. Engagement with local stakeholders is critical as is provision of local employment, a social programme, improved working conditions, prompt payment of rent and taxes and the ability to fulfill promises made. A good reputation takes years to develop but can be lost very quickly by poor management.

Starting with 90 hectares of potatoes in 2006, CFG now have a holding of 30,000ha using a proven model from their Polish business. This is a model with a sound integrated crop rotation of potatoes, wheat, oilseed rape, sugar beet and maize. This model is globally unique, characterised by combining high input/high hectarage with modern technology in contrast to Australian/US/Russian models of low input/high hectarage and the Western European model of high input/low hectarages. This is possible because of the opportunity presented by large tracts of fertile land at very competitive rent, low labour costs and a good climate.

There are no support payments in Ukraine and wheat and oilseed rape are produced and sold at lower than global prices. Yields are significantly above the national averages, e.g. 38 t/ha compared to 13 t/ha nationally for potatoes and 5.5 t/ha compared to 2 t/ha for wheat. As well as outperforming local farmers by up to 300%, the production systems implemented also outperforms other Western Farming companies in the region by 60-100%. In Poland wheat yields have risen to 8t/ha average on land managed under this model, compared to a national average of 3.2t/ha (Dawson 2011). The production systems used are based on an Integrated Farm Management (IFM) and Linked Environment and Farming (LEAF) model combined with a programme developed by the Scottish Government countryside agency Scottish Natural Heritage (SNH) called TIBRE (Targeted Inputs for a Better Rural Environment). IFM brings together varietal and soil management, use of rotations and cultural control, use of modern crop nutrition and protection, building and environmental techniques, use of diagnostics, treatment thresholds and informatics to optimise production processes. The proven system has a fourfold mission statement of:

1. Growing the highest yields
2. Of a product quality that the market wants
3. At the lowest cost of production per tonne
4. With a high level of Environmental and Social Responsibility

There have been significant difficulties along this upward trajectory with challenges in land amalgamation and registration, cultural differences, staff training, knowledge transfer and climate. New technology, particularly in improved (GM where possible and conventional) genetics is important and highlights the crucial role of genomics and investment in research and development. The Eastern European initiative described above is a textbook example of

the TIBRE approach. It is critical to success to develop best working practices and quality assurance coupled with a high degree of environmental stewardship. There is a clear "Leapfrog Effect" from horsepower in the Ukraine to 400hp GPS controlled tractors pulling large cultivators or computer controlled one pass planters. This allows both economies of scale and reductions in greenhouse gas emissions and significant organic matter return to the soils. Lack of infrastructure and machinery has required significant investment in storage, transport and drying to improve marketing. This has led to them being a significant employer in an area of high rural unemployment. They are used by the regional Oblast government as a textbook example of foreign direct investment and have embarked on a process of technology transfer with local farmers in partnership with the local regional government, which involved a sponsored visit to Scotland by senior Ukrainian officials during the summer of 2011 to visit JHI, LEAF farms and modern Scottish production facilities.

In addition to an analysis of the Ukraine project, the involvement of Scottish experts in a Saudi backed irrigation project in Northern Sudan, using abundant water from the Nile to irrigate 12,000ha, highlights such challenges. A third Scottish project in Cuba is also informative as a developing Agri-Energy project. This utilises remediated land as a source of feedstock for renewable energy plants, allowing subsequent cultivation for food crops with both domestic and export potential. This former agricultural sugar basket is now importing 85% of its domestic food requirements despite abundant agricultural land and a good climate, due to lack of markets, capital and land rights exemplifying many of the issues in global food security.

CONCLUSION

There are great opportunities for Scotland and Scottish technology in increasing production in emerging markets with low productivity using Scottish investment, technology and expertise. The case studies highlight the importance of meeting technical, economic and environmental objectives, together with the need for local involvement and social partnership alliances in successful outcomes for all stakeholders. Meeting these needs will be key in achieving global food security using Scottish skills.

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THE VALUE OF EFFECTIVE COMMUNICATIONS IN DELIVERING SUSTAINABLE ARABLE PRODUCTION

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In times of change, learners inherit the Earth, while the learned find themselves beautifully equipped to deal with a world that no longer exists. (Eric Hoffer).

Summary: Effective communication is a key component of sustainable agriculture; theoretical perspectives from communication science, such as learning approaches, key messages and understanding barriers, as well as different types of communication are all included. Communication about sustainable agriculture, which creates political-economic and social environments that promote development of sustainable systems, must more clearly define sustainability and what is to be sustained and must help producers and the public "think agro-ecologically." Communication of sustainable agriculture standards creates and disseminates information about how to farm and consume sustainably. Increasing farmer participation in production research and easing farmer access to media to disseminate on-farm trial and research findings will enhance this process. Already a lot is being done and it is critical that we learn and build on these activities to build a more robust and sustainable arable system. This paper will highlight some of the key findings from LEAF's (Linking Environment And Farming) work in communicating sustainable farming techniques among farmers, industry and consumers.

INTRODUCTION

"What I hear I forget, what I see I remember, what I do I understand."
Xunzi (340-245 BC),

Much of the current discussion on sustainable arable production reflects concerns about how the goals of agricultural research and production are to meet growing efficiency and production needs whilst protecting and enhancing the environment and engaging consumers. Increasingly the sustainability of agriculture has become a prime focus in many processes such as negotiating prices, setting economic goals, setting standards for agricultural producers and processors, whilst also achieving environmental enhancement and balancing societal goals of family, community and society. This negotiation has created opportunities for the non-agricultural public to express their views of agriculture's economic, environmental and societal roles, as well as for those in agriculture to better articulate their own goals and values to the general public.

The above currently demonstrates that communicating sustainable agriculture is considered as a social process and is not confined to the media, but also to our interaction with food, our values, rural environment considerations and our network of social relationships.

This paper is divided into four key areas:

1. Why there is a need to communicate sustainable arable production approaches
2. Different learning styles and techniques
3. Farmer to farmer and industry communications
4. Farmer to consumer communications

1. WHY THERE IS A NEED TO COMMUNICATE SUSTAINABLE ARABLE PRODUCTION APPROACHES

When the UK Government's Chief Scientist, Professor John Beddington (Government Office for Science, 2010), warned of a 'perfect storm' with food shortages, scarce water, insufficient energy resources and mass migration as people flee from the worst-affected regions, many farmers across the world listened hard as they considered the implications of crop failures to grain prices.

With 80% of the population consuming a diet of principally four main crops: maize, wheat, potatoes and rice, given the projected population growth it is critical that we increase yields rapidly. Indeed Rothamsted Research has set targets to grow the potential of wheat crop to 20 tonnes per hectare within 20 years. Such growth targets demand innovation and clear messages, and for these to be communicated effectively among farmers, researchers and industry. It also requires the public to accept both the objectives and new techniques needed to achieve them. The future is not doing more of the same; it is about increasing sustainability at all levels. The real element of change is about growing production, whilst enhancing environmental health, and societal well-being in a fully integrated approach.

2. DIFFERENT LEARNING STYLES AND TECHNIQUES

The same communication approaches do not work for all. Individuals learn best in different ways. We all have different learning styles and our choices are made on a mixture of experience, tradition, culture, perceptions and prejudice.

Over the last 20 years LEAF has experience of using a wide range of learning approaches. LEAF was set up in 1991 to emphasise the need for practical, realistic and achievable solutions to farmers, consumers, processors and retailers, through management tools, demonstration and a food assurance scheme. Delivering food security with supply chain-led initiatives is only part of the solution. The role and potential of Integrated Farm Management (IFM) in provision of more sustainable food security has been consolidated by the participation of representatives from growers, environmentalists, consumers and companies throughout the agriculture and food supply chain. These groups have engaged and encouraged the implementation of more sustainable measures through demonstration and communication and other appropriate means, including farm assurance (IACPA, 1995). The emphasis has been on grower involvement by creating added value to collaborating farmers, and for consumers to purchase products produced with certified environmental benefits.

Today LEAF focuses its work on four areas to deliver the above:

- Technical know how
- Demonstration and communication
- Market opportunity
- Political influence

LEAF contributes to meeting these global challenges through the provision of simple practical ways for the farmers to deliver sustainable agriculture. Part of an effective communication strategy is the promotion of acceptance of new technology by society, challenging ingrained habits, and openness. Part of delivering this change is providing a range of communication approaches in environments conducive to learning. Style considerations should include:

- Structure - logical, clear, structured, created
- Sociological - group activities, self learners, group/peer learning, cooperative learning
- Auditory - listening, class discussions, lecturing
- Visual - visual colours, text
- Tactile - most people learn best with hand on activities

The variety of approach in communication is essential to understand and influence changes in behaviour.

"We've gone from being exposed to about 500 ads a day back in the 1970s to as many as 5,000 a day today." Jay Walker-Smith, Yankelovich Consumer Research

Long gone are the days when simply a letter or a conversation is sufficient to change behaviour. Today we experience advertising, social media and blogs, e-mails, websites, radio, television, the list goes on. The challenge for delivering the sustainable agriculture message is finding the right combination of communication media and messages. The sophistication of communication continues to grow, tapping in to our senses, tugging at our conscience and feeding our perceptions. It is not only what is said that is crucial, it is how it is said and how we communicate depends on the audiences that we wish to influence, encourage and change.

3. FARMER TO FARMER AND INDUSTRY COMMUNICATIONS

In 2009, LEAF, with support from Defra, carried out an extensive study of the effectiveness of knowledge transfer of beneficial management practice, legislation, innovation, innovation systems and "sticky" knowledge. Specifically, LEAF looked to identify the strengths and weaknesses of the current demonstration and knowledge transfer approaches, where there were gaps in knowledge, and identification of more effective ways to work with industry, consumers, farmers and researchers. This was to ensure that an effective and co-ordinated approach was used to prepare the farming and food sector for the impacts of climate change and market forces.

Recommendations

The LEAF recommendation was that there needs to be more understanding of the systems in which the various actors operate, and, above all else, the need to form coalitions or alliances of

actors with shared interests to enable change to occur. The importance of intermediaries in knowledge transfer was emphasised, and of 'sticky' knowledge and the process for making information reach farmers in a memorable and lasting way. It is worth stressing that knowledge is formed by building on research with experience, skills and practical application to develop innovation and solutions.

For this work we also interviewed and sought the opinions of over 120 individual farmers, those representing companies, and government agencies. We asked them where and how they currently received information, the effectiveness of the LEAF Demonstration Sites, gaps in knowledge and what communication mechanisms would help support their business in the future.

It is clear that from this work that there is a need to provide a framework for a more effective exchange of knowledge and information, building clearer linkages between researchers, commercial companies and farmers. Following this study LEAF is now carrying out and developing the two recommendations it highlighted in the study:

1. To develop and strengthen the current network of demonstration sites and establish a stronger regional network bringing together researchers, farmers and commercial businesses as well as environmentalists, retailers and consumers, with a view to investigating new approaches to sustainable agriculture, and demonstrate tried and tested approaches to beneficial resource management practices and encourage change in farming practice.

2. To develop an online information set of 'wikis' and illustrated beneficial practices (video clips and podcasts) – a 'LEAF'ipedia. We have already started this work developing blogs, and video clips relating to some of the key messages from our corporate members and individual farmers. One such member is Jake Freestone, the farm manager at Overbury Estate. His success in using social media has benefited his business, clearly demonstrating their personal commitment to more sustainable farming techniques as well as providing their customers with up-to-date information about what they are doing on-farm. With over 1700 tweet followers he is able to communicate up-to-date information and has already provided new contract opportunities for their business.

Aside from the management tools that LEAF has developed it is also clear that farmers welcome and value the LEAF Innovation and Demonstration activities. This is clear from consistent feedback from farmers attending LEAF Field Days (2007 and 2009) detailed below:

Do you plan to attend another LEAF event?

74% - Yes, definitely *26% - Possibly, I don't know yet*
0% - Probably not *0% - No, definitely not*

As a result of visiting the event, will you be changing farm practices?

11% - Yes, we will be introducing changes straight away
80% - Probably, after taking advice
9% - No, we will not be making any changes

As a result of attending the event, has your awareness of your whole farm's environment increased?

18% - Yes and we are making changes to how we farm as a result
76% - Yes, it has made us think of what we might do
8% - No, we were already aware of all the issues discussed

Demonstration Farms. Demonstration has been a key part of LEAF's work. We have established over 100 demonstration farms from the 2300 farmer members and are now strengthening this network. At these sites, the promotion of beneficial practices, through the adoption of IFM, helps and supports other farmers to improve their business and environmental performance.

Future developments. Our knowledge base is developing into an online 'LEAFipedia' to host technical information, practical solutions, and industry examples. What has been fundamental to the success of this approach is to engage in broad communication and collaboration with stakeholders, and others to ensure a co-ordinated and comprehensive approach to sustainable agriculture issues.

Market Standards. The other key success criteria have been to encourage a step-by-step approach that is structured with a defined end goal, i.e. market opportunity alongside a sustainable business. The LEAF Marque standard (LEAF, 2011) is based on IFM. The standard is developed with the help of many organisations including Waitrose and other retailers, RSPB, Natural England, farmers, Environment Agency, WWF and others; there is a technical advisory board and a management committee to ensure good governance. The standard is whole farm, dynamic and requires farmers to demonstrate continual improvement in environmental management.

4. FARMER TO CONSUMER COMMUNICATIONS

As with any environmental management strategy or corporate social responsibility, communication with the public is critical. With a growing urban population, we have the potential to loose the understanding of nature, food, our culture and many traditions. Part of LEAF's remit is communication with the public. The demonstration farmers have been instrumental in building trust and understanding with a range of consumer, environmental and educational groups. From this has sprung the successful and interactive "Speak Out" (LEAF, 2005) training courses we offer, the range of notice boards that provide information in-field to consumers and walkers, the Virtual Farm Walk, development of LEAF Marque now on over 20% of our fresh produce, and more recently to projects such as Open Farm Sunday and Let Nature Feed Your Senses.

Engaging consumers.

It is critical that, not only do we deliver more sustainable production, but also that consumers understand where they can play a part. This can be through the market and making sustainable choices easy, or through engaging them on-farm. Research has shown that when children, especially under the age of 11, experience the wild and learned basic outdoor skills, this increases their self-confidence and encourages free-thinking and environmental awareness. (Louv, 2005).

This is the seventh year of LEAF Open Farm Sunday (LEAF,2010), encouraging farmers to welcome the public out on to farms. In June 2011 over 360 farmers opened their gates and with the help of some 6100 people in the industry, welcomed some 120,000 consumers out on to farms on one day, building more understanding and trust between farmers and their customers. Of these visitors some 36% were under 18. Over the last 6 years over three quarters of a million people have attended Open Farm Sunday with over 900 farms taking part and at least 10,000 individuals being involved. Over 80% of people attending thought the day was brilliant or amazing, helping to demonstrate how valued farming can be. Visitor knowledge and understanding of food production and how farmers care for the environment has therefore been extensively enhanced.

In 2011 the Countryside and Community Research Institute (CCRI) (Mills, 2010) conducted a study of the benefits of LEAF membership. Aside from improved business performance through adopting IFM practices, significant social benefits were identified. Improved communication appears to be a critically important benefit of LEAF membership, which is likely to have been under-reported in the previous member surveys:

- Over 90% members interviewed agreed or strongly agreed that LEAF membership had "improved understanding among the local community"
- Members feel they are seen as *'more approachable'*; this increases people's appreciation & tolerance of farming practices, and improves understanding

CONCLUSION

There is much value to individual businesses, and the farming industry as a whole in communicating sustainable arable practices. Sustainable agriculture is critical for businesses to ensure the ability to demonstrate flexible, practical, innovative, knowledge rich and technically astute farming strategies, such as IFM. LEAF has much evidence to demonstrate that communication is key and we will increasingly evaluate the importance of communication alongside the need to practically engage consumers through all communication channels, such as LEAF Demonstration Farms. Open Farm Sunday, Let Nature Feed Your Senses and market opportunities like LEAF Marque.

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ECOLOGICAL TOLERANCE: CHANGING OUR APPROACH TO CROP PROTECTION

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Summary: Leaf-associated microbial communities of the field-grown winter barley cultivar Sumo were monitored for a year. The phyllosphere were colonised by many micro-organisms that interact with pathogens such as barley leaf blotch, caused by *Rhynchosporium secalis* (Rs). Crop protection treatments applied in the field alter the natural microbial populations, which could in turn affect the ecological competition of the phyllosphere. Crop protection strategies may therefore need modifying to favour beneficial microbes and disadvantage both pathogens and helper organisms.

INTRODUCTION

Crop protection against fungal diseases is normally achieved through fungicides that are highly effective at killing most fungi and are toxic to some non-target organisms. The non-targets that populate the leaf surface can include many fungal and bacterial species that may be beneficial, benign or pathogenic. Inevitably, many become part of the disease complex and may enhance symptoms such as with *Septoria tritici* on wheat (Newton *et al.*, 2004). Others may reduce symptoms, thereby offering the potential for biocontrol such as against powdery mildew (Kiss, 2003). Therefore the non-target effects of crop protection treatments should be considered in order to enhance biocontrol organisms and inhibit pathogenicity "helper" microbes (Newton & Toth, 1999).

Crop cultivar, agronomy, location, weather and many other factors affect the leaf microbial community composition (Lindow & Brandl, 2003). Crop protection treatments superimpose changes on top of these either by differentially killing components using conventional agrochemicals, and/or priming or inducing the plant's own defence mechanisms using "resistance elicitors" or "plant activators" (Walters *et al.*, 2005), which will also differentially inhibit different microbes. Epiphytes and endophytes (i.e. microbes living on or in the leaf respectively) are modified similarly based on the applied treatment. We have carried out trials to find out how treatments could challenge leaf-associated micro-organisms to disadvantage pathogens, focusing on barley leaf blotch.

MATERIALS AND METHODS

In 2010, the barley cultivar Sumo was grown on site in a randomised split plot three replicate trial with plots measuring 1.6 x 4.2 m sown at a density of 320 seeds/m^2. A range of treatments was applied including fungicides and combinations of elicitors on three occasions corresponding to normal fungicide treatment times T1, T2 and T3: beta-aminobutyric acid, acibenzolar-S-methyl and cis-jasmonate were combined as described by Walters *et al.* (2011), whereas fungicides treatments included cyprodinil and spiroxamine, prothioconazole and pyraclostrobin, epoxiconazole (Oxley & Burnett, 2008) and applied at T1, T2 and T3 respectively. Leaves were sampled at regular intervals between each treatment application and were either used fresh for microbial isolation or stored at -20°C for molecular characterisation. Disease was visually scored according to Newton and Hackett (1994).

After weighting them, five leaves were placed in a scintillation vial with 10 ml of 0.1 M potassium phosphate buffer (pH 7.0) and sonicated for 7 min. Micro-organisms were isolated by plating appropriate serial dilutions on selective complex media: nutrient agar supplemented with rifamycin (30 µg/ml) or CzV8CM supplemented with streptomycin (100 µg/ml). Colony forming unit (cfu) count was estimated after incubating plates for three (bacteria) to five (fungi) days at 18°C. Washing solutions were pelleted and stored at -20°C together with the sonicated leaves for further molecular characterisation of epiphytic and endophytic communities respectively.

The 16S ribosomal DNA region of 44 isolated bacteria was sequenced and isolates were identified by comparing the sequences to online database using BLAST. Subsequently, sequences were aligned to sequences of known related species using CLUSTALW and phylogenic trees were constructed based on the maximum-likelihood algorithm (bootstrap value: 100). The effect of culturable bacteria was assessed with a dual-inoculation plate assay and was carried out with *Rhynchosporium secalis* (Rs – strain L2A) and one isolate of each identified species.

DNA was extracted from washing pellets and sonicated leaves using a method described in Fountaine *et al.* (2009). DNA fingerprinting of the bacterial population was assessed qualitatively by Ribosomal Intergenic Spacer Analysis (RISA) and semi-quantitatively by Terminal-Restriction Fragment Length Polymorphism (T-RFLP). Briefly, RISA is based on the variable distance between the 16S and 23S rDNA genes of bacterial species (Fountaine *et al.*, 2009), whereas T-RFLP is based on variable position of *HhaI* restriction site within the conserved 16S rDNA gene (George *et al.*, 2009). Only fluorophore-labelled terminal fragments, also referred to as operational taxonomic units (OTUs), with a fluorescence value greater to 1 % of total fluorescence were used to determine the OTUs relative abundance.

RESULTS

The leaf surface of field-grown barley was shown to be a highly colonised habitat. The most common microbes present were bacteria and yeast, but filamentous fungi still represented a large epiphytic population (Table 1). The overall culturable bacterial population of the phylloplane was largely dominated by *Pseudomonads* (Table 2). Phylogenic study identified that most *Pseudomonads* grouped either with *P. syringae* or *P. fluorescens*, but two other species were also identified: *P. graminis* and *P. migulae*. Finally, isolates related to *Erwinia tasmaniensis* were also detected.

Table 1. Colony forming unit count of micro-organisms (mean ± SE) from various phyla isolated from the leaf surface of the field-grown winter barley cultivar Sumo.

	Bacteria	Yeast	Filamentous fungi
$\log_{10}$ of cfu per leaf fresh weight (g)	5.52 ± 0.056	5.56 ± 0.063	4.17 ± 0.028

Table 2. Identity, proportion and theoretical OTU size of epiphytic bacterial isolates (N.D.: not determined).

Identified species	Percentage	OTUs size (bp) T-RFLP	OTUs size (bp) RISA
Pseudomonas syringae (Ps)	42	209	815
Pseudomonas fluorescens (Pf)	34	157 - 207	796
Pseudomonas graminis (Pg)	8	207	798
Pseudomonas migulae (Pm)	4	206	N.D.
Erwinia tasmaniensis (Et)	12	371	862
Pectobacterium atrosepticum (Pba)	PCR detected	373	767

Even though the phyllosphere was highly colonised with culturable micro-organisms, they represented a fraction of the whole leaf-associated ecological diversity (Figure 1). Bacterial richness was estimated by counting the number of bands, which correspond to one OTU. DNA fingerprinting revealed that epiphytic bacteria population had a much greater richness than when using culturable technique. Furthermore, an OTU can group many closely related species (Table 2). The molecular method enabled differentiation of epiphytic from endophytic bacteria and showed that both habitats share some OTUs, but others are unique to one or the other. However, variability within each habitat was also observed between biological replicates.

In order to characterise microbial interactions occurring in the leaf, a dual-inoculation plate assay was set up using Rs and one isolate of each identified culturable bacteria. *Pectobacterium atrosepticum* (Pba) was also tested as its presence in the field had previously been correlated with reductions of Rs symptoms (Newton *et al.*, 2004). Furthermore the bacterium was detected in the field using species-specific PCR (data not shown). All bacteria hindered Rs growth *in vitro* but this could be explained partially by competition for nutrients (Table 3). However, both *P. atrosepticum* and *P. syringae* impaired even more Rs growth compared to other bacteria, suggesting they possess antifungal properties.

Table 3. Effect of bacterial isolates on Rs growth *in vitro* (LSD: 0.100)

Treatment	Control	Mock	Et	Pba	Pg	Pf	Pm	Ps
Rs growth (cm^2)	2.429	2.138	1.498	1.257	1.606	1.564	1.473	1.072

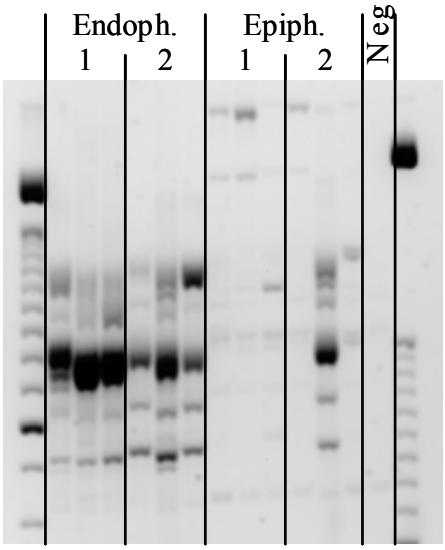

Figure 1. RISA profiles from endophytic and epiphytic bacterial populations from two field plots, run in triplicate

In the field, fungicide treatment prevented leaf blotch developing in treated plots. Elicitor treatments resulted in a substantial reduction of leaf blotch symptoms (Table 4). However, treatments did not only affect the fungal disease, but also the endophytic bacterial population. The overall richness was similar (data not shown) but the relative abundance was modified (Figure 2). Endophytic bacteria are largely dominated by the 204 bp OTU that is likely to correspond to the Pseudomonads (Table 2). Treatment application resulted in a great reduction of the relative abundance of Pseudomonads, increase of other OTUs (55, 90, 93 and 135) and appearance of others (294). Furthermore, treatments affected differently OTUs.

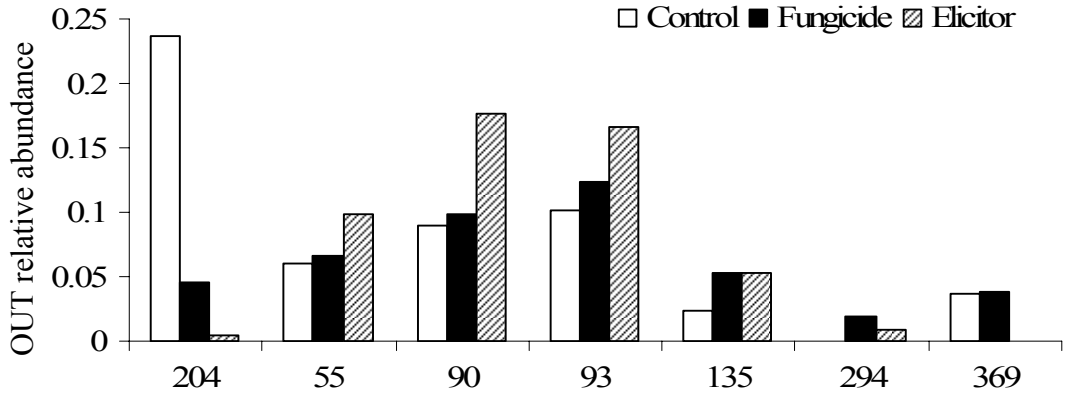

Figure 2. Relative abundance of a selection of T-RFLP fragments

Table 4: Effect of various treatments on Rs symptoms on field-grown Sumo (mean ± standard deviation) ($P<0.001$)

	Rs infection (%)		
	Control	Elicitor	Fungicide
GS25	0.47 ± 0.46 [a]	0.43 ± 0.49 [a]	0.43 ±0.29 [a]
GS30	4.23 ± 3.71 [b]	3.4 ± 2.77 [b]	0.03 ± 0.06 [a]
GS39	20 ± 4.33 [c]	6.67 ± 1.44 [b]	0.03 ± 0.06 [a]

DISCUSSION

The barley phyllosphere is largely colonised by many different micro-organisms. Bacteria and yeast are the most abundant. The culturable bacterial population is dominated by *Pseudomonads* species, which are commonly detected in the phyllosphere of other plant species (Lindow & Brandl, 2003). Both *P. syringae* and *P. atrosepticum* are well-known plant pathogens, whereas *P. fluorescens* and *E. tasmaniensis* are well characterised biological control agents (BCA). Even though the roles of these bacteria on the barley leaf are unknown, they possess many adaptive features to manipulate the host and/or surrounding microfauna. The presence of a high inoculum of *P. atrosepticum* in a barley field was already correlated with a reduction in Rs symptoms (Newton *et al.*, 2004). *In vitro* assays have demonstrated that *P. atrosepticum* and *P. syringae* hinder Rs growth more than other leaf-associated bacteria. The mechanisms involved in this interaction remain unknown, but suggest diffusible compounds, such as non-ribosomal peptides (NRP) (Mendes *et al.*, 2011).

Both fungicide and elicitor applications reduced Rs symptoms compared to the control, but fungicide treatment reductions were much greater. However, endophytic bacterial communities were also altered by the treatments, but differentially based on the treatment applied. The effects of such changes in the bacterial communities on Rs control and on the plant are unknown. Determining the roles and functions of most of the bacteria remains difficult but necessary and would require deep-sequencing technology. Interestingly though, the 204 bp OTU corresponds to *Pseudomonads*, according to *in silico* T-RFLP, and includes *P. syringae* which was shown to hinder Rs growth *in vitro*. As this OTU relative abundance is much lower after treatment application, this would lead to the elimination of these particular natural Rs competitors. Promoting such groups of micro-organisms could improve the reliability of crop protection treatments.

Using a BCA from a different ecological niche has multiple disadvantages (Kiss, 2003) whereas managing the natural plant-associated microbial population offer considerable potential for improving the sustainability of crop protection (Newton *et al.*, 2010). Due to European restrictions on pesticide usage (e.g. EC 850/2004), elicitors represent a valuable complement to the use of fungicide in integrated pest management programmes. Elicitors and fungicides differentially alter the leaf-associated bacteriome, which could lead to new integrated disease control strategies. Additives in treatment formulations could also help to manage the phyllosphere.

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MANAGEMENT OF *PYRENOPEZIZA BRASSICA* IN OILSEED RAPE IN NORTHERN BRITAIN

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Summary: Prothioconazole and tebuconazole, alone or in co-formulated mixtures, have been in commercial use extensively for many years, as foliar sprays, to control diseases of oilseed rape. These data show the current situation, relating to light leaf spot (*Pyrenopeziza brassicae*) control, as seen in five trials conducted during the season 2010-2011 in Northern Britain Two spray programmes of prothioconazole and/or tebuconazole gave more effective disease control and yield improvements compared to flusilazole.

INTRODUCTION

Pyrenopeziza brassicae (Light leaf spot) is the major oilseed rape disease in northern Britain. Although the disease occurs in the rest of Britain, Phoma is more dominant in southern Britain; however, the scale of light leaf spot in southern Britain is probably underestimated due to latent or indistinct visual symptoms (Gladders *et al*., 2006).

Light leaf spot causes infections on leaves, flower buds and pods, often seriously diminishing rape yields in susceptible crops, with yield losses of up to 40% in the worst case. Average yield losses range between 0.5 and 1.2 t/ha, depending on the severity of infection (Sandsford *et al*., 1996).

In order to maximize returns from oilseed rape crops in northern areas of the UK it is necessary to implement a robust disease management strategy involving cultural measures alongside effective chemistry. In recent years control of the disease in areas of northern Britain has become difficult as a result of specific conditions very favourable to the development of the disease, difficulty in making well timed applications due to climatic conditions and possible shifts in sensitivity to azole fungicides. This paper presents recent trials data from northern England and Scotland to demonstrate the effectiveness of the key light leaf spot azoles, prothioconazole and tebuconazole, and to identify best practice for management of light leaf spot in oilseed rape crops in northern areas of the United Kingdom. These data follow on from a previous paper presented at this conference by Godley and Bush in 2006.

Prothioconazole is a broad-spectrum fungicide discovered and developed by Bayer AG belonging to the triazolinthiones, which are demethylation inhibitors (DMI). It is systemic and provides protective, curative and long lasting anti-fungal activity (Mauler-Macknik *et al.,* 2002). It is active against the important fungal diseases in oilseed rape, Sclerotinia (*Sclerotinia sclerotiorum*)**,** Stem canker (*Leptosphaeria maculans*) and light leaf spot.

Tebuconazole is an azole fungicide discovered by Bayer in the early 1980's and is now a well-established active ingredient, used commercially for many years to control a wide range of crop diseases throughout the world. It is used straight and in mixture in a range of foliar formulations and seed treatments.

MATERIALS AND METHODS

Field trials using fully a randomised block design were conducted on winter oilseed rape in the season 2010-11. There were five sites and locations which are noted in table 1. The minimum plot size was $36m^2$. Fungicide treatments were applied to field trials with pressurised knapsack equipment through flat fan nozzles calibrated to deliver 200 l/ha. Prothioconazole as Proline® was applied as a 275 g/l emulsifiable concentrate (EC), tebuconazole as a 250g/l emulsion(oil in water) formulation as Folicur®, Prosaro® was used as an EC containing 125g/l prothioconazole and 125g/l tebuconazole, all three were compared to Sanction 25® which was as a 250g/l emulsion (oil in water). Two spray applications were applied, one timed in autumn and the second in winter/early spring. All trials were naturally infected with light leaf spot. Other agronomic inputs accorded with local practice.

Foliar disease was assessed visually with the % leaf or plant infection recorded, Seed yields were assessed using small plot combine harvesters and corrected to 9% moisture content. Data were analysed statistically using an analysis of variance and LSD test at the 5% probability level.

RESULTS

Field trials data is presented to demonstrate the effects of tested fungicides on light leaf spot over the five trial series throughout northern Britain. .

Results in Table 1 show the activity of prothioconazole and/or tebuconazole against light leaf spot in 5 trials conducted in 2010-11. Assessments were carried out over a protracted time scale, due to bad weather conditions in late winter into the spring.

A summary of yields is presented in Table 2. Yield responses from prothioconazole were generally rate related whilst the responses with tebuconazole were often uneven or flat, particularly when comparing the moderate or higher dose (188 gai/ha and 250gai/ha) treatments.

Table 1. Control of light leaf spot leaf infections in winter oilseed rape.

	% light leaf spot severity					
Trial	SAC	CW	DH01	MB01	NDSM	Mean
Date	*7/4*	*3/5*	*8/3*	*23/3*	*28/3*	
Location	Lothian	Perth	N. Yorks.	N. Yorks.	N. Yorks.	
Treatment g a.i./ha						
prothioconazole 124	10.8	1.4	3.5	12.8	13.8	8.5
prothioconazole 160	11.3	1.3	1.1	4.5	12.5	6.1
prothioconazole 173	8.3	1.1	0.6	2.8	8.8	4.3
tebuconazole 125	3.0	1.9	3.8	9.7	18.8	7.4
tebuconazole 188	1.6	1.7	3.5	4.0	17.5	5.7
tebuconazole 250	1.5	1.1	1.8	2.6	11.3	3.7
tebuconazole 125 + prothioconazole 125	5.3	1.4	1.0	1.2	10.0	3.8
flusilazole 100	7.5	1.6	1.5	14.3	22.5	9.5
Untreated control (% disease index)	23.3	6.6	11.8	20.9	46.3	21.8
LSD (*P*=0.05)	7.24	0.97	1.7	7.55	7.49	

Table 2. Oilseed rape seed yield,(t/ha), after fungicide applications against light leaf spot.

Trial	CW	SAC	DH01	MB01	NDSM	Mean
Date	*7/4*	*9/9*	*17/8*	*1/8*	*17/8*	
Location	C. Angus	Perth	N.Yorks	N.Yorks.	N.Yorks.	
Treatment g a.i./ha						
prothioconazole 124	5.47	4.47	4.14	4.44	4.20	4.54
prothioconazole 160	5.56	4.7	4.21	4.70	4.27	4.69
prothioconazole 173	5.59	5.02	4.20	4.82	4.28	4.78
tebuconazole 125	5.43	4.61	4.16	4.3	4.05	4.51
tebuconazole 188	5.41	4.88	4.30	4.46	4.05	4.62
tebuconazole 250	5.52	4.68	4.44	4.5	4.05	4.63
tebuconazole 125 + prothioconazole 125	5.62	4.7	3.95	4.79	4.13	4.64
flusilazole 100	5.47	4.24	4.02	4.41	3.87	4.40
treated control	5.21	3.86	3.87	4.2	3.61	4.15
LSD (*P*=0.05)	0.312	0.645	0.65	0.268	0.210	-

DISCUSSION

The data demonstrated good activity of prothioconazole and tebuconazole against light leaf spot, an important yield robbing disease of oilseed rape. The levels of disease control and yield responses were above those of flusiazole.

Prothioconazole and tebuconazole were both equally effective against light leaf spot in these trials, independent of disease levels. Mean disease data from the sites shows a dose response with higher control levels achieved at higher doses. Mean disease levels across sites were highest in the flusilazole treatment.

Whilst a clear disease dose response with tebuconazole is seen across the five sites, the same is not true for the yield responses, here tebuconazole gave a lack of mean dose response across the five sites, when used at the two highest dose rates tested. With prothioconazole the disease control and yield improvement more closely follow the dose rate, with prothioconazole providing generally superior yield delivery to tebuconazole in most cases.

Margin over input costs were best from prothioconazole treatments, with the highest dose treatment, 173gai/ha, giving the greatest return of £168/ha, based on oilseed rape @ £360/tonne. Flusilazole gave only a £65/ha return. Insensitivity of *P. brassicae* to azole fungicides in crops in Scotland has resulted in growers now needing to increase their application rate to two thirds to three-quarters of full label rates of commercial fungicides for effective control (Burnett, 2004). Poor control of light leaf spot in recent years is likely to be related to a combination of difficulty in achieving correct application timing and favourable conditions for disease development, as much as fungicide insensitivity, particularly in a season like 2010-11, when weather factors made it difficult to apply products at the correct timing.

Light leaf spot is a trash borne disease and as such is likely to become more problematic in years to come, if the trend for minimal tillage and closer rotations continue. Effective fungicides, like prothioconazole and tebuconazole, in conjunction with improved agronomic practices and new varieties with improved varietal resistance, can offer growers the opportunity to control light leaf spot, increase yield and improve oilseed rape profitability.

Prothioconazole and tebuconazole, like other fungicides approved against *P. brassicae* are azoles, but results in this paper demonstrate that both actives can be part of an effective integrated approach to light leaf spot control. However, to maintain the performance of both prothioconazole and tebuconazole in the future, growers should adhere to resistance management principles.

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CONTROL OF LIGHT LEAF SPOT ON WINTER OILSEED RAPE USING RESISTANCE ELICITORS

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Summary: Field trials were conducted in two seasons to examine the effect of resistance elicitors on light leaf spot on oilseed rape. An elicitor combination comprising acibenzolar-S-methyl (ASM), β-aminobutyric acid (BABA), and *cis*-jasmone (CJ), was used, and was compared to standard fungicide treatments. In both years, the elicitor combination applied on its own, provided effective control of light leaf spot, superior, in most cases, to that provided by fungicide treatments. None of the treatments produced a significant effect on yield. Based on these data, and in view of the poor performance of fungicides in regions at high risk from light leaf spot, resistance elicitors offer the potential for a new approach to controlling light leaf spot on oilseed rape.

INTRODUCTION

Resistance can be induced by application of various agents to plants (Reglinski & Walters, 2009) and can be split into two main types: systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR involves a restriction of pathogen growth and a suppression of disease symptom development, and its onset is associated with salicylic acid (SA) accumulation at sites of infection and systemically, and with the coordinated activation of a specific set of genes encoding pathogenesis-related (PR) proteins. Application to plants of SA or one of its functional analogues e.g. acibenzolar-S-methyl (ASM; marketed in Europe as Bion®), induces SAR and activates the same set of *PR* genes. In contrast, ISR develops as a result of colonisation of plant roots by plant growth-promoting rhizobacteria (PGPR) and functions independently of SA and activation of *PR* genes, requiring instead, jasmonic acid (JA) and ethylene (ET) (Reglinski &Walters, 2009).

Induced resistance offers the prospect of broad spectrum disease control using the plant's own resistance mechanisms. As a result, there has been considerable interest in the development of agents (known as resistance elicitors or plant activators) which can mimic natural inducers of resistance (Lyon, 2007). These include elicitor molecules released during the early stages of the plant-pathogen interaction, the signalling pathways used to trigger defences locally and systemically, as well as various compounds with no reported role in host-pathogen interactions. Examples of elicitors include ASM, which has been shown to elicit SAR in a wide range of plant-pathogen interactions, the non-protein amino acid β-aminobutyric acid (BABA), and the oxylipin, *cis*-jasmone (CJ) (Lyon, 2007).

The use of induced resistance in practical crop protection faces several obstacles, including its variable efficacy under field conditions. Insufficient attention has been paid to investigating the mechanisms underlying variable efficacy and approaches that might be adopted to incorporate elicitors into crop protection practice, such as use of elicitors and fungicides together in the same disease control programme, and use of combinations of elicitors. Here, we report the results of field experiments over two years, undertaken to determine the potential for use of an elicitor combination to control light leaf spot *(Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*). Light leaf spot is a major disease of oilseed rape, the third most important crop in the UK, and can result in yield losses of 1.4 t/ha per annum (Oxley & Evans, 2009). Triazole fungicides applied in the autumn can provide effective control, although control can be considerably less effective in areas at high risk from *P. brassicae.*

MATERIALS AND METHODS

Field experiments were conducted in 2008/2009 and 2009/2010 at Boghall Farm, Edinburgh, Scotland, UK, on a site which was cropped with spring barley in the 2006/2007 and 2007/2008 seasons. The winter oilseed rape cultivar Castille was used, which has a resistance rating to light leaf spot of 5 (HGCA, 2009). Seeds were sown in a randomised block design at a seed rate of 100 seeds m^{-2} and an individual plot size of 22 m x 2 m. Three replicate plots were planted per treatment. Plots received standard fertiliser and herbicide regimes.

The field experiments compared the effects of the elicitor combination with standard fungicide treatments used to control foliar pathogens of winter oilseed rape in Scotland. Treatments were applied in the autumn (GS1.6) and spring (GS3.3) using a knapsack sprayer using an equivalent spray volume of 200 L ha-1. The elicitor and fungicide treatments used in the field trials were:

<u>2009</u>
Elicitor 1: ASM (0.175 g l^{-1}) + BABA (0.1 g l^{-1}) + CJ (0.625 g l^{-1}), applied autumn and spring.

Fungicide: Proline® (Bayer; prothioconazole, 250 g/l) applied autumn and spring.
<u>2010</u>
Elicitor 1: ASM (0.175 g l^{-1}) + BABA (0.1 g l^{-1}) + CJ (0.625 g l^{-1}), applied autumn and spring
Fungicide 1: Caramba® (BASF; metconazole, 60 g/l) applied autumn and spring

Fungicide 2: Proline® (Bayer; prothioconazole, 250 g/l) applied autumn, Prosaro® (Bayer; prothioconazole + tebuconazole, 125:125 g/l) applied in the spring.

ASM was a gift from Syngenta (Bion®, 50 WG), BABA was purchased from Sigma Chemical Company, Poole, Dorset, UK, CJ was purchased from Sigma Aldrich, Dorset, UK.

Control plots (1 plot per cultivar per block) received no elicitor or fungicide treatment and experimental plots were not artificially inoculated, but relied on natural inoculum. Symptoms of light leaf spot were assessed visually in each plot at GS 1.9, GS3.1 and GS4.5. Ten plants from each plot, chosen at random, were used for assessments. Plots were harvested at normal harvest time and yields expressed as tonnes/ha 91% DM.

All data were subjected to ANOVA using the GenStat Release 11.1 statistical program. Percent leaf area diseased values from field experiments were log-transformed prior to analysis and back-transformed values are shown in graphs. Comparison of treatment means was performed using Fisher's protected least significant difference (LSD) test.

RESULTS

Most effective control of light leaf spot on oilseed rape at GS1.9 in 2009, was achieved using the elicitor combination, which provided complete protection against infection (Fig. 1). In contrast, the fungicide, prothioconazole, reduced light leaf spot levels by 49% (Fig. 1). The mixture of the elicitor combination and prothioconazole also provided excellent control, reducing light leaf spot by 98% compared to untreated plants (Fig. 1). At GS4.5, both the elicitor combination and prothioconazole reduced light leaf spot by 93% (Fig. 1).

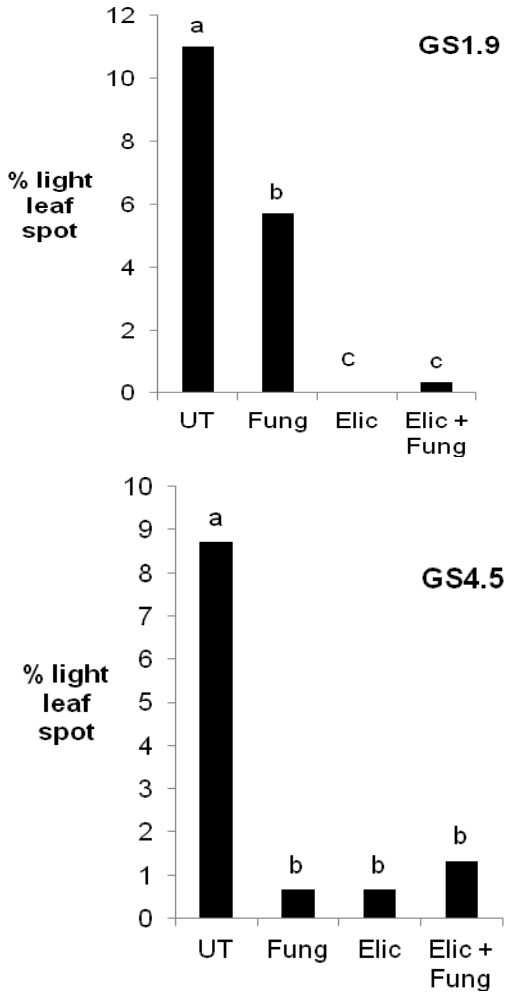

Figure 1. Effects of elicitor and fungicide treatments on light leaf spot on winter oilseed rape in a field experiment in 2008/2009. Treatments were applied in the autumn and spring, and light leaf spot levels assessed at GS1.9 and GS4.5. Details of treatments are described in the Materials and Methods section. Bars with different letters are significantly different at $P < 0.05$ (LSD).

The pattern was similar in 2010, with the elicitor combination again providing effective control of light leaf spot (Fig. 2). However, in contrast to 2009, the two fungicide treatments (prothioconazole/tebuconazole, and metconazole) reduced light leaf spot severity more substantially (73% and 71%, respectively) at GS1.9 than the elicitor combination (42%; Fig. 2). At GS4.5, the elicitor combination reduced light leaf spot by 93%, and prothioconazole/tebuconazole and metconazole reduced disease by 22% and 65%, respectively (Fig. 2). At this stage, best control of light leaf spot was obtained with the mixture of elicitor and metconazole (97%; Fig. 2).

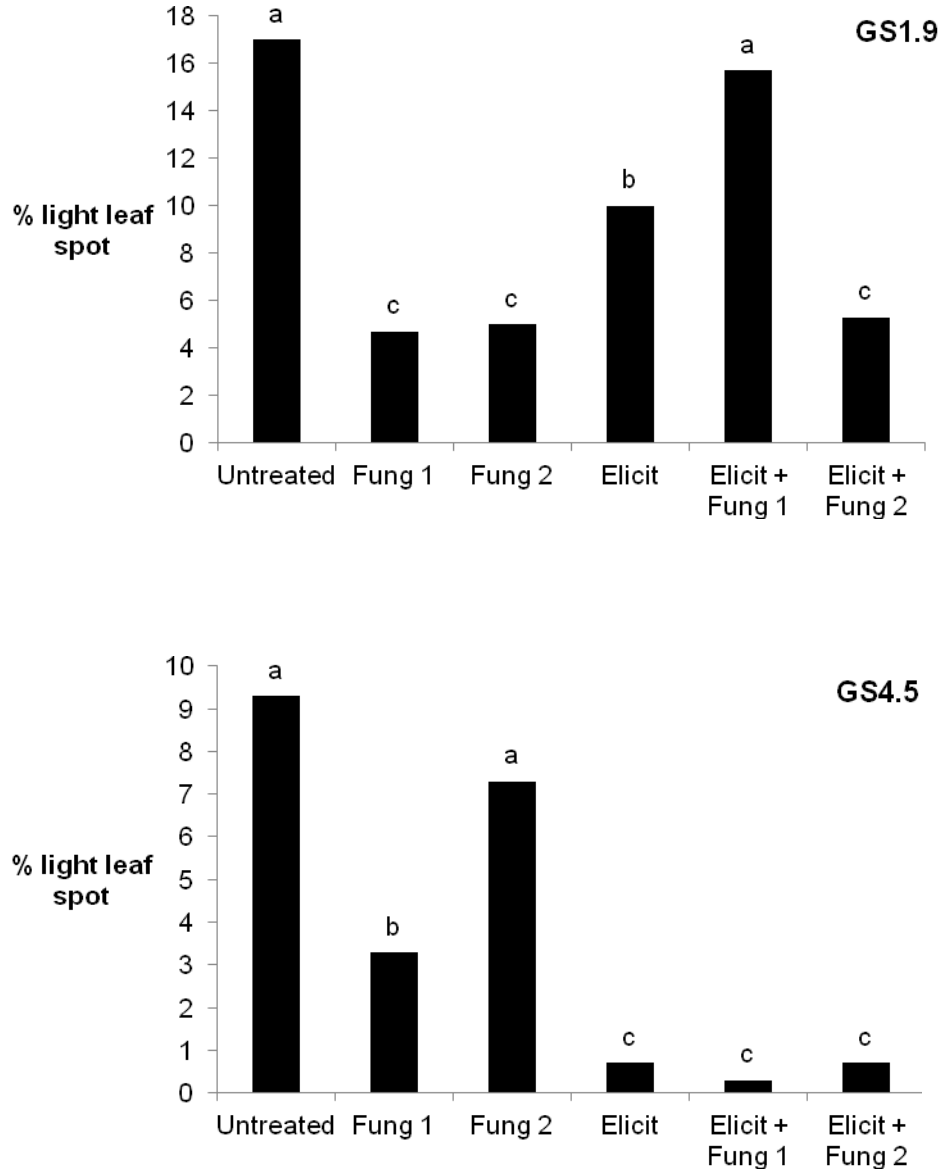

Figure 2. Effects of elicitor and fungicide treatments on light leaf spot on winter oilseed rape in a field experiment in 2009/2010. Treatments were applied in the autumn and spring, and light leaf spot levels assessed at GS1.9 and GS4.5. Details of treatments are described in the Materials and Methods section. Bars with different letters are significantly different at $P < 0.05$ (LSD).

In both 2009 and 2010, there was no significant effect of treatment on yield and none of the treatments affected plant vigour adversely (data not shown).

DISCUSSION

The data presented here show that a combination of resistance elicitors provided control of light leaf spot on oilseed rape that was, in many cases, superior to that provided by two commercial fungicides. In order to control light leaf spot effectively, fungicides need to be applied in the autumn (Figueroa et al., 1994). Triazole fungicides can provide good levels of disease control, although under high risk conditions, levels of control can be considerably lower than expected (Oxley & Evans, 2009). Indeed, the results presented in this paper showed levels of light leaf spot control of 70% or less with fungicide treatments applied in the autumn and again in the spring. The elicitor combination used in the present work has been shown recently to control *Rhynchosporium secalis* on spring barley, especially if used with reduced rate fungicide (Walters et al., 2010). However, while the levels of control of *R. secalis* achieved on barley were moderate when the elicitor combination was applied on its own, on oilseed rape, control of light leaf spot ranged from 80 – 100%. Moreover, in spring barley, whereas high levels of disease control were provided when the elicitor combination was applied first, followed later by application of fungicide, either at full or reduced rate (Walters et al., 2010), in oilseed rape, combined application of elicitor and fungicide was, in most cases, less effective than elicitor applied on its own. Importantly, the data presented here suggest that use of the elicitor combination is not associated with yield reductions.

Elicitors have been shown previously to control foliar pathogens on oilseed rape under field conditions. Thus, ASM applied in the autumn, was shown to reduce both the incidence and severity of phoma stem canker on oilseed rape in field experiments (Liu et al., 2006).

Recent work has suggested that this elicitor combination activates SAR in barley (Walters et al., 2011), although whether it does so in oilseed rape awaits investigation. Irrespective of the defence signalling involved in oilseed rape, the levels of disease control obtained in this study suggest that induced resistance is worthy of investigation as an approach to disease control in this important crop.

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THE CURRENT AND FUTURE RISKS OF FUNGICIDE RESISTANCE IN BARLEY DISEASE MANAGMENT

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Summary: Fungicides, alongside varietal resistance, are one the key components in disease control strategies in barley cropping systems. Without the effective use of this group of agrochemicals the quantity and quality of grain produced in northern Britain would significantly decline as a direct consequence of increased disease burden and through an erosion of varietal resistance which would be over exposed to pathogen pressure without the use of supporting fungicides. In this paper the current and future risks of fungicide resistance development in barley cropping systems are discussed alongside the implications for the major chemical classes in relation to current pathogen risks.

INTRODUCTION

Fungicide usage is an integral part of disease management in barley cropping systems. Grain quantity and quality relies on the effective use of these active compounds (Hardwick *et al.,* 2002). The appropriate selection of active ingredient is therefore vital to overcome the threat from yield losses. Before application, the advisor or grower should first consider the status of fungicide resistance. This information can be found in numerous locations e.g. in the UK, the independent Fungicide Resistance Action Group (FRAG-UK) is the main source.

The development of fungicide resistance in pathogens of crops is directly influenced by the biology and variability of the pathogen and the target site and mode of action of the fungicide (Hollomon & Brent, 2009). Fungicides, by their very nature, can be highly specific chemicals that target fungal pathogens. This specificity can often result in rapid pathogen evolution. The target site of the fungicide is one of the most important aspects driving pathogen evolution, as fungicides that have a single target site often tend to evolve fungicide resistance quickly e.g. in the case of methyl benzimidazole carbamates (MBC) and (QoI) fungicides. This phenomenon is termed ‘qualitative’ resistance and can extend to all compounds with the same mode of action. This resistance can not be readily reversed, even if treatments are withdrawn. However, other fungicide groups such as the sterol demethylation inhibiting (DMI) fungicides tend to develop ‘quantitative’ resistance, which builds up over time giving gradual shifts in the fungal population that can decline when fungicide is withdrawn. The occurrence of either type of fungicide resistance can have a significant impact on sustainable food and fibre production (Hollomon & Brent, 2009).

REVIEW OF OCCURANCE OF FUNIGICIDE RESISTACE IN BARLEY CROPS

Fungicide resistance has been studied in barley since the introduction of these agrochemicals in the 1970’s. Initially this was in the form of seed treatments, with foliar applications soon

following. During the 1970s and 80s, *Rhynchosporium secalis* was controlled by the use of two groups of fungicides, the MBCs and the DMIs. Both of these fungicide groups were successfully used to control *R. secalis* either alone or in a mixture up until the early 1990s, after which MBC fungicide resistance was first detected (Kendall *et al*., 1994). The frequency of MBC resistant isolates rapidly increased especially in Northern Ireland, where in 1991-1995 approximately 50 % of the population was resistant. This level of resistance meant that MBC fungicides were no longer effective in controlling disease (Taggart *et al*., 1994; Locke & Phillips, 1995) and as a result, their use declined. Other barley pathogens were also affected by resistance to MBCs either directly or indirectly, but there is little documented evidence on the current resistance status in major barley diseases to this fungicide group.

The DMI fungicides, otherwise known as 'azoles', are still the most important group of chemicals for the control of all diseases in barley production. However, in all of the important barley pathogens, shifts in the sensitivity to some of the older DMIs have been observed. These shifts in DMI efficacy have been studies more often in *R. secalis,* with several papers highlighting the decline in efficacy over time. This was first reported by Hollomon (1984) and Jones (1990), who were studying the DMI seed treatment, triadimenol. There are several subgroups within the DMIs, for example imidazoles and triazoles and during these studies; it was observed that there were differences in the population towards the sensitivity to two chemically related DMIs, triadimenol and propiconazole (both triazoles). Triadimenol showed a bimodal population distribution, but propiconazole showed a gradual shift in the population following a unimodal distribution. However, when the same *R. secalis* isolates were tested with the imidazole, prochloraz, there was no loss in fungicide efficacy. Therefore, it was concluded that there was cross-resistance between the different triazoles, but there was no cross-resistance observed between the imidazoles and triazoles (Kendall *et al.,* 1993). Subsequent work on the mutations responsible for shifts in other pathogens i.e. *Mycosphaerella graminicola* (Fraaije *et al.,* 2007) would suggest that this cross-resistance pattern is unlikely to be very stable if either subgroup were used extensively. Since this work, routine monitoring has shown further small shifts in the efficacy of some of the older DMI type fungicides, such as epoxiconazole (Cooke *et al.,* 2004; Oxley *et al.,* 2008). The loss of efficacy in DMIs has been gradual over successive growing seasons, rather than the complete loss of efficacy shown by MBC fungicides (Cooke *et al*., 2004). The reason for this gradual decline has been investigated by Hawkins *et al*. (2010). This study demonstrated that any point mutations in the target *CYP*51 gene were not related to the decline in fungicide sensitivity, as the fungicide to protein binding is not directly affected by the nucleotide change. However, unlike other well-studied pathogens such as *M. graminicola* which has only one *CYP*51 gene, *R. secalis* has two functional genes (*CYP*51A and *CYP*51B) and it is believed that the interaction, function and expression levels of these genes is a possible reason for the decline in sensitivity of some DMI fungicides.

Other barley fungicides have also been found to develop a slight decline in efficacy in the control of diseases such as powdery mildew (*Blumeria graminis*), yellow and brown rusts, (*Puccinia hordei* and *Puccinia striiformis*), Ramularia leaf spot (*Ramularia collo-cygni*) and net blotch (*Pyrenophora teres*) (FRAG-UK 2011). However, these studies are limited and do not appear to show significant impacts on field performance. Despite the partial loss of DMI efficacy in some parts of the UK and Europe, they remain one of the most important fungicide groups for the control of barley diseases. However, when these fungicides are mixed with other partner chemistry with a different mode of action, the yield benefits due to improved disease control can be significant. The recommended mixing partners are the Quinone outside

Inhibitors (QoIs), anilinopyrimidines, chlorothaonil and the newer succinate dehydrogenase inhibitors (SDHI) fungicides.

The QoI fungicide group is an important partner fungicide for disease management. Resistance to this group has been quick to develop and is now common in many plant pathogens. The first barley pathogen to develop resistance was powdery mildew. Resistance was first detected in Germany within a few years of commercial use (Brent & Hollomon, 2007). This resistance was the result of a point mutation found in the cytochrome *b* gene. This G143A mutation results in the exchange of glycine to alanine at amino acid position 143, and produces a high resistance factor (Sierotzki *et al.,* 2000). This single point mutation gives complete resistance to all QoI fungicides and has been subsequently observed in a large number of plant pathogens e.g. *R. collo-cygni* which was first reported by the Fungicide Resistance Action Committee (www.FRAC.info) in 2006. However, analysis of the Rothamsted Hoosfield spring barley archive using molecular techniques indicated that the development of resistance occurred much earlier. Fountaine and Fraaije (2009) demonstrated that resistance developed during 2002 in the UK (Figure 1). The shift in the resistant populations was also very quick with the resistant population going from almost zero to 100 % within two seasons. A corresponding decline in efficacy was seen in fungicide performance trials (Oxley & Burnett, 2009). This is also comparable to the situation observed in *M. graminicola* (Fraaije *et al.,* 2005) which is closely related to *R. collo-cygni* (Crous *et al.,* 2001). Therefore, this pathogen is now considered a medium to high-risk for future fungicide resistance development according to the FRAG-UK management guidelines (FRAG-UK, 2011).

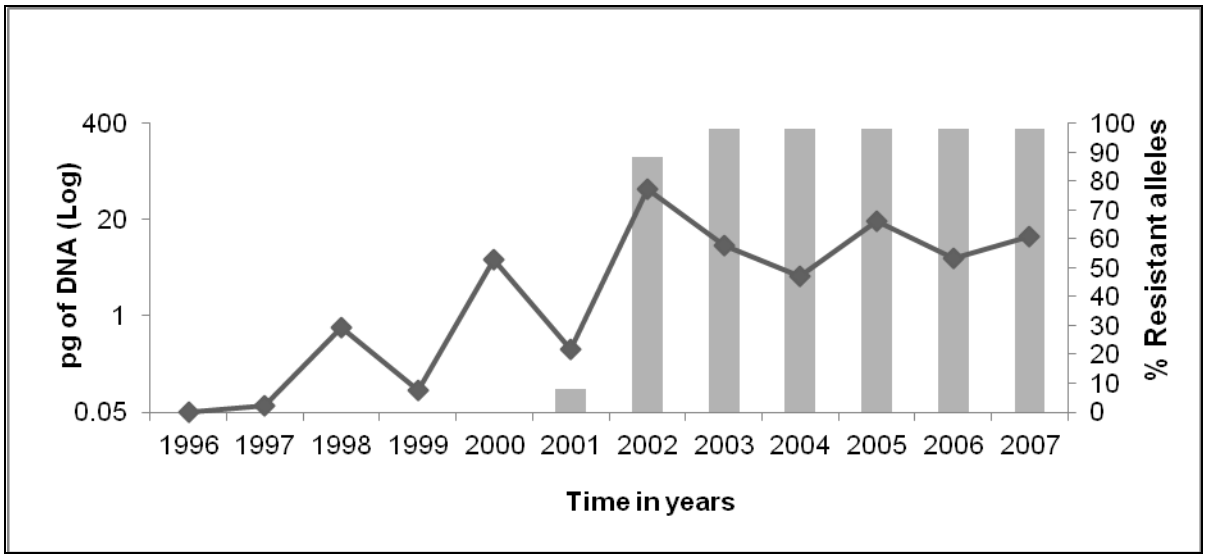

Figure 1. *Ramularia collo-cygni* DNA levels and % resistance, detected by allele specific real-time PCR, in the Hoosfield spring barley archive.

Resistance to QoI fungicides has also developed in *R. secalis.* However, the development did not follow the pattern observed in other plant pathogens. Initial resistance was first reported by FRAC during 2008 in northern France. The mutation detected was the same G143A observed in other pathogens. However, since the initial report no other resistant isolates have been observed in either northern France or other parts of Europe. In the UK, a large screening project looked at isolates from around the UK but no isolates where found to contain the

mutation causing resistance (Fountaine *et al.,* 2011). A simple PCR-RFLP test was used to screen UK isolates for the mutation causing fungicide resistance (Fountaine *et al*., 2011). Therefore, whilst it is expected that resistance may well develop in the future, the current levels of QoI resistance appear to remain very low and has not affected *R. secalis* disease control. Resistance management strategies encourage the mixing of different fungicide partners and the application of no more than two sprays of chemicals with the same mode of action in any one growing season.

Resistance issues can also be observed in net blotch disease, caused by *P. teres*. Initially, the efficacy of DMI fungicides was observed to be declining in some locations, but there have been some fluctuation between seasons and it now appears that the sensitivity across Europe is stable (FRAC). The QoI fungicides are important mix partners for DMIs, therefore, when moderately resistant isolates were first detected this could have had a significant impact on the field control of disease. However, the single point mutation that has occurred in the fungus *P. teres* is a mutation at codon 129 (Semar *et al.,* 2007). This mutation leads to leucine replacing phenylalanine at position 129 of the cytochrome *bc* 1 complex. This mutation confers a partial resistance factor rather than the complete resistance produced by the G143A mutation in other plant pathogens (Semar *et al.,* 2007). This limited effect therefore, only causes a slight decrease in efficacy and this can be overcome by following the recommended guidelines when mixing a combination or fungicide groups to make a tank mix.

The development of the G143A mutation and subsequent high levels of resistance is not expected to occur in *P. teres, P. hordei* and *P. striiformis*. This is due to the occurrence of an intron in the coding sequence of the DNA after codon 143 in the cytochrome *b* gene, which would result in a lethal gene splicing situation. Therefore, the risk of a high level of QoI fungicide resistance is currently much reduced. The presence of this intron has fatal consequences to individual fungi which develop the mutation (Grasso *et al.,* 2006).

DISCUSSION

Fungicide resistance will continue to develop in pathogens as long as the selection pressure, due to fungicide use, is applied to the population. Therefore, integrated management systems are required to be implemented in barley disease control in order to slow down the loss of active ingredients. The most effective methods currently adopted are i) the application of the appropriate dose at the correct timing ii) mixing different chemical with different modes of action in combination and iii) the use of resistant cultivars. Varietal resistance is under pressure and the twin approaches of fungicide use and the use of resistant varieties may preserve the longevity of efficacy of each approach. It is also extremely important that independent fungicide resistance testing is maintained in order to give important impartial advice on the performance of these products. The testing of some pathogens can be difficult and the sample sizes too low or the precise mode of action unknown before a new product is launched, allowing the possible risk of resistance developing without appropriate risk reduction strategies. The decline in DMI fungicides is still a cause for concern, as these are the most important chemical class for general disease control. However, recently introduced members of the SDHI fungicide group are also potentially at a high risk of resistance development due to the target gene and its high level of activity. As a result, SAC has an active research interest in this area and has developed a number of rapid screening tools in an important number if pathogens to detect for any changes in the resistant status of UK populations.

ACKNOWLEDGEMENTS

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EPIDEMIOLOGY OF *RAMULARIA COLLO-CYGNI*

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Summary: *Ramularia collo-cygni* is now one of the most economically important fungal pathogens which attack barley (*Hordeum vulgare)* crops in Northern Britain. The epidemiology of this disease is slowly being elucidated. Seed borne infection has been revealed to be the primary source of early *R. collo-cygni* infection in crops. Later *R. collo-cygni* spore release events were found to be related to periods of maximum leaf surface wetness. A prediction scheme for *R. collo-cygni* severity in a cropping season based on surface wetness at GS 31 has been devised. The effect of *R. collo-cygni* on yield has been studied by analysing Area Under Disease Progress Curves (AUDPC) and yield figures from untreated crops over a number of years and sites. A relationship between yield and AUDPC was established for winter and spring barley crops. Information derived from experiments and has been incorporated into risk assessment models aimed at optimisation of disease control.

INTRODUCTION

Ramularia collo-cygni is an increasingly important late season pathogen of barley (*Hordeum vulgare*) both in Europe and the United Kingdom (Sachs *et al*., 1998, Pinnschmidt & Hovmøller 2003, Walters, 2008). It is the causal agent of Ramularia Leaf Spot (RLS) in barley. Disease symptoms appear on foliage after the emergence of the ear and contribute to premature loss of green leaf area (Havis *et al*., 2004). Yield losses of between 0.1 and 0.9 t/ha have been observed in Scottish trials (Oxley & Havis, 2004). The exact relationship between disease severity over a growing season and yield has not been fully established. Disease severity over time can be quantified by calculating Area Under Disease Progress Curves (AUDPC). AUDPC values have been used in many plant-pathogen interactions to evaluate disease management practices (Jeger, 2004).

The accurate prediction of RLS symptoms has not been possible as the conditions conducive to disease severity over a growing season could not be accurately forecast. However, Salamtai &Reitan (2006) reported that a correlation existed between the duration of maximum surface wetness in a spring barley crop during the first two weeks in June and disease severity. The greater the levels of surface wetness the higher the disease levels in the crop. This was based on observations at one site in Norway.

The development of molecular based diagnostic tests for R. collo-cygni has allowed more detailed studies of the ecology, aetiology and epidemiology of this pathogen (Havis *et al*.,

2006; Frei *et al*., 2007, Taylor *et al*., 2010). Recent work has shown that pathogen levels are much lower in the canopy during the growing season and only increase in the upper leaves and ears after flowering (Havis *et al*., 2010). The development of a method for transforming the fungus to express a fluorescent protein offers the potential for even greater understanding of fungal movement within the host plant (Thirugnanasambandam *et al*., 2011)

MATERIALS AND METHODS

Monitoring of *R. collo-cygni* spores in the environment

Burkard 7 day spore samplers were set up at the Bush Estate, Midlothian and Drumalbin Farm, Lanark. This machine samples air from the environment which is drawn through a small aperture and passes over coated Mellinex tape. After 7 days the tape is removed and divided into segments which correspond to 24 hour periods. These were then halved lengthways and stored at -20 °C. DNA was extracted from the tape using the method described in Fountaine *et al.* (2007). *Ramularia* DNA levels were quantified using a real time PCR test recently developed in our laboratory (Taylor *et al.,* 2010). An automatic weather recording station was situated next to the spore sampler to provide local meteorological data. Weather parameters and DNA levels were analysed by Biomathematics and Statistics Scotland (BIOSS).

Establishment of a relationship between AUDPC and yield

A number of yielded field trials have been carried out on spring and winter barley crops at SAC trial sites over the previous ten years. Regular assessments of RLS infection over the course of the trials was carried out. Data from untreated plots within these trials was extracted and AUDPC values calculated using the trapezoidal rule (Whittaker & Robinson, 1967). Yield data was extracted and regression analysis carried out to establish a potential relationship between the two parameters.

Validation of a prediction scheme for RLS severity

Weather data was collected from automated meteorological stations located at SAC trial sites in 2008. The number of minutes of surface wetness was calculated at each site for the last two weeks in April and the first two weeks in June, since these dates corresponded to GS31 in both crops at Bush and Drumalbin. At the end of the season AUDPC values from untreated plots in winter and spring barley trials at each site were calculated and mean values taken. A regression analysis was undertaken to establish if a relationship existed. Over the next three cropping seasons surface wetness figures were calculated and a prediction of the risk of RLS symptoms at each site made.

RESULTS

Plotting daily Ramularia DNA levels against surface wetness in the crop indicated that spore release events occurred 24-48 hours after periods of maximum surface wetness in the crop (Fig 1). Analysis by BIOSS indicated the following conclusions could be determined from *R. collo-cygni* spore release events. Spore release reaches a maximum in July or August depending on the season. and increases with surface wetness on the preceding and same days. In addition, spore release increases as temperatures increases between 5 and 15 deg Celsius.

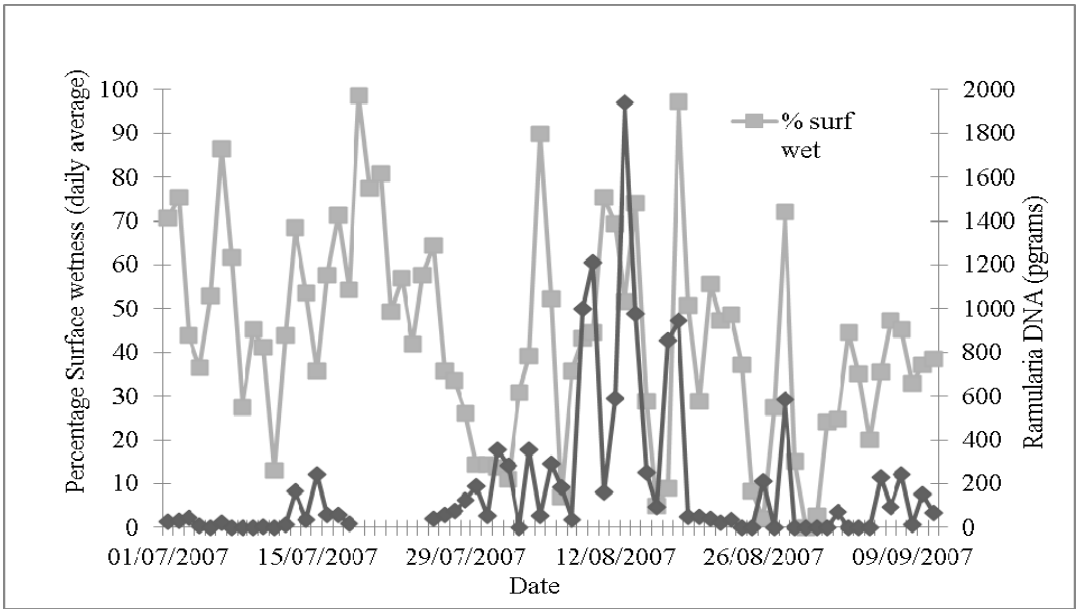

Figure 1. Ramularia DNA plotted against percentage daily surface wetness (Bush, 2007)

Yield from winter and spring barley SAC trials were plotted on a graph against RLS AUDPC and a regression analysis carried out. The analysis showed the same yield response in winter and spring crops to increasing AUDPC.

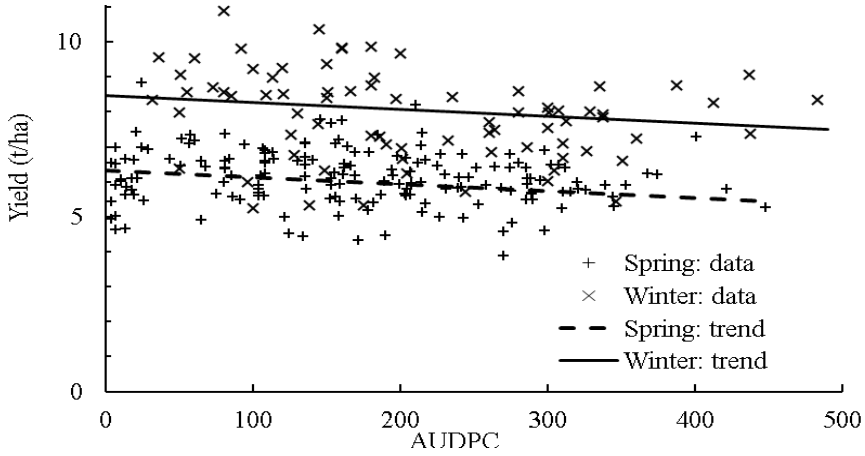

Figure 2. Relationship between Ramularia AUDPC (Winter and Spring Barley) and Yield

The yield loss can be plotted against AUDPC. Assuming a price of £128t for feed barley, a reduction in AUDPC of 96 will give an economic benefit. Assuming a price of £147t for malting barley, a reduction in AUDPC of 74 would give an economic benefit.

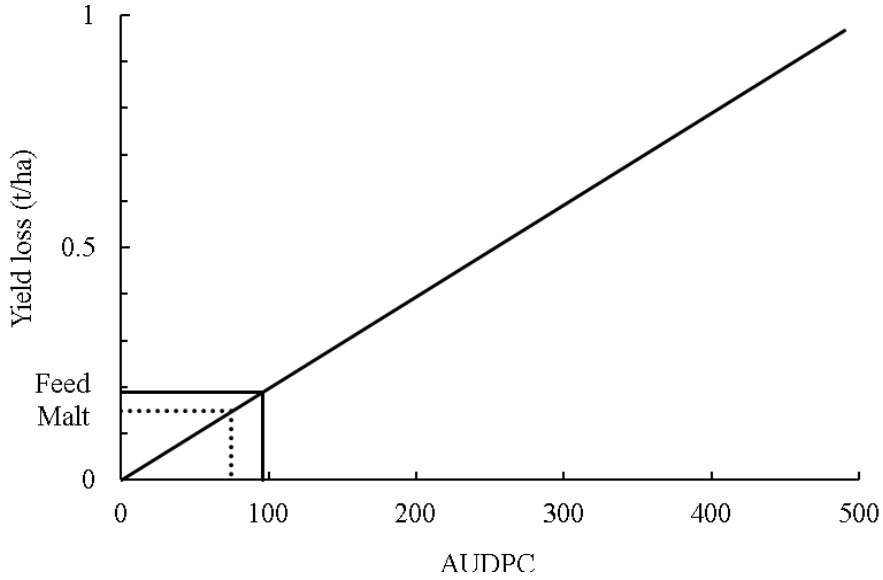

Figure 3. Relationship betweeen Ramularia Yield Loss and AUDPC

Leaf wetness figures for the first 2 weeks in June were calculated and plotted against the mean Ramularia AUDPC in untreated plots at four trial sites. Regression analysis indicated a correlation exists between the amount of surface wetness and AUDPC values.

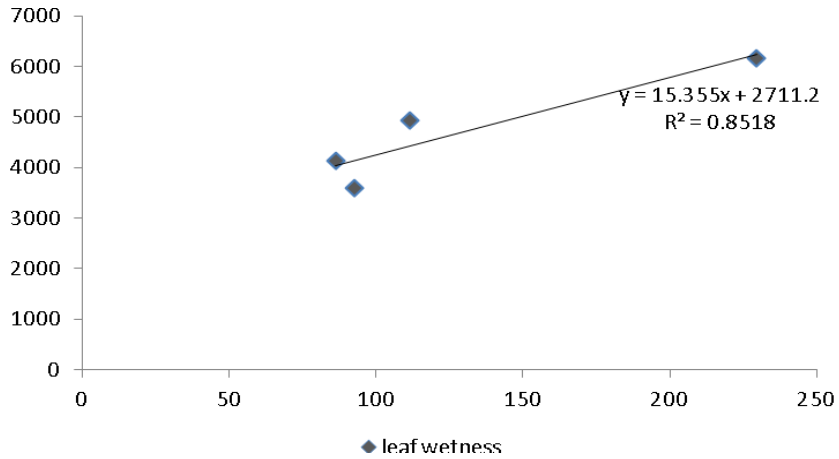

Figure 4. Surface wetness in early June vs Ramularia AUDPC in untreated spring barley plots at four sites (2008)

DISCUSSION

Studies examining the release of fungal spores and meteorological data have indicated that the distribution patterns vary depending on the spore type and conditions (Troutt &Levetin, 2001). Our analysis indicates that spore release is related to surface wetness in the crop and temperature (Fig 1). The Ramularia species which attacks sugar beet (*R. beticola*) also releases

spores which can be detected above the crop. Spore production was related to the amount of relative humidity in the crop in the previous 24 hours (Hestjberg & Dissing, 1995).

Research in Germany has also indicated that spore release events late in the season and weather conditions are not the main influences on the development of RLS in barley crops in a growing season (Schützendübel *et al.,* 2008). The results from the analysis of the spore sample tapes appear to support this conclusion. Previous work at SAC has shown that epidemics begin prior to any the detection of spore movement in the crop (Havis *et al.,* 2010). However, heightened levels of Ramularia spores in the environment could lead to higher levels of seed infection and potential epidemics in following seasons (Oxley *et al.,* 2008).

AUDPC has been used in modelling other disease borne diseases e.g. Alternaria leaf spot in sesame (Jeger, 2004). Higher seed infection levels gave increased AUDPC levels and a faster rate of disease development. More detailed analysis of levels of seed infection on Ramularia AUDPC is required. Higher AUDPC levels have been associated with yield loss in a number of pathogen-host interactions. In barley crops very high yellow rust levels have been associated with extremely high AUDPC values and yield losses of over 70% (Marshall and Sutton, 1995). Our results indicate that an increase in Ramularia AUDPC values will contribute to a yield reduction in both winter and spring barley. The threshold AUDPC levels at which fungicide treatments become economically viable are below 100 for feed and malting barley. Oxley *et al.* (2008) reported that a well timed fungicide programme gave an economic return in spring barley trials in between 2005-7.

The prediction scheme for Ramularia severity has allowed practical advice to be given to growers during the growing season, at a point when fungicide programmes can still be altered. The optimal timing for Ramularia control remains at GS 45-49 (Oxley *et al*., 2008). The reasons for the effect of surface wetness on fungal movement and subsequent disease expression are currently under investigation and the transformation of the fungus now allows even more detailed experiments to be undertaken (Thirugnanasambandam *et al.,* 2011)

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ADVANCES IN CONTROL OF *RAMULARIA COLLO-CYGNI*

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Summary: *Ramularia collo-cygni* has become an increasing problem for barley growers throughout Europe in recent years and the pathogen continues to spread in cereal producing areas. Control measures against Ramularia are based on a combination of varietal resistance, seed treatments and foliar sprays. The work presented demonstrates that at present foliar fungicides represent the most effective strategy. The introduction of a second generation of succcinate dehydrogenase inhibitors (SDHI) fungicides has increased the options to growers. However, changes in EU legislation, resulting in removal of fungicides from the market, will present a serious challenge to growers and advisors.

INTRODUCTION

Ramularia collo-cygni is the major biotic component involved in the development of Ramularia Leaf Spot (RLS), which attacks barley crops in the late growing season (Walters *et al*., 2007). RLS is most commonly observed on foliage after flowering in the crop. Initial signs of infection are small brown to blackish spots, 1-2mm long. The spots develop a chlorotic halo and eventually neighbouring lesions may coalesce to form a larger necrotic region. The subsequent loss of green leaf area leads to deleterious effects on yield quantity and quality (Walters *et al*., 2007).

Control programmes in Austria in the late twentieth century were based on the triazole fungicide, tebuconazole (Huss & Sachs, 1998) When RLS was first identified in the UK, control programmes were developed which were based on strobilurin fungicides and chlorothalonil applied at the booting stage (Havis *et al.,* 2002). However, strobilurin fungicides ceased to give effective control after 2000 (Oxley *et al.,* 2006; McCabe, 2009). Subsequent analysis of seed samples from the Rothamsted Research continuous spring barley experiment indicated that the G143A mutation, which confers resistance to strobilurin fungicides, could be detected in 2002 (Fountaine & Fraaije, 2009). HGCA funded research into fungicide performance (RD-2008-3462) to control fungal pathogens in barley has allowed regular monitoring of fungicide efficacy in controlling RLS. Combined data from 2005-9 indicated that a number of fungicides could give very significant control of Ramularia. The only treatment which had no effect on RLS was pyraclostrobin (Comet 200 ®) (Oxley & Burnett, 2010). The Fungicide Performance curves are available on the HGCA website (HGCA, 2011).

R. collo-cygni was first shown to be present in seed in 2006 (Havis *et al.,* 2006; Frei *et al.,* 2007). Early experiments indicated that conventional fungicide based seed treatments are not completely effective in reducing RLS, with significant control achieved only in susceptible varieties (Havis *et al.,* 2008). The deep seated nature of the fungus in the seed does make effective control more difficult. A LINK project was established to look into the control of Ramularia via varietal resistance and seed treatments. The efficacy of novel seed treatments

has been reported previously (Havis *et al.,* 2010). Recent research at SAC has indicated that the application of heat, in the form of microwaves, can reduce the level of *R. collo-cygni* within seed.

MATERIALS AND METHODS

Monitoring of varietal susceptibility

Spring barley from the 2010 and 2011 recommended list (HGCA, 2011) was sown in trial plots (5m x 2m) at the Bush Estate, Midlothian and Drumalbin Farm, South Lanarkshire. The plots received no fungicide inputs during the trial. Other agronomic inputs were used as per local practice. Disease assessments were taken at regular intervals after ear emergence in the crop and the amount of leaf area affected by RLS recorded. The mean figures for each variety and least significant differences were calculated using Genstat.

Control of *R. collo-cygni* using seed treatments

Seed samples from three varieties (Cocktail, Optic and Decanter) grown in 2010 spring barley trials were treated with experimental seed treatments. The treated seed was sown in field trials (10m x 2m plots) at the Bush Estate, Midlothian in April 2011 in a randomised block design.

The seed treatments used were prothioconazole + tebuconazole + triazoxide (pro +teb + triaz) (Raxil Pro ®), hot water (2 hours at 52 °C followed by 72 hours at 25 °C), steam (Thermoseed treatment), and microwaving for 20 or 35 seconds. In both trials the plots received one foliar fungicide application at GS 25-30 (75 g/l metrofenone (Flexity ®) and 80 g/l pyraclostrobin (Insignia ®). These fungicides were selected as they had no efficacy against RLS but controlled mildew or rhynchosporium respectively. Leaf layers were assessed for the severity of RLS throughout the growing season and area under disease progress curves (AUDPC) values were calculated using the trapezoidal rule (Whittaker & Robinson, 1967). The plots were taken to yield and treatment means and least significant differences calculated using Genstat.

Control of RLS using foliar fungicide sprays

Spring barley (cv. Prestige) was sown in field trial plots (10m x 2m) at the Bush Estate, Midlothian and Carlow in Ireland in April 2010. The plots received one fungicide application at GS 45-49. A number of fungicides and dose rates were applied. Treatments were Bontima® (cyprodinil [187g/l] + isopyrazam [63g/l]), Siltra® (bixafen [60 g/l] + prothioconazole [200 g/l]), Proline® (prothioconazole [275 g/l]), Tracker® (boscalid [233 g/l] + epoxiconazole [60 g/l]) and Bravo® (chlorothalonil [500 g/l]). Fungicides were applied at twice the full label rate, full label rate, half the full label rate and one-quarter of the full label rate (Table 2). RLS disease levels were assessed three weeks and six weeks post-fungicide application and dose response curves generated.

RESULTS

Disease assessments on the 2010-11 untreated varieties indicate that there are no resistant lines available for farmers to grow. RLS levels in the plots ranged from 2 to 14 per cent. Mean figures from varieties at two sites ranged from 4.5 per cent to 8.5 per cent. There was a significant difference between the worst variety (Odyssey) and the best (Cromwell) in 2011 (Fig 1).

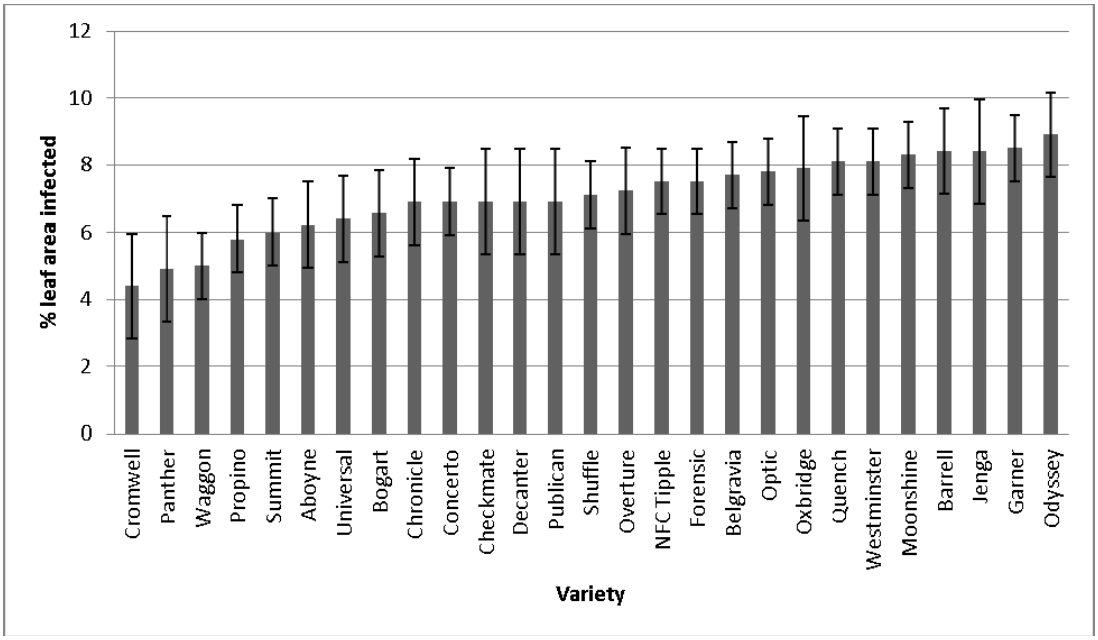

Figure 1. Late season Ramularia lesions in SAC spring barley trials 2011 . LSD (P=0.05) = 3.45

None of the seed treatments gave a significant decrease in RLS AUDPC in the varieties Cocktail and Decanter. However, both the (pro+teb+triaz) and hot water treatment gave a significant reduction in AUDPC in the variety Optic. No treatment gave a significant increase in yield (Table 1).

Table 1. Effect of seed treatment on RLS disease severity and yield (Bush Estate, 2011).

Variety	Seed treatment	RLS AUDPC	Yield (t/ha at 85% DM)
Cocktail	Untreated	113	7.95
Cocktail	Pro+teb+triaz	105	7.72
Cocktail	Hot water	127	7.28
Cocktail	Steam	112	8.29
Cocktail	Microwave 20 s	108	7.52
Cocktail	Microwave 35 s	116	7.38
Decanter	Untreated	100	7.64
Decanter	Pro+teb+triaz	78.0	7.85
Decanter	Hot water	74.9	7.22
Decanter	Steam	97.8	8.06
Decanter	Microwave 20 s	84.4	7.60
Decanter	Microwave 35 s	92.3	7.37
Optic	Untreated	116	7.66
Optic	Pro+teb+triaz	84.8	7.16
Optic	Hot water	60.5	7.49
Optic	Steam	100	7.89
Optic	Microwave 20 s	92.2	7.93
Optic	Microwave 35 s	89.2	7.78
	LSD (P=0.05)	30.6	0.76

The results from the dose response curve trials from 2009-10 indicate that all of the products tested give protection against the formation of RLS in spring barley. The bix + pro treatment produced the curve with the steepest slope indicating it is the most effective at lower doses.

Table 2. Chemicals used in fungicide performance trials.

Product name	Label rate (L/ha)	Abbreviation in Fig 1
Bravo®	2.0 l/ha	chlor
Bontima®	2.0 l/ha	cypr + isopyr
Siltra®	1.0 l/ha	bix + pro
Tracker®	1.5 l/ha	bosc + epoxy
Proline®	0.72 l/ha	pro

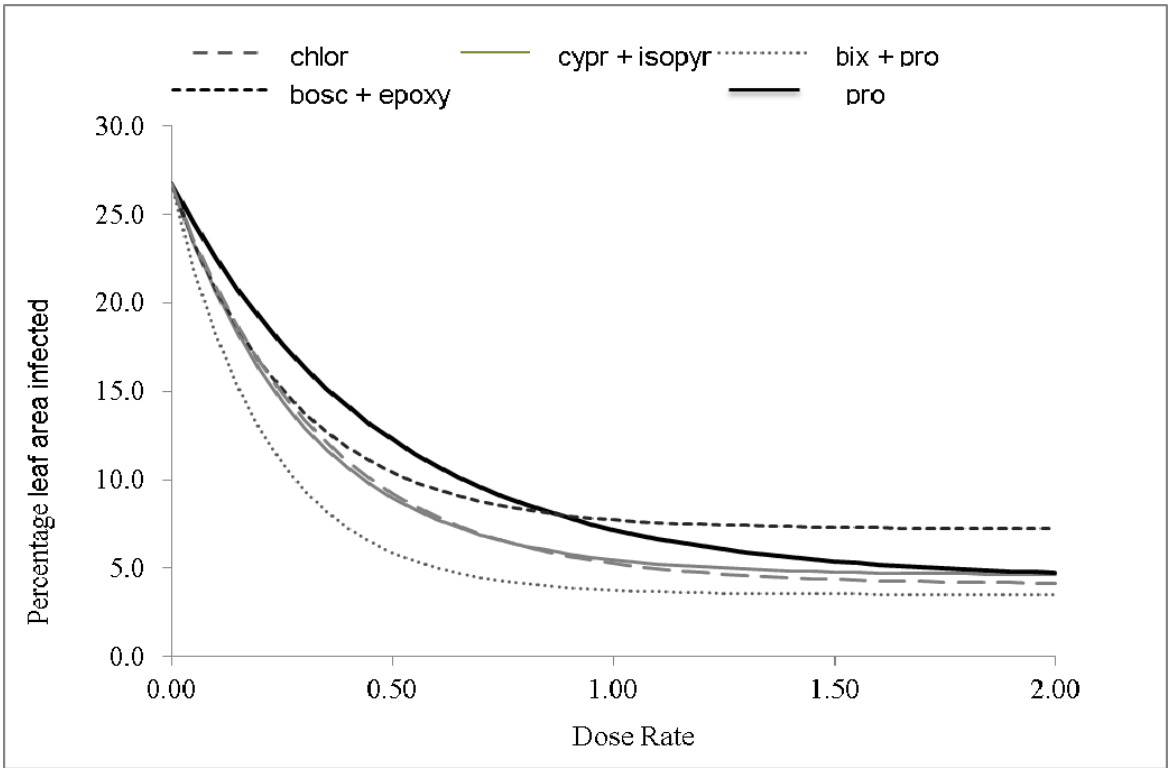

Figure 2. Dose response curves for Ramularia protection in spring barley 2009-10

DISCUSSION

The current varieties available to growers in the UK do not have complete resistance to RLS. Some varieties are more tolerant to disease symptoms than others. Cromwell, Panther and Waggon were three of the more tolerant varieties tested in 2011. Varieties can become less tolerant to RLS. The variety, Optic used to show moderate tolerance but it is now exhibiting increasing RLS symptoms (Oxley & Havis, 2009). Results from Denmark indicate that there is a correlation between varietal resistance to powdery mildew and rust and increased susceptibility to RLS (Pinnschmidt & Sindberg, 2009). The current LINK funded project is

examining the potential of suitable barley crosses to show increased tolerance to RLS symptoms.

Seed treatments alone do not give consistent reliable control of RLS. The hot water and (prothioconazole + tebuconazole + triazoxide) treatments gave a significant reduction in Ramularia AUDPC (Table 1). However, the results from this trial confirm earlier findings that seed treatments may reduce Ramularia DNA levels in seed but pathogen load increases over theseason and RLS symptoms are not significantly reduced (Havis *et al.,* 2010). Seed treatments may be best incorporated into an integrated control programme. Resistance elicitors have also been tested against RLS (Walters *et al.,* 2009). These compounds elicit the defence response in the host plant and offer the prospect of broad spectrum disease control by utilising the plants natural defence mechanisms. Results from a number of trials suggest that these compounds may not have a role to play as a solo treatment but can produce significant reductions in RLS when applied early (GS 24) followed by reduced rate fungicides at GS 31 and 39 (Havis *et al.,* 2009).

Fungicides have been shown to give control of RLS in barley crops. Triazole fungicides have continued to show activity against *R collo-cygni* (Oxley *et al.,* 2006). Prothioconazole and epoxiconazole are still useful components of a fungicide programme (Fig 2). The appearance of the G143A mutation in *R. collo-cygni,* both in the UK and other European countries, means the strobilurins no longer play an active part in RLS control (Fountaine & Fraaije, 2009). The SDHI fungicides were first discovered over 40 years ago but a new generation have been shown to be very active against plant pathogens (Russell, 2009). The curves produced from the fp trials indicate the high efficacy of the SDHI fungicides against RLS (Fig 2). Bixafen and prothioconazole gave the most effective reduction in RLS at low doses. However, there a number of mutations recorded in other fungi which would confer resistance to SDHI fungicides (Russell, 2009). The use of these fungicides on barley requires careful management in order to ensure resistance does not appear as rapidly as resistance to strobilurins. Many of the SDHI fungicides are co-formulated with triazole fungicides as part of an anti-resistance strategy (Oxley & Burnett, 2010). In addition, the use of chlorothalonil at booting stage reduces RLS and physiological leaf spotting (PLS). The loss of some or all of the triazole group of fungicides as a result of EU legislation and the similar threat to chlorothalonil presents a great challenge to barley growers. It is important for all potential alternative treatments to be tested prior to the loss of any active compounds, in order preserve grain yield and quality in barley crops and in order to protect the remaining SDHI fungicides from resistance development.

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RHYNCHOSPORIUM COMMUNE EFFECTORS AS POTENTIAL ACTIVATORS OF NOVEL RESISTANCES IN BARLEY

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Summary: The development of sustainable strategies for the management of Rhynchosporium depends on an improved understanding of the biology of the causal fungus and its interaction with barley. Recent genome sequencing of *Rhynchosporium commune* provided a unique opportunity to identify the putative effector population mediating interactions with host plants and activating barley resistance. Screening of barley cultivars, landraces and mapping populations for recognition of *R. commune* effectors can have a direct impact on Rhynchosporium disease resistance breeding programmes by providing rapid identification of effective resistance sources. It can also help with predicting durability of individual resistance genes.

The fungal pathogen *Rhynchosporium commune,* formerly known as *R. secalis* (Zaffarano *et al.,* 2011), causes 'Rhynchosporium', barley scald or leaf blotch. It is one of the most destructive diseases of barley, especially in areas with cool temperate climate. Barley scald can decrease yield by 30-40% as well as reduce grain quality (Shipton *et al.,* 1974). The disease regularly occurs in wetter parts of the UK, particularly southwest and northern England, as well as Scotland and Northern Ireland (HGCA, 2000/2001). *R. commune* can complete its infection cycle asymptomatically, thus the disease threat may remain hidden, only to appear and cause crop damage when conditions favour the pathogen. Populations of *R. commune* can change rapidly, defeating new barley resistance genes and fungicides after just a few seasons of widespread commercial use (Newton *et al.,* 2001). New EU regulations may lead to the loss of the most effective triazole fungicides, making Rhynchosporium control even more problematic. As climate change predicts wetter winters for northwest Europe, Rhynchosporium may become an even bigger threat for winter barley.

The development of sustainable strategies for the management of Rhynchosporium depends on an improved understanding of *R. commune* biology and the interaction of this fungal pathogen with barley. All pathogens trigger non-host resistance (NHR) in plants. Successful pathogens can suppress or manipulate NHR by secretion of small proteins called ‘effectors’. Once a pathogen has suppressed NHR, plants deploy a second layer of defence in the form of resistance proteins. Resistance proteins detect certain pathogen effectors, termed ‘avirulence’ (Avr) proteins, and activate resistance responses. Pathogens can avoid recognition by some of the resistance proteins by losing either the expression or function of a non-essential (redundant) effector with no apparent cost to pathogen fitness. Both of these strategies have been deployed

by *R. commune* to completely overcome *Rrs1*-mediated resistance in under 10 years (Rohe *et al.,* 1995; Houston & Ashworth 1957).

We aim to understand redundancy within *R. commune* effectors. Redundant effectors like *AvrRrs1* are known to be readily lost by the pathogen, resulting in lower durability of host resistance genes recognising these effectors. Therefore, breeding should aim to target introgression of resistance genes recognising pathogen effectors which are non-redundant, less variable in pathogen populations, and essential for pathogenicity.

Recent sequencing of the *R. commune* genome provided a unique opportunity to identify the putative effector population mediating interactions with host plants. The Rothamsted Research team has developed a system for screening barley germplasm for recognition of *R. commune* effectors. This method is based on *in planta* expression of *R. commune* secreted effectors in barley leaves using binary BSMV vector. Screening of the James Hutton Institute (JHI) extensive collection of barley cultivars, landraces and mapping populations for recognition of *R. commune* effectors can have direct impact on *R. commune* disease resistance breeding programmes by providing rapid identification of effective resistance sources. It can also help with predicting durability of individual resistance genes. This information can also assist the selection of resistance genes or combinations of genes that are more likely to prove durable.

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GENETIC CONTROL OF INFECTION AND SYMPTOM EXPRESSION BY *RHYNCHOSPORIUM COMMUNE* IN BARLEY

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Summary: Traits associated with resistance to *R. commune* were investigated in a mapping population from a cross between a winter and spring barley. In addition, its ancestral lines were studied using GFP-expressing *R. commune* isolates and confocal microscopy. Visible disease symptoms were closely associated with independent measures of pathogen growth. However, two QTL were identified as being significant once this relationship had been accounted for, indicating some genetic control of symptom expression separate from pathogen growth.

INTRODUCTION

Rhynchosporium commune (formerly *R. secalis*) is one of the most economically significant barley pathogens worldwide, affecting both yield and grain quality (Zhan *et al,* 2008). The pathogen causes the disease known as 'rhynchosporium', 'barley leaf blotch' or 'scald', and is associated with cool humid environments. Rhynchosporium management relies primarily on fungicide treatments, but the cost of such treatment, coupled with restrictions on available fungicides, make cultivar resistance increasingly important for sustainable disease management.

A number of major genes for resistance to *R. commune* have been identified (Zhan *et al.,* 2008). However, due to the pathogens ability to respond quickly to the widespread use of new, resistant cultivars, the identification of novel resistance sources is an ever more important objective. It has been observed that winter barleys are generally more resistant to *R. commune* (Newton *et al.,* 2004). As such, winter barley may represent a valuable resource for the improvement of spring barley, although it is not clear whether this advantage could be partially a pleiotropic effect of winter growth habit or vernalisation requirement, or a reflection of real genetic differentiation between the two gene pools.

R. commune is a polycyclic pathogen with initial inoculum from infected crop debris, seed coat infection, or airborne ascospores. Penetration of the leaf is made directly through the leaf cuticle, followed by a period of extensive sub-cuticular growth without visible disease symptoms (Jones & Ayres, 1974). Due to the long period of the disease epidemic when symptoms are not visible, the development of *R. commune*-specific PCR primers for real-time qPCR (Fountaine *et al.,* 2007), and the production of GFP-expressing isolates

(Thirugnanasambandam *et al.,* 2011), offer a valuable opportunity to study interactions between *R. commune* and barley, during this asymptomatic phase.

The aim of this study was to investigate the genetic basis of variation in resistance between winter and spring barley types. A further objective was to use molecular phenotyping methods to examine the relationship between infection by *R. commune* and the expression of visible symptoms, in particular, the effect of cultivar resistance on this relationship.

MATERIALS AND METHODS

Field trials

A doubled-haploid (DH) population was derived from a cross between the spring cultivar 'Cocktail,' and a winter parent selected from the F7 of a first backcross between the winter varieties 'Leonie' and 'Pearl' (where Pearl was the recipient parent and the *R. commune* resistance of Leonie, was combined with the malting quality of Pearl). The DH lines were winter sown (along with the three ancestors) in the James Hutton Institute (Invergowrie) rhynchosporium disease nursery over two seasons (2008, 2009). Visual symptoms were scored on a 1-9 scale (Newton & Hackett, 1994) at decimal growth stage 50. Plot disease scores were log transformed prior to further analysis. In addition leaf material was collected at GS 50 for subsequent qPCR analysis.

The upper three leaves from five randomly selected plants from each plot were collected. Corresponding leaves from across plots were combined for joint analysis. 50ng of total DNA from each sample was quantified for *R. commune* DNA using a protocol described in Fountaine *et al*., (2007).

A third trait, relative disease expression (RDE), was defined to account for the relationship between visible symptoms and *R. commune* DNA. A standardised major axis linear regression model was fitted to the relationship between *R. commune* DNA and visible symptoms. The residuals (measured as orthogonal deviations from the model) were taken as measurements of relative disease expression.

Map Production and QTL Analysis

DNA from each of the DH lines was extracted for genotyping with an Illumina BeadXpress® assay utilising the 384 representative set of Barley Oligo Pooled Array 1 SNP markers described by Moragues *et al*., (2010). Genotypic data were used to construct a genetic map using a regression mapping procedure in the software package Joinmap4 (Van Ooijen, 2006).

Multi environment QTL mapping of resistance traits was performed using the Biometris QTL mapping procedure library (Boer *et al.,* 2007) implemented in Genstat 12 (Payne *et al.,* 2009).

Confocal Microscopy

The interaction between a GFP-expressing isolate of *R. commune* and three ancestral lines was studied using confocal microscopy, as described by Thirugnanasambandam *et al.,*(2011). Leaf segments 3cm long of the 1st leaf were collected from seedlings at the three leaf stage. Leaf

sections were placed in sealed perspex boxes containing 120 ppm benzimidazole agar, and inoculated with 10µl of $1x10^5$ /ml spore solution. Five confocal laser scanning microscope images, from each of four leaf sections per line, were taken at 2, 3, 8, and 9 days post-inoculation (dpi). Confocal images were converted to binary images, with foreground pixels representing fungal mycelium. The size of colonies was recorded as the total number of foreground pixels per image.

RESULTS

Ancestral Line Characterisation

In the three ancestral cultivars, the percentage of spores present on the leaf surface that were germinated 2 dpi was consistent with field resistance ratings (HGCA, 2011) and with disease scores from the field trials (Figure 5A), with Leonie showing lowest levels of spore germination, and Pearl and Cocktail showing similar and higher levels of spore germination. At 8 dpi, cv Leonie had the smallest mean colony size and Pearl had the largest, Cocktail was intermediate, but more similar to Leonie than Pearl.(Figure 5B**Error! Reference source not found.**).

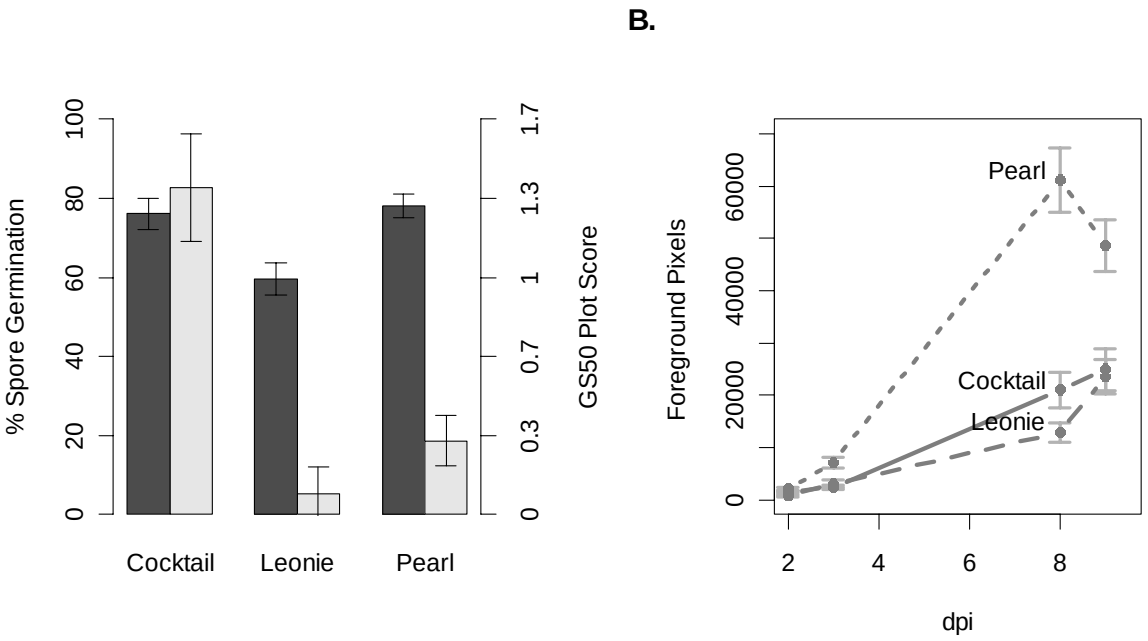

Figure 5. A. Percentage of germinated spores at 2dpi (solid bars) against field trial disease scores (empty bars) for each of the three ancestral lines. Vertical bars indicate standard errors. B. Mean colony size at 2, 4, 8 and nine dpi for each of the three ancestral lines.

QTL Mapping

Multi-environment QTL genome scans identified three significant QTL effects for visible disease symptoms in the mapping population. These were located on chromosomes 2H, 3H and 7H (Figure 6; Table). The QTL effect on chromosome 3H co-locates with the previously identified position of a semi-dwarfing gene (*sdw-1*) which is known to be to be present in Cocktail and therefore segregating in this population. The other two QTL effects did not correspond to known morphology genes, or previously reported resistances to *R. commune*. In all cases, the winter parent contributed the resistant allele.

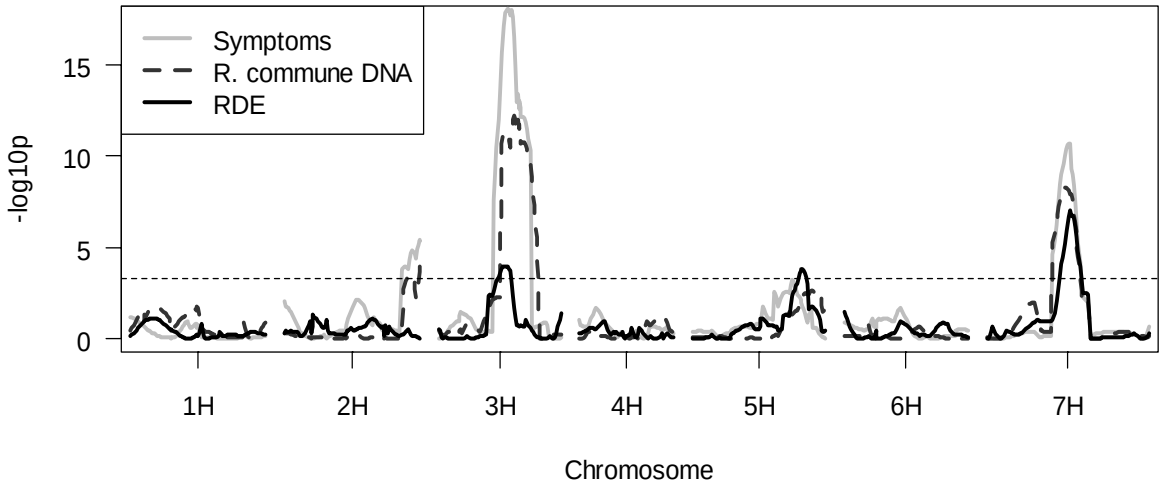

Figure 6. Results of QTL genome scan for all three traits examined in this analysis. For each trait, the line indicates the strength of association between trait and genotype across each of the 7 chromosomes. The horizontal line indicates the significance threshold.

Table 1. Final QTL models for the three disease traits examined showing the chromosome and map position. In each case, the parent contributing the resistant allele is indicated.

Locus	Chr	Position (cM)	Resistant Allele
a: Visual plot scores[a]			
1	2H	179.1	Winter
2	3H	90.5	Winter
3	7H	110.9	Winter
b: R. commune DNA[b]			
1	2H	180.6	Winter
2	3H	99.6	Winter
3	7H	107	Winter
c: Relative disease expression[c]			
1	3H	86.6	Spring
2	5H	145	Winter
3	7H	111	Spring

Significant QTL effects for *R. commune* DNA were in all cases identical to those identified for visible symptoms, with the winter parent contributing the resistant allele for each QTL. The QTL mapping identified 3 QTL for RDE. Two of these (3H and 7H) were in similar positions to QTL for the two primary traits, with an additional QTL on chromosome 5H.

DISCUSSION

The identification of a strong QTL effect for disease resistance at the known position of *sdw-1* suggests that this effect is a pleiotropic effect associated with plant height. This is consistent with previous results (Thomas *et al.,* 2010) and reinforces the importance of disease escape in host defence against *R. commune*. The remaining 2 QTL, identified on chromosomes 2H and 7H, appear to be novel and were not associated with loci that determined seasonal growth habit or morphology traits. In all cases, the winter parent supplied the resistant allele. This is consistent with the observation of generally higher resistance levels in winter varieties, and demonstrates the value of winter barley as a resource for the improvement of *R. commune* resistance in spring barley.

Analysis of patterns of fungal growth on the ancestral lines showed that these were not simple reflections of field resistance scores. In particular, the mean colony size on the most susceptible ancestor (Cocktail) was similar to the most resistant (Leonie), whilst mean colony size on cv. Pearl was substantially higher. However, the levels of spore germination recorded on the susceptible ancestors were higher. This suggests that prevention of spore germination may be an important component of resistance in these lines.

The close relationship between visible disease symptoms and *R. commune* DNA, both in the strength of the genetic correlation, and the co-location of significant QTL effects suggest that the severity of epidemics is primarily determined by the amount of pathogen present on the leaf. This is also supported by the observation that the resistant ancestor shows lower levels of spore germination, and smaller colonies than the susceptible ancestors. This result is not surprising, given the nature of the relationship between the two traits. However, the genetic analysis of residuals from this relationship (RDE) revealed significant QTL, suggesting that the control of symptom expression has a degree separate genetic control. Two of these QTL were co-located with QTL for the two primary traits. Whilst the QTL for RDE at the position of *sdw-1* is not expected (assuming that this locus acts solely through disease escape), the significance is greatly reduced. For the QTL effect on 7H however, the significance remained high. At this locus, and in contrast with the primary traits, the spring parent contributes the resistant allele, suggesting that increased expression of disease symptoms may be a pleiotropic expression of the resistance locus. This suggestion is further supported by the observation that the most susceptible ancestral cultivar (Cocktail) shows pathogen growth (as measured by confocal microscopy) that is similar in magnitude to the most resistant (Leonie).

Detailed analysis of DH lines carrying alternative alleles at each of the resistance loci detected in this study, and in particular observation of patterns of fungal growth using confocal microscopy should provide a more complete understanding of the nature of the resistance QTL identified in this work.

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PREDICTING THE NEED FOR EYESPOT TREATMENT IN WINTER WHEAT

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Summary: Eyespot is common in intensive cereal rotations but disease development and yield loss vary between sites and therefore predicting the need for management intervention is difficult. This paper presents a new, two phased method of assessing disease risk. In the autumn an assessment can be made based on agronomic information that would allow the grower to identify high risk situations and so select a variety with eyespot resistance or modify other criteria such as site selection, with a view to risk reduction. In the second phase a crop disease incidence assessment in the spring is combined with the earlier assessment to predict the need for a fungicide application with specific eyespot activity. This is a development from previous risk assessment methods which depended on spring assessments to predict eyespot risk and consequently were only suitable for fungicide interventions and could not encompass more integrated methods of eyespot management such as varietal resistance.

INTRODUCTION

Eyespot is common in intensive cereal rotations. The causal organisms, *Oculimacula yallundae* and *O. acuformis* infect the stem base and reduce water and nutrient uptake, causing yield losses, whiteheads and reduced specific weights. In more extreme cases the stem is weakened to the point of lodging. Severity and yield loss, however, vary significantly between sites (Burnett & Hughes 2004; Fitt *et al.*, 1988) and consequently it is hard for growers to predict which sites require management intervention. Previous risk assessments for eyespot were based either on disease assessment in the spring (Anon, 1987) or on a risk score of agronomic, weather and disease information in the spring (Burnett & Hughes, 2004). Until recently the main management intervention was the application of fungicides in the spring, timed to coincide with stem extension in the crop. Such risk assessment methods however did not allow for more integrated ways of managing disease or, more specifically, for growers to identify sites where they could benefit from drilling varieties with eyespot resistance. There are two genes for eyespot resistance currently in use in varieties in the recommended list variety tables (HGCA, 2011). The *Pch2* gene confers a moderate level of resistance and is assumed to be present in most current varieties. The *Pch1* gene confers a higher degree of resistance and has only been successfully included in commercial varieties since 2004 with the introduction of the variety Hyperion, since outclassed, and now present in varieties like Grafton and Battalion

Varietal resistance reduces the risk of eyespot but does not always negate the need for treatment (Burnett & Hughes, 2004) and in many cases varietial choice can be limited by the market available to the grower. Fungicides therefore remain an effective and necessary management input but represent an additional cost and input to a standard spray programme.

As such there was a need to identify sites prior to drilling that were at risk from eyespot and to combine this with disease progress information prior to fungicide decisions being made. The aim of the work described here was to develop such a system.

MATERIALS AND METHODS

Field trials

Data was collected from trials carried out between 2000 and 2010. The use of the historical data sets provided large amounts of information. A previous HGCA eyespot model data set from 2000 – 2004 (Burnett & Hughes, 2004)) was an important component of the current project. In addition to this original data set comprising 341 wheat crops untreated for eyespot (but treated to control foliar disease), data from a further 324 untreated wheat crops were available for analysis from fungicide efficacy trials carried out by research partners and funded by agrochemical companies between 2004 and 2010. The two data sets were not combined. As for the original data set, the new data set was incomplete in the sense that over the whole series of trials, not all risk factors were recorded for each crop. In fact, no single crop had all risk factors recorded, and in general, the sub-set of the overall data that comprised the 324 untreated crops was more unbalanced than the original data set.

A randomised block design was used in all trials, with four replicates per treatment. The trials were mainly drilled (preferable) or superimposed in a predominantly cereal rotation. Trials were over-sprayed at GS39 and GS55-69 (Zadoks, 1974) to minimise the effects of foliar disease on yield. These over-sprays varied by site but were selected to offer robust control against the main foliar disease threats perceived at each of the sites.

Information was recorded for each site on environment-based factors either as discrete variable (D) or continuous variable (C): Region (D), Tillage method (D), Sowing date (C), Straw removal (D), Soil type (D), Soil pH (C), Soil P (C), Soil K (C), Soil Mg (C), Previous crop (D), Mean temperature during September/October/November (C), Mean temperature during December / January / February (C), Mean temperature during March / April / May (C), Total rainfall during September / October / November (C), Total rainfall during December / January / February (C), Total rainfall during March/April/May (C).

Assessments were made as follows:-

GS31-32	Eyespot visual assessment over trial site
Pre GS39	Before flag sprays applied, foliar disease assessment all plots
GS45	Eyespot visual assessment
GS70-80	Eyespot visual assessment, lodging and whiteheads if present
Harvest	Lodging and whiteheads, yield

Application Details

Treatments were applied with a hand held CP3 Knapsack sprayer or Azo plot sprayer in approximately 200 L of water per ha.

Sampling methods

At GS25 to GS32, 25 plants per plot were sampled and the stem bases assessed for eyespot which was recorded as % incidence. At later growth stages 25 tillers per plot were sampled and the stem base diseases scored. Eyespot was recorded as 0 = no symptoms, 1= lesions affecting less than 50% of the stem circumference, 2 = lesions affecting over 50% of the stem circumference and 3 = lesions affecting over 50% of the stem circumference and tissue softened so that lodging would readily occur.

A % stem base index was then be calculated for each disease :- (((no of score 1) + (no of score 2 x 2) + (no of score 3 x 3)) / no of stems) x (100 / 3).

Statistical methodology

Logistic regression was used to identify important risk factors. In the original data set the risk factors identified were:- soil type, previous crop, tillage method, sowing date, eyespot at GS31-32 and March/April/May rainfall (Burnett & Hughes, 2004). Individual risk factors were combined into a risk algorithm, following Yuen *et al*. (1996). A risk points score was calculated for each level of each factor, such that the maximum risk score was 50 points. Risk points were tabulated in such a way that, once the score for each factor was known for a particular crop, the risk points total could be calculated. This total was then compared with a threshold points score as a guideline for the need for fungicide application. In fact, two thresholds were determined: a higher one for risk-tolerant users and a lower one for risk-sensitive users.

This methodology allows the calculation of risk accumulation over a series of diagnostic steps. An example (from a clinical perspective) of the sequential diagnostic approach can be seen in Van den Ende *et al.* (2005), who present a figure showing the evolution of probability following consecutive diagnostic steps. The approach of Van den Ende *et al*. (2005) allows decision-makers to apply Bayesian logic without formal calculations. That is to say, an initial estimate of disease risk is modified as evidence related to a sequence of risk factors accumulates. That approach has influenced the way we have chosen to update the risk assessment method presented in Burnett and Hughes (2004).

For a crop, there are some disease-related risk factors, such as those associated with geographic location, site topography and soil physical properties, over which a decision-maker can exert little or no control. Then, the level of risk associated with factors such as previous cropping, tillage method, variety choice and sowing date is already decided at the start of the growing season. Subsequently, only risk factors relating to the environmental conditions during the growing season and the level of disease observed in the growing crop remain for the decision-maker to take into account. However, while it is possible to identify factors relating to the host, the pathogen and the environment (i.e., the classic ‘disease triangle’) that are important contributors to crop disease risk, it is also the case that individual decision-makers may respond differently to a specified risk accumulation. Thus we must also allow some flexibility for individual decision-makers to calibrate accumulated risk according to their personal circumstances.

The underlying principle on which we have based the update of the risk assessment method presented in Burnett and Hughes (2004) is to assign as much of the accumulation of disease risk as possible to factors that can be assessed prior to the crucial eyespot disease assessment at

GS31-32. Note that the pre-disease risk accumulation is actually an assessment of *conditional risk*. That is to say, we know (referring again to the disease triangle) that even if a susceptible variety is being grown in conditions conducive for the spread of disease, that in the absence of the pathogen the disease will not develop. So while the pre-disease risk accumulation categorises a predisposition to risk, this is not realised as an actual risk until the outcome of the eyespot disease assessment at GS31-32 is known.

RESULTS

Because of the severely unbalanced nature of the new data set, most of the analysis of pre-disease risk accumulation was carried out using the original data set. Where cross-checking was possible, no incompatibilities were found in the analysis of the individual risk factors selected for inclusion in the risk algorithm. There may be internal correlations between risk factors that are not apparent when they are analysed separately. If two risk factors are correlated (i.e., they account for the same component of the overall risk), then there is no need to include both of them when making a prediction of disease, and one is eliminated. This was the case with the risk factors *region* and *March/April/May rainfall*. Previously *region* was excluded from the eyespot risk algorithm and *March/April/May rainfall* included. We have now included *region* and excluded *March/April/May rainfall*. The main reason for this change is that it allows us to classify all the risk accumulation except for the component associated with the crucial eyespot disease assessment at GS31-32 as 'pre-disease' (conditional) risk. We also note that *region* (levels: East, North or West) is likely to be regarded as easier to determine than *March/April/May rainfall* (levels: less than or equal to 170 mm or greater than 170 mm).

Table 1 shows the pre-disease risk factors together with the calculated odds ratio (a measure of relative risk, see HGCA Report No. 347) and the $\log_{10}$(odds ratio) (an additive measure of risk). The revised risk points scale is derived by transforming the calculated $\log_{10}$(odds ratio) values to integer values on a scale from zero to twenty-five points.

Table 1. Pre-disease risk factors

Factor	Level	Odds ratio (OR)	Log_{10}(OR)	Risk points
Region	East	1	0	0
	North	1.149	0.0603	1
	West	1.788	0.2524	5
Soil type	Light	1	0	0
	Medium	1.071	0.0298	1
	Heavy	1.559	0.1928	4
Previous crop	Non-host	1	0	0
	Other cereal	2.245	0.3512	7
	Wheat	2.420	0.3838	8
Tillage	Minimum Till	1	0	0
	Plough	2.044	0.3105	6
*Sowing date**	Late	1	0	0
	Early	1.336	0.1258	2

* Before or after 6 October

Table 2. Pre-disease risk categories (conditional risk)

Pre-disease risk points	Verbal description of pre-disease risk category*
1-4	Low risk (L)
5-9	Low-medium risk (LM)
10-14	Medium risk (M)
15-19	Medium-high risk (MH)
≥20	High risk (H)

* The descriptions are advisory rather than prescriptive.

A field, once drilled, will come out of the winter with this pre-disease score determined. A decision then needs to be made about the need to treat with a fungicide with eyespot efficacy. Table 3 describes the risk of eyespot developing in that crop by combining the appropriate pre-disease risk category in Table 2 with the level of risk based on the disease visible in the crop at the GS31-32 assessment, from which an overall risk category can be determined (see Table 3).

Table 3. Eyespot disease risk categories*

Pre-disease risk points (conditional risk)	Eyespot disease assessment % incidence at GS 31-32				
	1-4	5-9	10-14	15-19	≥20
1-4	L	LM	M	MH	H
5-9	LM	M	M	MH	H
10-14	M	M	MH	MH	H
15-19	MH	MH	MH	H	H
≥20	H	H	H	H	H

*Verbal description of category: Low risk (L), Low-medium risk (LM), Medium risk (M), Medium-high risk (MH), High risk (H).

The decision to treat or not is then made by the operator. They may choose to do this based on the guidelines provided by Table 3, with the overall risk category determined by pre-disease risk category and level of disease risk determined by the GS31-32 assessment. In addition, they may wish to take into account their perception of eyespot as an important problem or a minor one, in which case they would be described as risk-sensitive or risk-tolerant respectively.

DISCUSSION

The two phase approach to risk assessment that has been developed is novel in crop protection and allows growers to assess options prior to drilling and, based on the pre-disease conditional risk score determined in the autumn, either select an alternative field for drilling or decide to drill a variety of wheat with eyespot resistance.

The second phase of the risk assessment allows growers to decide on the need for fungicide treatment in the spring by combining this pre-disease autumn score with information on the incidence of eyespot at stem extension. Here a methodology borrowed from medical literature was used to ascribe a risk status to crops based on low, low moderate, moderate, moderate high and high categories (see, e.g., Swets, 1988). This allows for more sensitivity in deciding on the need to treat than would a more simple low, moderate or high description. The two phased risk

assessment described allows for eyespot to be managed in line with integrated crop management principles, through the selection of resistant varieties or other management options taken in the autumn to reduce risk, such as delayed sowing or alternative field selection. This contrasts with previous risk assessments for eyespot which focused solely on spring assessment as a means of judging the need for fungicide inputs.

A logical development of this new methodology would be to develop a web-based / on-line tool that would allow calculations on eyespot risk to be made by entering relevant parameters.

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PESTICIDE USE PATTERNS IN SCOTTISH CEREAL CROPS 2000-2010: POTENTIAL IMPACT OF EU THEMATIC STRATEGY FOR PESTICIDES

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Summary: Whilst there has been little variation in the proportion of Scottish cereal crops treated with pesticides, both the number and weight of applications have increased over the past decade. The overall trend primarily reflects changes in fungicide applications. The number of herbicide applications has also increased although the quantity applied has remained static, whilst the quantity of insecticides applied has greatly decreased over time. The implementation of the EU thematic strategy for pesticides may significantly reduce the number of pesticides available to Scottish cereal growers, particularly in relation to fungicides.

INTRODUCTION

Cereals account for over 80% of Scottish arable cultivation, with a total market value of *ca*. £400 million per annum (Anon, 2010). Around 450,000 ha are grown annually, predominately consisting of spring barley, winter wheat, winter barley and oats (ca. 55, 25, 15 and 5% of the crop respectively). Fluctuations in overall area, and of constituent crops, are associated with factors such as climatic conditions at planting, grain prices and changes in agricultural subsidies. The aim of this paper is to present pesticide application data for Scottish cereal crops over the last decade and to discuss the influence that the newly introduced EU thematic strategy for pesticides may have on future pesticide use patterns.

METHODS

Pesticide application data were obtained from the dataset collected by SASA during biennial arable pesticide use surveys (Kerr & Snowdon, 2001; Snowden & Thomas, 2003; Snowden *et al.*, 2005; Struthers, 2007; Reay, 2009; Reay *et al*., 2011). The surveys are conducted by collecting data from a random sample of farms classified by size and geographic region. National estimates of pesticide use are produced from the sample data by applying raising factors based on crop census areas. Pesticide use expressed as ratio of spray area to treated area is the total area of pesticides applied, including multiple applications during the growing season, divided by the area of crop treated, this does not equate to number of applications.

EU Council Directive 91/414/EEC, concerning the placing of plant protection products on the market, has been replaced with a thematic strategy for pesticides. This consists of authorisation and statistics regulations (1107/2009/EC and 1185/2009/EC respectively) and an associated Sustainable Use Directive (2009/128/EC). A major implication of this legislation is that the pesticide approval process will become hazard as well as risk based. This may result in some active ingredients currently on Annex I being withdrawn or substituted over time. Whilst the

full impact of these changes is not yet known, the potential influence of this legislation has been investigated by comparing current pesticide use with predicted future approval status based on a Pesticides Safety Directorate (PSD) impact assessment (Anon., 2009).

RESULTS AND DISCUSSION

Almost all Scottish cereal crops receive pesticide applications and the proportion treated has been fairly constant over the last ten years (Table 1). However, both the ratio of the total spray area to treated area and the average quantity applied has increased over the last decade. These trends are discussed in relation to each of the main pesticide groups.

Table 1. Pesticide Application Data 2000-2010.

	2000	2002	2004	2006	2008	2010
	Percentage of Crop treated					
All pesticides	98	97	98	98	94	97
Fungicides	90	92	90	93	90	95
Herbicides	97	96	98	96	93	95
Insecticides	15	14	14	19	26	20
	Ratio of spray area to treated area (spray area/treated area (ha))					
All pesticides	10.6	10.9	12.1	12.0	14.1	13.9
Fungicides	4.8	4.7	5.9	5.8	6.9	6.5
Herbicides	3.1	3.2	3.4	3.5	3.6	3.9
Insecticides	1.1	1.1	1.1	1.1	1.1	1.1
	Average dose rate (kg/ha treated area)					
All pesticides	2.17	2.20	2.72	2.60	2.75	2.52
Fungicides	0.58	0.54	1.07	0.98	1.11	0.98
Herbicides	0.96	0.88	1.06	1.03	0.99	0.98
Insecticides	0.19	0.11	0.12	0.08	0.06	0.08

Fungicide Use Patterns

Whilst there has been little change in the proportion of cereal crops treated with a fungicide since 2000, the ratio of spray area to treated area increased by 35%. i.e. a greater number of products were applied to the crop. The quantity of fungicide applied has also increased, particularly between 2002 and 2004, when average dose rates doubled (Table 1). The 2004 increase in quantity was primarily due to greater use of chlorothalonil, a chloronitrile fungicide, which is applied at higher rates (Figure 1). Chlorothalonil use increased from 1 to 16% of total fungicide spray area (with a corresponding increase from 4 to 44% of total weight) between 2002 and 2004. This was primarily a consequence of the development of resistance to strobilurin fungicides in *Septoria tritici*. Consequently, the strobilurins show a drop in use over the decade, from 26% of total fungicide area in 2000 to 16% in 2010. As strobilurins are active at very low application rates, their decline also influenced the increase in dose rate. The amine fungicides (including fenpropimorph, and spiroxamine) have also shown a gradual decrease in use over the last decade (from 20 to 13% of total fungicide area) whilst use of triazole compounds has remained unchanged, accounting for 30 to 40% of fungicide applications.

Herbicide Use Patterns

Herbicides were consistently applied to more than 90% of cereal crops over the reported period. As with the fungicides a similar, albeit less pronounced, trend of an increasing spray area in relation to treated area is displayed (Table 1). Despite the increase in spray area the average quantity applied shows no significant variation. This is influenced by a 10% increase in use of the sulfonylurea compounds over the decade (Figure 2). Sulfonylurea herbicides are applied at low rates and their increase has coincided with a reduction in use of the higher dosage aryloxyalkanoic acid herbicides (in particular mecoprop-p), which have declined by a third over the same period. Another major change was the removal of the urea herbicide isoproturon from the market in 2009, which previously accounted for 9% of total herbicide application area.

Insecticide Use Patterns

The percentage of cereal crops treated with insecticides is considerably lower and more variable than fungicides and herbicides (Table 1). This is largely due to greater temporal variability in pest pressure and applications being more reactive than preventative. Whilst there has been no change in the relative proportions of spray area to treated area, dose rate has decreased markedly over the last decade. The particularly high quantity applied in 2000 was due to extensive use of organophosphates, which have very high application rates, for aphid control. Thereafter, the main trend has been a steady decrease in organophosphate use, from 27% of spray area (93% of weight) in 2000 to 8% spray area (85% of weight) in 2010. In contrast, use of pyrethroids, compounds which are applied at very low rates, has increased from 69 to 90% of total area (5 to 14% weight) over the same period. Carbamate insecticide use has shown little change over the reported period, remaining low throughout (ca 4% area).

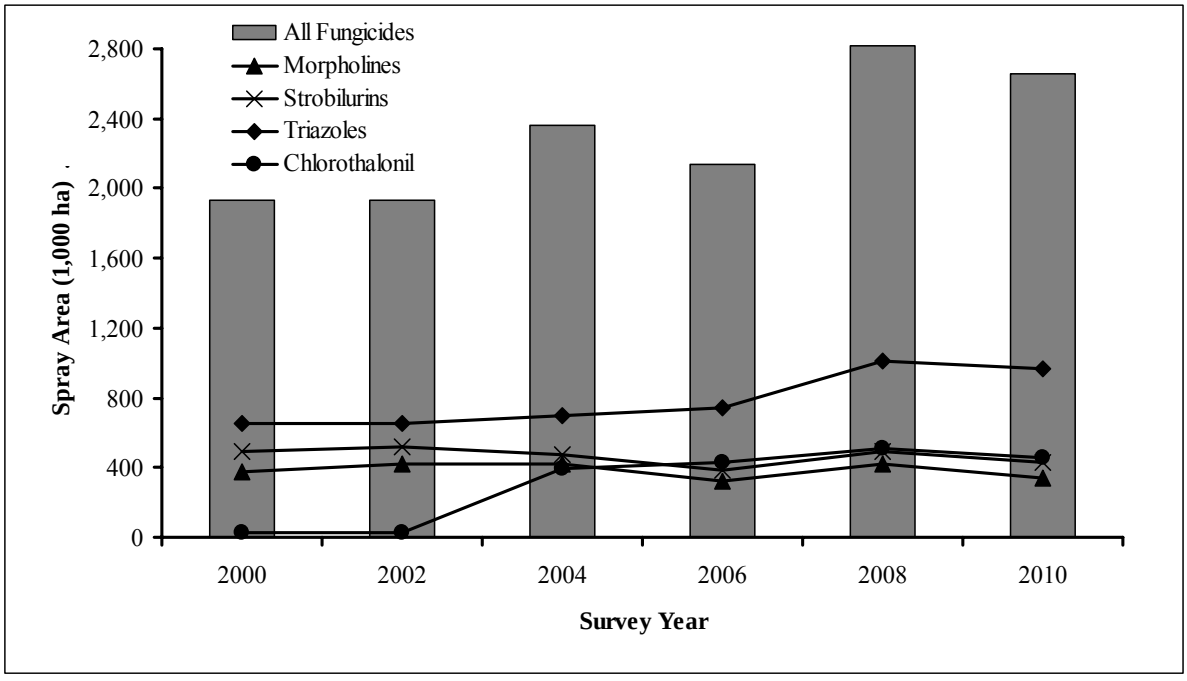

Figure 1. Spray area of total fungicides and main fungicide groups applied to cereal crops 2000-2010.

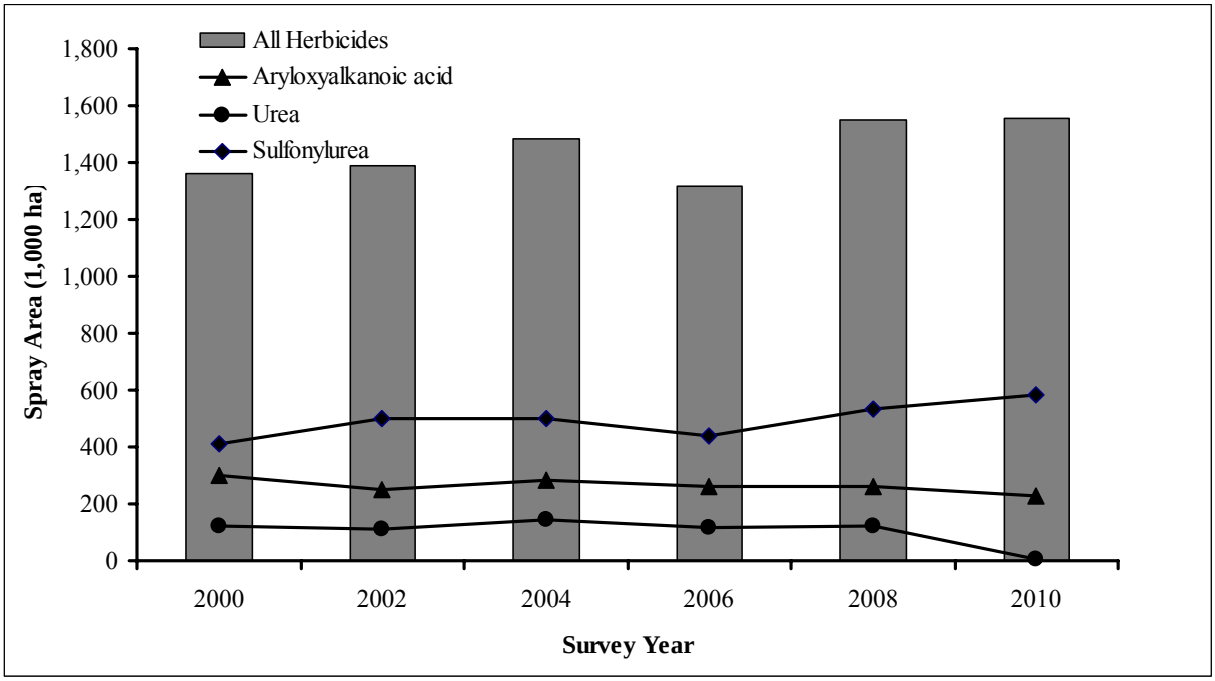

Figure 2. Spray area of total herbicides and main herbicide groups applied to cereal crops 2000-2010.

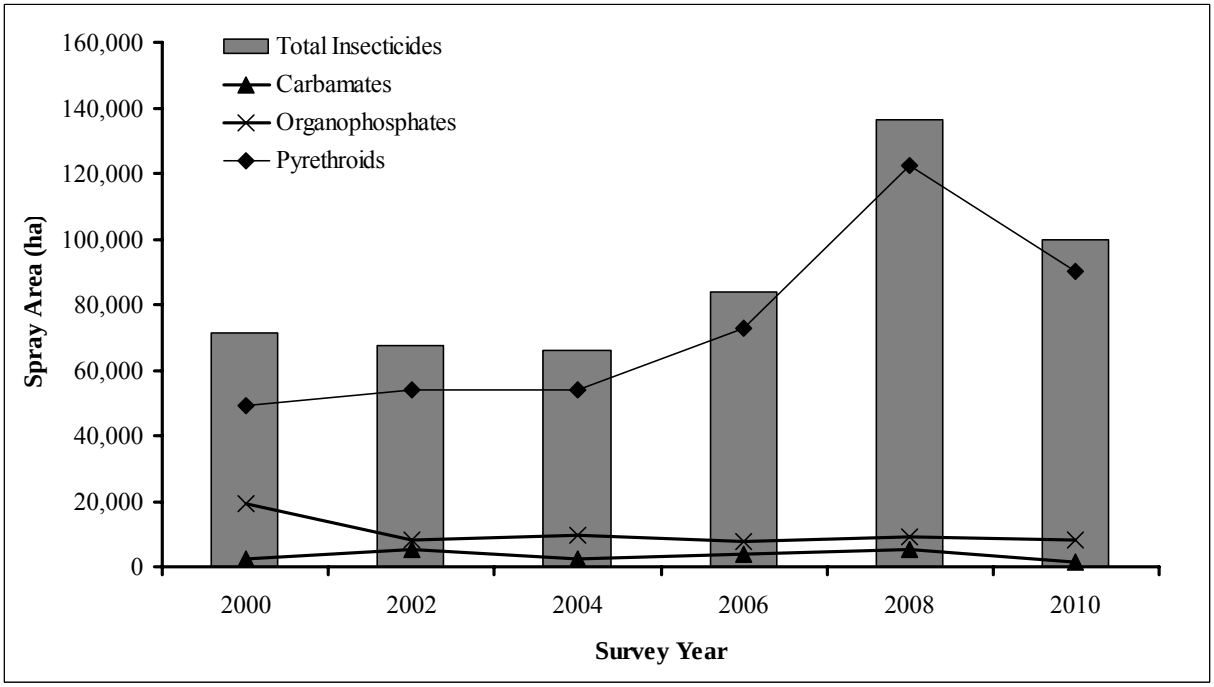

Figure 3. Spray area of total insecticides and main insecticide groups applied to cereal crops 2000-2010.

Potential Effect of EU Thematic Strategy on Pesticide Availability

The active ingredients most likely to lose approval status, according to the prediction of the PSD impact assessment, based on the EU thematic strategy, are listed in Table 2. Were these compounds to be removed from the market reduced fungicide availability would be the most serious threat to Scottish cereal production.

Many of the major cereal fungicides are listed as likely to lose approval under the new legislation. Five triazoles, notably epoxiconazole and tebuconazole, which cumulatively account for 20% of the 2010 fungicide spray area, are at risk due to their potential endocrine disruptor status. In addition, when compounds that the impact assessment state may come off the market are taken into account a further 4 triazoles (difenaconazole, propiconazole, prothioconazole and triadimenol) and an imidazole compound (prochloraz) are included. These compounds account for a further 16% of the fungicides applied in 2010 and are all Demethylation Inhibitors (DMI). DMI fungicides are an important group of broad spectrum compounds of economically important cereal pathogens, and are particularly important for *Septoria tritici* control. In addition, chlorothalonil is predicted to be a candidate for substitution. Chlorothalonil currently accounts for 17% of fungicide spray area and is an important component in control strategies for *Septoria tritici*, *Rhynchosporium secalis, Ramularia collo-cygni* and Fusarium *spp*. Overall, the loss of these fungicides would have major repercussions for both disease control and resistance management strategies, leading to resistance issues for the remaining compounds.

Table 2 Active ingredients most likely to lose annex I approval (PSD impact assessment, 2009) and 2010 use data

Active ingredient	Chemical group	Total spray area 2010 (ha)*	% of total application area 2010**
		Fungicides	
Epoxiconazole	Triazole	246380	9.2
Tebuconazole	Triazole	121697	4.6
Flusilazole	Triazole	84369	3.2
Cyproconazole	Triazole	60912	2.3
Metconazole	Triazole	33174	1.2
Mancozeb	Dithiocarbamate	46665	1.7
Carbendazim	Benzimidazole	2822	0.1
Quinoxyfen	Quinoline	1909	0.1
		Herbicides	
Pendimethalin	Dinitroaniline	66265	4.3
Ioxynil	Hydroxybenzonitrile	55792	3.6
Tralkoxydim	Cyclohexadione oxime	22713	1.5
Linuron	Urea	6074	0.4
		Insecticides	
Esfenvalerate	Pyrethroid	2120	2.1
Bifenthrin	Pyrethroid	727	0.7

* includes multiple applications to the crop area during the growing season

** for each separate group (fungicides, herbicides, insecticides)

Both the herbicides and insecticides are predicted to be comparatively less affected than fungicides. The herbicides most likely to lose approval cumulatively accounted for 10% of the 2010 herbicide application area. The most significant loss being pendimethalin, which was applied to 66 k spray hectares (4% of spray area) and is important for pre-emergence black grass control in cereals. When actives that may be removed, notably chlorotoluron, and those that are candidates for substitution, such as diflufenican, are considered, compounds applied to around a fifth of the current spray area are at risk and, as such, weed control options would be severely reduced.

In relation to insecticides, only two actives applied to cereal crops are predicted to be likely to be removed; bifenthrin and esfenvalerate. Both active ingredients, which cumulatively accounted for ca 3% of 2010 insecticide spray area, are at risk due to their classification as persistent, bioaccumulative and toxic (PBT) compounds. Deltamethrin and dimethoate, each accounting for ca 3% of total current spray area, may also be at risk as potential endocrine disruptors. Dimethoate is particularly important for control of wheat bulb fly in cereals and all four of these insecticides are important pest control options for winter wheat and barley. However, the most serious threat to cereal crops in relation to insecticide losses is the pyrethroid lambda-cyhalothrin, which is predicted to be a candidate for substitution. Lambda-cyhalothrin accounted for 40% of the total insecticide spray area in 2010 and is applied to all cereal crops.

In conclusion, pesticide input is integral to cereal production. The proposed changes to pesticide availability, particularly in relation to fungicides, have the potential to dramatically change current pesticide application regimes. Were the predictions from the PSD impact scenario implemented there would be significant difficulty maintaining both pest control and resistance management with the remaining compounds, leading to negative impacts on yield and the Scottish agricultural economy.

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THE CONTRIBUTION OF BASF SDHI CHEMISTRY TO CEREAL YIELD PERFORMANCE

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Summary: BASF's contribution to cereal yields with SDHI (carboxamide) chemistry started in 1974 with the introduction of benodanil as the product Calirus. Tracker, introduced in 2005 contains the broader spectrum carboxamide boscalid in joint formulation with epoxiconazole for increased disease control and mutual resistance management protection. This concept continues with the introduction of Adexar, based on the new highly active carboxamide Xemium®, containing a full registered dose of epoxiconazole. Data presented here predominantly from the north of Britain shows that Adexar controls the key wheat and barley diseases at least as well as recently introduced comparison products, and has particular strengths in the degree of curative and residual activity offered. Data is shown for the control of *Septoria tritici* and yellow rust in wheat, and net blotch and *Rhynchosporium* in barley. Tracker data is also presented which justifies its continuing position in wheat programmes at GS 32.

INTRODUCTION

The first foliar carboxamide from BASF, benodanil, was introduced in 1974. It was recommended for the control of cereal rusts, but was superseded with the introduction of broader spectrum triazole compounds. BASF described a broader spectrum carboxamide, boscalid, in 2002 (Ammermann, 2002), and this was introduced in cereals in 2005, this time in a co-formulation with epoxiconazole. The epoxiconazole partner compliments the carboxamide to provide reliable broad spectrum disease control with in-built resistance management. Trial results with this formulation in cereals in the north of the UK in comparison to commercial standards were reported at this conference in 2006 (Sykes *et al.*).

Xemium® is the third foliar carboxamide to be introduced by BASF. This novel and highly active carboxamide shows unusual uptake and movement characteristics in the plant, which leads to both rapid and continuous disease control over an extended period. Xemium® is formulated as an EC with epoxiconazole, and is now registered for use in all UK cereal crops where it shows good to excellent activity against all the key foliar disease targets. Its excellent performance comes from matched high levels of systemicity and residual activity from both components across the disease range, delivering consistent disease control and yield response, with in-built resistance management which is of increasing importance. Xemium® was first described and data published by BASF in 2010 (Semar *et al*.).

MATERIALS AND METHODS

All data shown in tables is from the 2011 harvest year and is taken from small plot fully randomised trials with three replicates per block, conducted in accordance with good experimental practice. Data presented in Figure 1 is from an unreplicated block trial with 26 varieties. In all trials agronomy inputs were applied by the farmer, and the reported experimental treatments were applied by trial contractors. Disease assessments were carried out using the standard method to assess the percentage leaf area infected on specific leaves. Yields were measured using small plot combine harvesters, with yields corrected to 15% moisture content. Disease assessments were carried out on the dates and leaves, and by the contractors specified in Table 1.

Table 1. Assessment details for data presented in Tables 3-8.

Table	Trial site	Date	Leaves	Trial contractor
3	Balgonie	25/6	3,4	Scottish Agronomy/SAC
3	Herefordshire	05/7	1	ADAS
4	Carlow, Ireland	26/6	1	Teagasc
5	Rawcliffe Bridge	specified in table		Agricultural Trial Services
6	Borders	09/7	3	Cropworks
6	Angus	01/8	2	Cropworks
7	Chirnside	15/6	2	Cropworks
7	Northants	07/6	2	Envirofield
8	Balgonie	06/5	1	Scottish Agronomy/SAC
8	Herefordshire	08/6	2	ADAS

All products used are registered for use in the UK, hence commercial product names are used and all trademarks are acknowledged. The formulation details of each product used are listed in Table 2. The higher ratio bixafen+prothioconazole product was used in wheat.

Table 2. Products used in the experiments.

Product	Company	Active ingredient(s)	gai/l	Label dose (l/ha)
Opus	BASF	epoxiconazole	125	1.0
Ignite	BASF	epoxiconazole	83	1.5
Comet 200	BASF	pyraclostrobin	200	1.25
Tracker	BASF	boscalid+epoxiconazole	233+67	1.5
Adexar	BASF	Xemium+epoxiconazole	62.5+62.5	2.0
Aviator 235 Xpro	Bayer	bixafen+prothioconaxole	75+160	1.25
Siltra Xpro	Bayer	bixafen+prothioconazole	60+200	1.0
Seguris	Syngenta	isopyrazam+epoxiconazole	125+90	1.0
Bontima	Syngenta	isopyrazam+cyprodinil	62.5+187.5	2.0

RESULTS

The activity of Xemium®+epoxiconazole against S. tritici in comparison to market standards is shown in Table 3. Overall, Xemium®+epoxiconazole gave superior disease control and yield response in comparison to the market standards tested.

Table 3. *S. tritici* control and yield response from GS 39 sprays in wheat.

Treatment	% S. tritici		Yield1 (t/ha)		
	Balgonie	Herefordshire	Balgonie	Herefordshire	Mean
Untreated	31.7	27.1	7.15	10.38	8.76
Xemium®+epoxiconazole 2 l/ha	17.5	0.8	0.87	2.83	1.85
Xemium®+epoxiconazole 1 l/ha	26.7	2.5	0.26	2.33	1.29
bixafen+prothioconazole 1.25 l/ha	22.5	3.1	0.98	2.29	1.63
bixafen+prothioconazole 0.625 l/ha	22.5	9.0	0.13	1.32	0.72
isopyrazam+epoxiconazole 1 l/ha	21.7	8.8	1.0	1.63	1.31
isopyrazam+epoxiconazole 0.5 l/ha	25.8	9.5	0.33	0.68	0.50
epoxiconazole(83.5gai/l)+ pyraclostrobin (1.25+0.4 l/ha)	24.2	4.2	0.46	1.19	0.82
LSD	8.65	5.97	0.70	0.55	

[1] Yields are shown as absolute t/ha in untreated, and the relative t/ha increase in treated

Disease pressure in northern Europe in 2011 was highest in Ireland, and the results from Teagasc are included to show product performance under higher pressure (Table 4). When optimally timed at GS 39, both Xemium®+epoxiconazole and bixafen+prothioconazole perform well, substantially better than the commercial standard of epoxiconazole (83gai/l). However, when products were applied late (GS39+20days), the superior curative activity of Xemium®+epoxiconazole became clear. The magnitude of this difference is shown by the parity between late-applied Xemium®+epoxiconazole and optimally timed epoxiconazole.

Table 4. Effect of fungicides applied under high disease pressure in Ireland.

Treatment	Applied at GS39		Applied at GS39+20days	
	% S. tritici	Yield (t/ha)	% S. tritici	Yield (t/ha)
Untreated	51.0	9.2		
Xemium®+epoxiconazole 1.33 l/ha	17.0	11.4	29.0	11.0
bixafen+prothioconazole 0.83 l/ha	18.0	11.3	39.0	10.7
isopyrazam+epoxiconazole 0.66 l/ha	37.0	10.9	48.0	10.5
epoxiconazole (83.5 gai/l) 1.0 l/ha	27.0	10.9	47.0	10.0
LSD	12.80	0.53	12.80	0.53

Improved yield performance of Xemium®+epoxiconazole in comparison with an azole+ pyraclostrobin programme where timings and doses were equivalent, was shown in a 24 variety trial at the BASF Rawcliffe Bridge demonstration site (Fig. 1). The programme based on Xemium®+epoxiconazole outyielded the standard programme on 19 of the 24 varieties, with a mean overall yield advantage of 0.58t/ha.

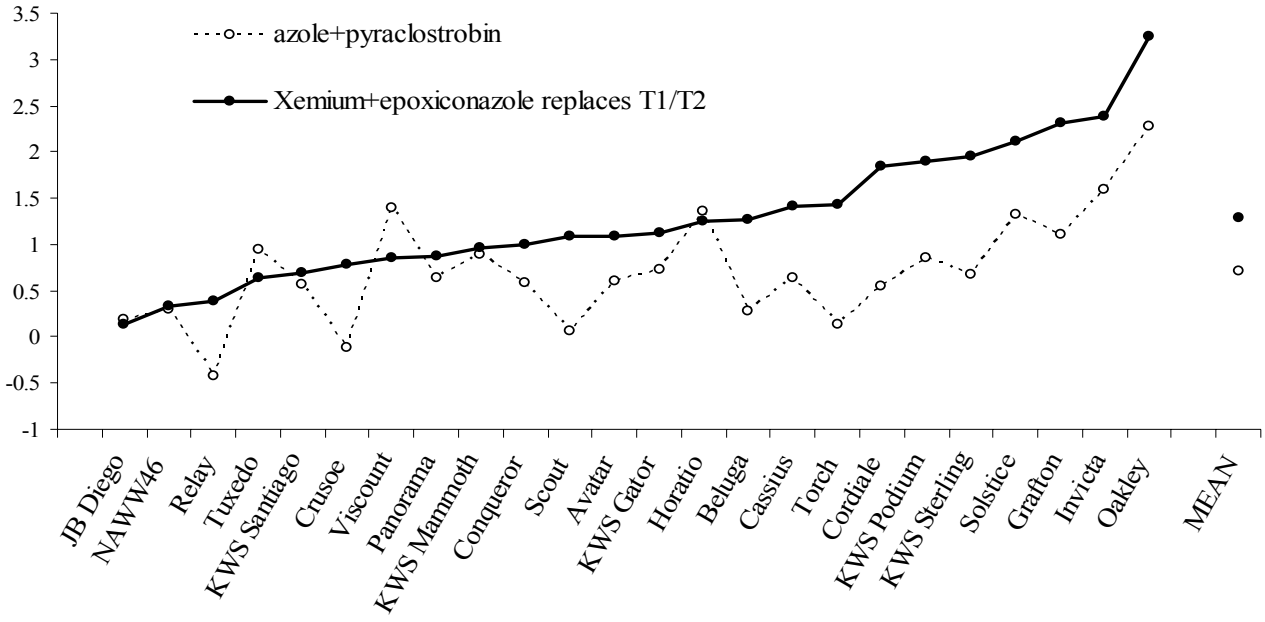

Figure 1. Yield response of 24 varieties to two fungicide programmes in wheat at Rawcliffe Bridge.

Table 5. Yellow rust control from fungicides applied at GS 32 (6th May).

Treatment	% y.rust 6th June Leaves 1-4, GS 63	% y.rust 20th June Leaves 1-2, GS 69-75	Yield (t/ha)
Untreated	34.7	13.4	6.67
boscalid+epoxiconazole 1 l/ha	2.3	6.5	9.31
Xemium®+epoxiconazole 1 l/ha	2.1	6.9	10.11
bixafen+prothioconazole 0.625 l/ha	3.4	17.1	8.69
isopyrazam+epoxiconazole 0.5 l/ha	4.2	9.8	9.27
LSD	5.83	7.08	0.62

Also at Rawcliffe Bridge, a trial on the variety Oakley carried a serious yellow rust infection (Table 5), and where a good measure of the relative persistence of the fungicides was obtained. The earlier assessment 4weeks after application showed similar activity from all products, but after a further 2 weeks significant differences appeared between treatments, with boscalid +epoxiconazole and Xemium®+epoxiconazole the most effective products at this time. Large and significant yield responses to treatment were correlated to the degree of disease control obtained.

Bosclid+epoxiconazole is established as a T1 (GS32) input in the UK wheat crop, and trials in programmes with Xemium®+epoxiconazole at T2 confirmed the value of the additional boscalid at this application timing, in comparison to straight epoxiconazole (Table 6).

The value of Xemium®+epoxiconazole in barley is shown in trials with net blotch and Rhynchosporium. Trials in Scotland in 2011 carried lower disease pressure than those at sites in Herefordshire and Northamptonshire, therefore data is included from these southern locations. In these trials, Xemium®+epoxiconazole showed improved activity against net blotch in comparison to recent introductions (Table 7), and at least matched these standards when tested against Rhynchosporium (Table 8).

Table 6. Effect of GS32 sprays followed by Xemium®+epoxiconazole at GS39.

Treatment	% *S.tritici*		Yield (t/ha)		
	Borders	Angus	Borders	Angus	Mean
Untreated	44.2	98.2	9.67	6.55	8.11
epoxiconazole (125 gai/l) 0.5/ Xemium®+epoxiconazole 1 l/ha	2.2	4.2	12.33	8.57	10.15
boscalid+epoxiconazole 1.0/ a Xemium®+epoxiconazole 1.0 l/h	1.1	2.5	12.66	8.66	10.35
epoxiconazole (125 gai/l) 0.5/ Xemium®+epoxiconazole 1.5 l/ha	2.8	1.7	12.2	8.83	10.52
boscalid+epoxiconazole 1.0/ Xemium®+epoxiconazole 1.5 l/ha	0.3	0.6	12.58	8.82	10.70
LSD	0.19	0.56	0.66	0.39	

Table 7. Effect of fungicides on net blotch control in winter barley.

Treatment	% net blotch		Yield (t/ha)		
	Chirnside	Northants	Chirnside	Northants	Mean
Untreated	24.8	54.4	9.17	7.7	8.43
Xemium®+epoxiconazole 1.2 l/ha GS31	0.2	8.3	10.2	8.4	9.32
bixafen+prothioconazol 0.6 l/ha GS31	2.0	16.9	10.4	8.4	9.41
isopyrazam+cyprodanil 1.2 l/ha GS31	6.3	27.5	9.87	7.8	8.83
Xemium®+epoxiconazole 1.2 l/ha GS39-45	0.8	6.4	10.5	8.3	9.41
bixafen+prothioconazole 0.6 l/ha GS39-45	1.1	11.5	10.3	8.3	9.28
isopyrazam+cyprodanil 1.2 l/ha GS39-45	2.8	10.4	9.73	7.7	8.71
LSD	0.11	8.11	0.56	0.57	

Table 8. Effect of fungicides on Rhynchosporium control in winter barley.

Treatment	% Rhynchosporium		Yield (t/ha)		
	Balgonie	H'fordshire	Balgonie	H'fordshire	Mean
Untreated	5.7	19.2	10.1	6.11	8.09
Xemium®+epoxiconazole 1.2 l/ha GS3	13.5	1.9	10.6	7.65	9.12
bixafen+prothioconazole 0.6 l/ha GS31	3.33	2.4	10.6	7.0	8.8
isopyrazam+cyprodanil 1.2 l/ha GS31	5.67	9.2	10.6	6.57	8.56
Xemium®+epoxiconazole 1.2 l/ha GS39-45	4.67	7.5	10.8	7.81	9.32
bixafen+prothioconazole 0.6 l/ha GS39-45	3.67	14.0	10.8	7.38	9.06
isopyrazam+cyprodanil 1.2 l/ha GS39-45	4.0	9.9	10.6	6.92	8.78
LSD	2.8	7.8	0.28	0.78	

DISCUSSION

Starting with BASF's first foliar carboxamide fungicide introduction in 1974, this chemistry initially played only a small part in cereal fungicide programmes due to the proliferation of broad spectrum triazoles during the 80's and 90's. However, the discovery by BASF of boscalid, a broader spectrum carboxamide fungicide, re-established the contribution of this important group of chemistry. The introduction of boscalid in co-formulation with epoxiconazole into the UK cereal market in 2005 set the model for joint formulation of carboxamide combined with triazole chemistry for complimentary fungicidal activity combined with mutual protection against resistance risk for both of these key groups.

The introduction of BASF's third foliar carboxamide Xemium®**,** also in co-formulation with epoxiconazole, sets the overall standard for broad spectrum activity in cereals within this product type as shown by the results reported here, and which are supported by other data generated throughout Europe. In wheat, Xemium®+epoxiconazole has shown strong control of both key foliar targets, S. tritici and cereal rusts. Data presented here has confirmed both strong curative and residual activity, with associated yield responses. These properties give the farmer more consistency in performance, particularly under more testing conditions which are often encountered in practice, for example when applications are late, or when gaps between spray applications are longer than intended.

Xemium®+epoxiconazole has also shown excellent activity in barley against the 2 key yield limiting blotch diseases, Rhynchosporium and net blotch. Data presented here has shown that when tested against these diseases, Xemium®+epoxiconazole at least meets the standard set by recent product introductions.

Therefore in the one product, Xemium®+epoxiconazole presents the advisor and farmer with a highly active and flexible solution for broad spectrum disease control and yield performance in both wheat and barley, as well as other cereals (oats, rye, and triticale), in a formulation with in-built resistance management to prolong the effective life of both of these important groups.

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THE BENEFITS OF SPRAY DROPLET ANGLE FOR THE CONTROL OF BLACKGRASS WITH PYROXSULAM

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Summary: Improved product performances are often claimed from the angling of nozzles, either forward or backwards, in comparison to traditional vertical use. Pot studies were conducted to investigate two pyroxsulam containing products to test performance against *Alopercurus myosuroides* (blackgrass) when applied using a flat fan nozzle or air induction nozzle in either a vertical or an angled position. Results indicated that a forward facing nozzle was beneficial, recording higher control and greater deposition on small targets. Spring applied field studies were conducted and indicated no benefit from angling nozzles and it was concluded that in situations of small target weeds and minimal crop interception, as found in the autumn, then a forward facing, angled nozzle could be beneficial. For applications applied in the spring, where canopy interception was greater, the vertical nozzle may be optimal.

Key words: pyroxsulam, *Alopecurus myosuroides*, spray trajectory, angling, nozzles

INTRODUCTION

Growers are increasingly aware not only of the importance of spray solution coverage and spray quality but increasingly in the importance of presenting the spray solution in a manner that will benefit product performance. This has never been more important than in the area of graminicide usage where growers and researchers alike are investigating all methods to improve control or increase the consistency of grassweed control. Previous pot studies have identified the importance of spray quality and water volume of a spray solution of pyroxsulam and florasulam for the control of *A. myosuroides* and postulated that not only was spray coverage important for *A. myosuroides* control but that droplet deposition on the plant may also be key to influencing control levels (Harris *et al.,* 2010). Much effort has been involved in the use of angled nozzles and the benefits of presenting the spray solution at an angle relative to the target plant, with data published to show the increase in deposition of the spray solution when applied to vertical steel rods (Miller *et al.,* 2010).

Pot studies were initiated to investigate the influence of spray trajectories of Broadway Star[1] (70.8 g/kg pyroxsulam + 14.2 g/kg florasulam, WG, referred to as GF-1364) and Broadway Sunrise[1] (5.4 g/litre pyroxsulam + 314 g/litre pendimethalin, OD, referred to as GF-2010) for the control of *A. myosuroides*. Two further field studies were carried out to investigate

[1] Trademark of Dow AgroSciences LLC

whether the findings from the pot study data could be transferred to a field situation. Treatments used in the pot and field studies are listed in Table 1.

MATERIALS AND METHODS

Pot Study

A. myosuroides seeds were sown on September 2nd 2010 into a compost soil mixture contained in square 8 cm by 8 cm plastic containers. Twenty such containers were placed within 30 plastic trays and grown outdoors in a secure area. On the day of treatment (3rd October), the trays were removed, inspected to ensure that there were adequate numbers of plants at the chosen growth stages (GS11 to GS12 - one to two true leaves). Each tray to be sprayed was located midway between four adjacent spraying nozzles, midway down a 50 metre long spray track in a soil hall and recessed into the soil with the top of the container at the same height as the adjacent soil surface. Visual assessments to determine control were conducted on 24th November 2010 (52 days after application). Full, half and quarter label rates of each treatment were applied to try and induce greater differences between the nozzle type or angle.

Application was carried out using a Hardi NK 300 litre capacity conventional field crop sprayer with a 6 metre boom spraying at 12 km/h. Treatments were applied at one application volume of 130 litres/ha; a choice (as with the spraying speed) that reflects that at or close to normal practice today. Only the four more distal outer nozzle locations of one boom section were used to generate an effective, uniform swath width of 1.5 metres wide. Nozzles used were Spraying Systems TeeJet[2] 110-04 (the reference nozzle), and the Billericay Farm Services (BFS) Bubble Jet[3] 04. All nozzles used were angled in the direction of spraying, whilst the TeeJet 04 and the Bubble Jet 04 were also used vertically. One nozzle, the Bubble Jet 04 was also used angled away from the direction of travel (backwards).

A separate group of *A. myosuroides* plants of the same growth stage to the study described above were treated with a spray solution of a non ionic surfactant, Activator 90 at 0.1% v/v, to investigate the quantification of retained spray solution when applied with different nozzles. BFS Bubble Jet 04 and TeeJet 110-04 were all tested when angled forward, with the Bubble Jet and TeeJet tested in the vertical position and the Bubble Jet tested when angled away from the direction of travel. To facilitate the quantification of retained spray solution, fluoroscein dye was dissolved into water with Activator 90 at 0.1% v/v. Immediately after spraying, 40 plants were cut at soil level into groups of ten plants and placed into polythene bags, washed and analysed using a Perken Elsem LS30 calibrated to the linear range of 0-10.0 µl.

Field studies

Treatments were applied on the 29th April 2010 trial using a trailed sprayer with a forward speed of 12 km/h. The nozzles used were a Spraying Systems TeeJet Flat Fan 110-04 at a vertical position and also a 30° forward facing angle to give an application volume of 130 litres/ha. Treatments were applied at a crop growth stage of GS30-31(beginning of stem elongation to first node) and an *A. myosuroides* growth stage of GS14-37 (four leaves to flag leaf just visible). Trial design used a single plot size of 6 x 12 m. With no pre-emergence

[2] Trademark of Spraying Systems
[3] Trademark of Billericay Farm Services

treatment applied to the trial area, the practice was outside the label recommendation for optimising grass weed control. However it provided an opportunity to study grass weed control and the influence of droplet trajectory in a field situation. Considerable effort has been devoted to identifying and categorising herbicide resistance of *A. myosuroides* to understand variation in field performance. A rating system (R to RRR) has been developed such that a R rating indicates early evidence of herbicide resistance, a RR is confirmed resistance that will probably reduce herbicide performance and RRR is confirmed resistance and highly likely to reduce herbicide performance (Moss *et al.,* 1999). Testing of the seed indicated that the *A. myosuroides* was a RR metabolic biotype.

The 2011 trial applications were applied (18th March) via a TeeJet 110-03 nozzle facing vertically or forward facing, at a forward speed of 10 km/h using a quad bike mounted sprayer. Trial design was a three replicate, randomised complete block design and a plot size of 3 x 12m. Growth stage of the winter wheat at application was GS23-25 (three to five tillers) and an *A. myosuroides* growth stage of GS13-21(three leaves to one tiller). The trial area received an application of 0.6 litres/ha Liberator[4] (400 g/litres flufenacet + 100 g/litres diflufenican, SC) on the 19th October at pre-emergence of the crop. Field history indicated that the *A. myosuroides* was a RR metabolic resistant biotype.

Table 1. Treatments used in pot and field studies

Treatment name	Active substances	Rates (Full, 0.5 & 0.25)
GF-2010	5.4 g ai/litre pyroxsulam + 314 g ai/litre pendimethalin	3.5, 1.75 & 0.875 litres/ha
GF-1364	70.8 g ai/kg pyroxsulam + 14.2 g ai/kg florasulam	265, 132.5 & 66.3 g/ha
Atlantis WG[4]	30 g ai/kg mesosulfuron-methyl + 6 g ai/kg iodosulfuron-methyl sodium	400, 200 & 100 g/ha
Biopower4	adjuvant	0.5% v/v
Respond[5]	adjuvant	0.25% v/v
Activator 90[6]	adjuvant	0.1% v/v

RESULTS

Pot study

The pot study was un-replicated so no statistical analysis was conducted. Mean % visual control values for the three dose rates (full, half and quarter label rates) for GF-1364 are presented in Table 2 below. On the small weed size (GS12-14) the TeeJet nozzle at vertical or forward facing achieved the best control. The coarser spray quality of the vertical Bubble Jet nozzle appeared the least efficacious. Angling of the nozzle and the direction of the angle indicated some differences in control with the rearward facing Bubble Jet achieving lower control than the forward facing nozzle.

[4] Trademark of Bayer Cropsciences Ltd
[5] Trademark of AmegA Sciences plc
[6] Trademark of De Sangosse Ltd

Table 2. Influence of nozzle type and angle on the effect of *A. myosuroides* control by GF-1364; mean of the three dose rates as % visual control

Treatment	% control
Bubble Jet vertical	86
Bubble Jet rearward	91
Bubble Jet forward	93
TeeJet vertical	94
TeeJet forward	95

Mean % visual control values for the 3 dose rates of GF-2010 are presented in Table 3 and indicate no difference in control

Table 3. Influence of nozzle type and angle on the effect of *A. myosuroides* control by GF-2010; mean of the three dose rates as % visual control

Treatment	% control
Bubble Jet vertical	94
TeeJet vertical	94
Bubble Jet rearward	95
TeeJet forward	95
Bubble Jet forward	96

The pot results, though not replicated suggest that angling nozzles forward may offer higher levels of efficacy for GF-1364. *A. myosuroides* control was lower when applied via a Bubble Jet vertical or Bubble Jet rearward facing nozzle. The differences were not evident with GF-2010 and this may be due to the residual activity of pendimethalin on the small *A. myosuroides* plants. The analysis of the amount of a spray solution of Activator 90 retained on *A. myosuroides* leaves following removal and quantification of the fluorometric marker are presented in Table 4. The study was un-replicated and no statistical analysis was conducted.

Table 4. Influence of nozzle type and angle on spray retention on *A. myosuroides* leaves

Nozzle	µl Spray retained
TeeJet vertical	15.7
TeeJet forward	16.1
Bubble Jet forward	18.1
Bubble Jet vertical	13.9
Bubble Jet rearward	11.2

The TeeJet nozzle was effective either angled forward or as a vertical whilst sprays from the Bubble Jet angled forward appeared more effectively retained than those angled backwards or vertically.

Field studies

The initial, non replicated study into the use of angling nozzles was conducted in 2010 with the winter wheat at growth stage GS30-31 (stem extension to first detectable node). With a dense crop canopy the influence or potential benefits of angling the nozzles were unclear. Results are shown in Table 5.

Table 5. Influence of nozzle angle on *A.myosuroides* control; % visual control, 2010.

Treatment	Nozzle type/direction	% control
Mesosulfuron-methyl + iodosulfuron-methyl + Biopower	TeeJet 110-04 Vertical	65
GF-2010 + Respond	TeeJet 110-04 Vertical	65
GF-1364 + Respond	TeeJet 110-04 Forward	45

The 2011 trial was a three replicate study where post-emergent treatments were applied following a pre-emergence application of flufenacet + diflufenican. *A. myosuroides* population was a RR resistant population and control levels were lower than expected. Winter wheat growth stage was GS23-25 (early to mid tillering), with ground cover being complete. Results are shown in Table 6. There were no significant differences between treatments.

Table 6. Influence of nozzle angle on *A.myosuroides* control; % visual control, 2011.

Treatment	Nozzle type/direction	% control
Mesosulfuron-methyl + iodosulfuron-methyl + Biopower	TeeJet 110-03 Vertical	65.0 ab
GF-2010 + Respond	TeeJet 110-03 Vertical	68.3 ab
GF-2010 + Respond	TeeJet 110-03 Forward	58.3 ab

(P=0.05, NSD)

CONCLUSIONS

The pot study indicated that both GF-1364 and GF-2010 were highly effective in controlling the susceptible biotype of *A. myosuroides*, with a maximum control of 96% achieved. Of the two products, GF-1364 indicated more sensitivity to nozzle type and direction of the spray trajectory. The most effective nozzle on these small target weeds was the TeeJet with the forward facing setting being slightly more efficacious than the vertical setting (95% vs 94%), though the results are not statistically different. On the small, upright growth habit of the *A. myosuroides*, control from the vertical or rearward facing BFS Bubble Jet nozzles was lower than the forward Bubble Jet facing nozzles. For the GF-2010 the effects were less evident with the vertical nozzle settings appearing less effective (94%) with the angled settings recording 95-96% control (no statistical difference). Though the Bubble Jet forward facing achieved the

highest control, the rear facing Bubble Jet was only 1% lower. It was postulated that the difference responses to the nozzles from the two products is due to GF-1364 being a contact only material and GF-2010 being a mixture of a contact material and the residual component (pendimethalin). By presenting spray solution closer to the plant stems and meristematic points with an angled nozzle may better utilise the pendimethalin activity. The spray droplet retention study presented in Table 4 showed higher leaf deposition from the forward angling of the TeeJet nozzle (not statistically different) than the vertical position. This supports the assumption from other studies that with forward angling of nozzles, greater amounts of spray deposits are achieved on small, vertical targets. The Bubble Jet used as a forward facing position achieved higher amounts of spray deposits though perhaps unsurprisingly as a vertical nozzle the leaf deposits decreased, supporting the view that coarse droplets are less appropriate for use on small, vertical targets. The use of a rear facing Bubble Jet showed a pronounced reduction in leaf deposition and this may be due to the disruption of the spray droplet curtain by the forward speed of the tractor.

In both field trials the perceived benefits of increased spray droplet loading or retention were not evident and treatments of GF-2010 applied with a TeeJet flat fan nozzle in a vertical position achieved 10-20% increased control compared to a forward facing nozzle setting. The results from the 2011 trial recorded a 10% reduction in control from the forward facing nozzle, compared to the vertical nozzle however this was not a statistically significant difference. Field trials were applied in the spring with ground cover or crop canopy very advanced and it is postulated that in situations such as this a vertical nozzle setting offers improved performance compared to an angling of the nozzles. The evidence suggests forward angling may be beneficial under autumn conditions when the crop is small and would not interfere with the movement and penetration of the crop, however under spring conditions when the crop canopy is more advanced and crop interception of spray solution is greater, the conventional vertical nozzle position may be optimal.

ACKNOWLEDGEMENTS

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GRAIN STORE HYGIENE

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Summary: Reldan 22 (225 g/litre chlorpyrifos-methyl, EC) marketed by Dow AgroSciences Ltd is approved for the control of a range of insect pests of stored grain and is approved as a fabric treatment (applications to walls or floors) of grain stores or as a treatment of cereal grain (ad-mixture) entering store. Data are presented on ad-mixture treatments against *Sitophilus granarius*, grain weevil; *Cryptolestes ferrugineus*, rust red grain beetle; *Oryzaephilus surinamensis*, saw toothed grain beetle and *Acarus siro,* flour mite compared with pirimiphos-methyl. Results showed >99% control of organophosphorus (OP) strains of these pests up to 90 days after application and clearly demonstrated the benefit of using chlorpyrifos-methyl as part of grain store hygiene management.

INTRODUCTION

Growers are rightly meticulous in the attention to detail they give their crops whilst in the field. Selection of an appropriate product and timeliness of applications for herbicides to minimise crop competition, as well as fungicidal applications to minimise disease effects and protect crop yield and grain quality criteria, are well established and accepted. As global demand for grain remains high and with it the corresponding effect on price, the penalties for a lack of grain store hygiene has never been greater. The potential rejection of a load or loads of grain due to insect infestations has dramatic financial consequences. Whilst the financial consequences are at their greatest, the product choice to effectively control insect pests in grain stores is limited. Chlorpyrifos-methyl formulated as a 225 g/litre EC controls all of the key insects *Sitophilus granarius*, grain weevil; *Cryptolestes ferrugineus*, rust red grain beetle; *Oryzaephilus surinamensis*, saw toothed grain beetle and *Acarus siro,* flour mite that infest stored cereal grains and grain stores, with approvals for use as a pre-harvest treatment on the fabric of the building or as an application to cereal grains going into storage. The Central Science Laboratory (CSL), were commissioned to validate studies conducted by Dow AgroSciences Ltd into the effectiveness of chlorpyrifos-methyl for the control of grain store insects (Dow AgroSciences unpublished internal report).

MATERIALS AND METHODS

Chlorpyrifos-methyl applied at 4.5 mg ai/kg was compared with the standard treatment, pirimiphos-methyl at 4 mg ai/kg.

Pesticide-free winter wheat was used which had been stored in a freezer for a minimum of 21 days to ensure any pests on the grain were killed.

The beetles used were laboratory OP susceptible strains of *O. surinamensis*, *S. granarius* and *C. ferrugineus*. All pests were laboratory reared at CSL.

Grain in 13 kg batches was treated with each of the respective test treatments, with three replicate batches for each treatment. Subsamples of grain were used for treatment with either the mites or the beetle species. Assessments were conducted at 1, 7, 42 and 90 days after treatment.

The mite species, *Acarus siro* (flour mite) was an OP susceptible strain, reared in a laboratory at the CSL.

RESULTS

Results for activity against the key pests of stored grain (*O. surinamensis*, *C. ferrugineus, S. granarius* and *A. siro)* are presented in the figures below.

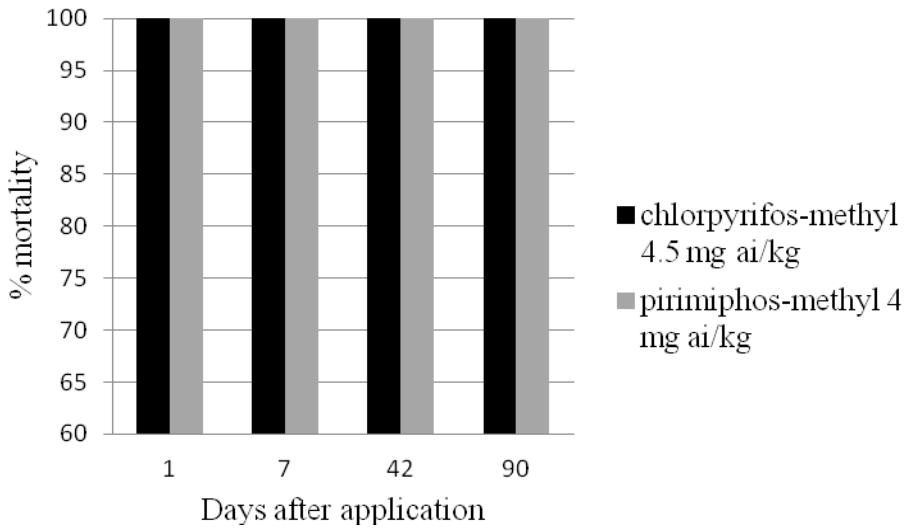

Figure 1. Percentage mortality of *O. surinamensis* (OP susceptible)

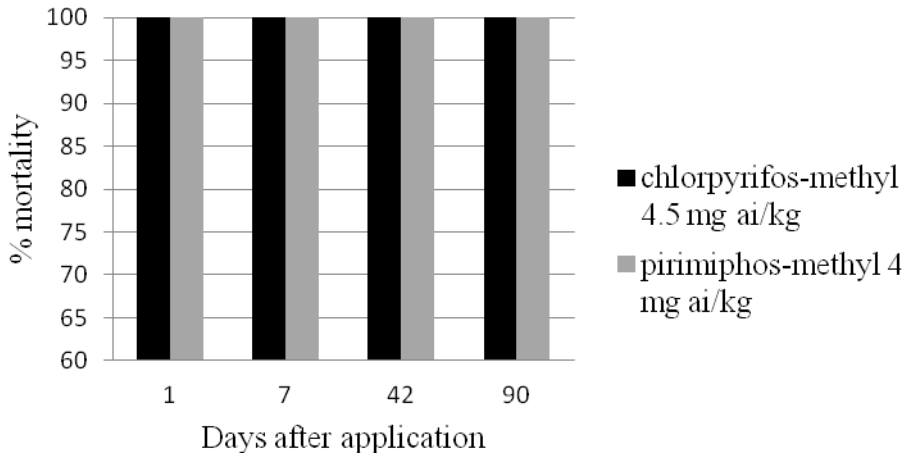

Figure 2. Percentage mortality of *C. ferrugineus* (OP susceptible)

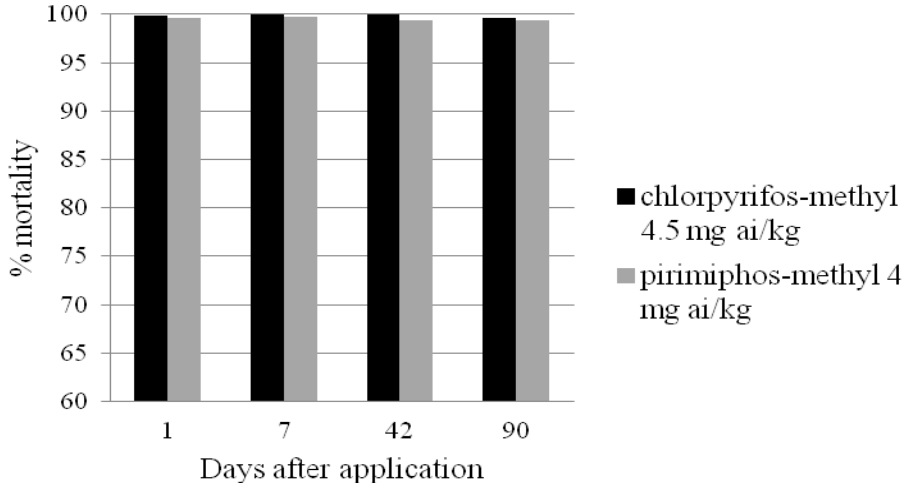

Figure 3. Percentage mortality of *A. siro* (OP susceptible)

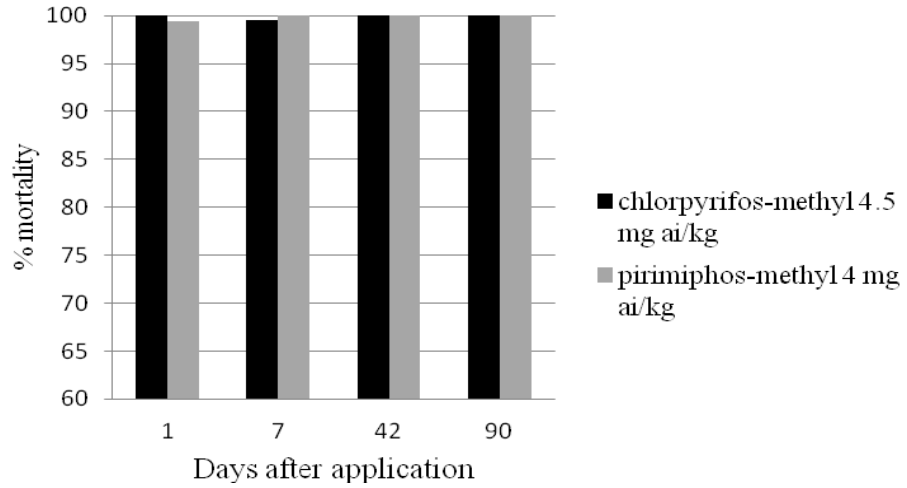

Figure 4. Percentage mortality of *S. granarius* (OP susceptible)

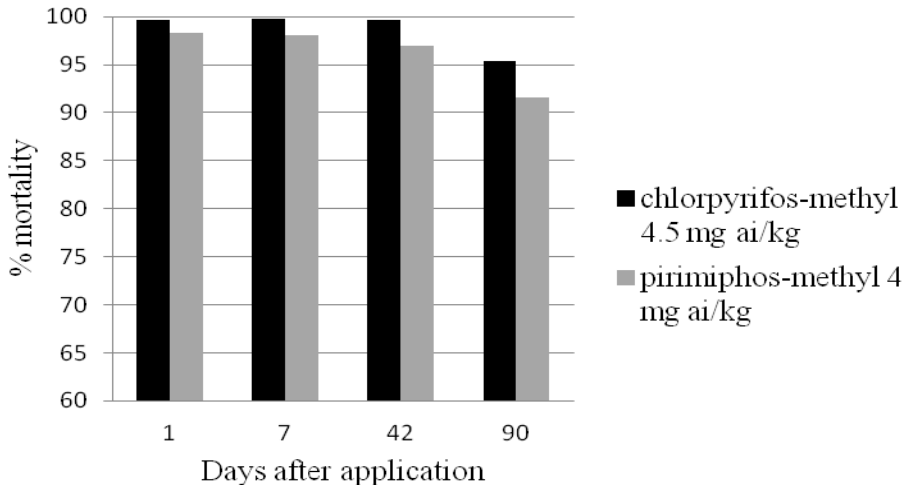

Figure 5. Percentage mortality of *S. granarius* (OP resistant)

DISCUSSION

Applications of either treatment to winter wheat grain achieved 100% mortality of both *O. surinamensis* and *C. ferrugineus* over the 90 day period. Against *A. siro* or OP susceptible *S. granarius*, equivalent control was observed between both treatments and control over the 90 day period exceeding 99% was observed. Against an OP resistant population of *S. granarius*, not unsurprisingly, both treatments were affected. Over the 90 day period the efficacy of pirimiphos-methyl was lower than that of chlorpyrifos-methyl at each assessment timing, with control at 90 days showing a statistically significant difference (P=0.05) in falling to 91.6% for pirimiphos-methyl compared with 95.4% for chlorpyrifos-methyl. Whilst in this test OP resistant strains were reasonably well controlled there are some strains of certain pests of stored grain that are resistant and treatments are unlikely to give satisfactory control.

With OP resistant populations of grain store pests identified it is prudent to take all reasonable actions to minimise insect pest infestations. Prior to harvest ensure the store is adequately cleaned and any grain removed from the store. Chlorpyrifos-methyl formulated as a 225 g/litre EC can be applied to the fabric of the grain store (at a rate equivalent to 200 mL in 5 litres of water per $100m^2$ of surface). This treatment should be applied one month prior to filling the store and will control pests for up to 6 months. Treatments to grain (ad-mixture) can be applied to cereals and are fully supported by both the British Beer and Pub Association (BBPA) and the National Association of British and Irish Millers (NABIM). Applications to oilseed rape or cereals intended for seed production are not supported.

CONCLUSION

As global demand for grain remains high and with it the corresponding effect on price, the penalties for a lack of grain store hygiene has never been greater. Chlorpyrifos-methyl, as either a fabric or an ad-mixture treatment, offers a cost effective solution to control the key insect pests of stored grain and so minimise the potential rejection of grain due to insect infestations and eliminate the associated financial consequences.

USE OF ARABINOXYLAN POLYMERS FOR PLANT DEFENCE

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Summary: The ability of a plant-derived arabinoxylan polymer to control foliar pathogens of barley (*Hordeum vulgare*) was studied in field experiments carried out in two consecutive seasons. The results obtained show a significant effect on disease level and progress, green leaf area and yield. The experimental data suggest an inducing effect of the polymer on plant resistance to pathogens. This discovery offers the potential for development of a novel, cost-effective and environmentally benign measure for crop disease control.

INTRODUCTION

Successful control of plant diseases is a key priority in agricultural research. Yield losses caused by pathogens not only cause economic damage but also threaten food security. Fungal pathogens in barley are responsible for up to 15% of total crop yield loss (Oerke & Dehne, 2004). Effective control of plant diseases, however, might require multiple fungicide applications, which often become ineffective due to development of resistance by pathogens (Walters *et al*., 2005).

Every plant has the potential to defend itself. Plant defences include expression of pathogenesis-related (PR) proteins, production and accumulation of phytoalexins, production of reactive oxygen species (ROS) and the oxidative burst, the hypersensitive response, fortification of cell walls and expression of enzyme inhibitors (Walters, 2010). Even plants that are genetically susceptible to certain pathogens can express enhanced resistance if their defence machinery is triggered by prior infection or the application of various agents. This phenomenon is known as induced resistance (Ton, 2006). Plant defence systems can be induced directly or "primed" for rapid response to a pathogen challenge. It seems likely that resistance induced by the application of resistance inducing agents, known as elicitors, will involve both direct induction of resistance and priming.

Research in SAC is exploring the effects of an arabinoxylan polymer, derived from maize cell walls, on disease control and fitness in spring barley under field conditions. Previous research showed that the polymer controlled a number of foliar pathogens under glasshouse conditions (Walters *et al*., 2008). The work presented here was carried out to assess the potential for the polymer to provide disease control in field-grown spring barley.

MATERIALS AND METHODS

Field trials were carried out in 2010 at Cauldshiel, East Lothian, and in 2011in Lanark, South Lanarkshire. The spring barley cultivar Optic was used, which has a resistance rating for Brown rust of 5 and for Rhynchosporium of 4 (HGCA, 2011). Seeds were sown at a rate of 360 seeds/m2 in randomised plots of 10m x 2m, with three replicates per treatment. Standard fertiliser and herbicide treatments were applied to the whole trial. The arabinoxylan polymer at a concentration of 0.08% was applied at a rate of 200 l/ha at GS24-25 and GS31-32. The standard fungicide programme for Cauldshiel in 2010 was: prothioconazole + fluoxystrobin (Fandango®) 1.0 l/ha +metrafenone (Flexity®) 0.25 l/ha at GS25 and prothioconazole (Proline^{275}) 0.36 l/ha + chlorothalonil (Bravo™ 500) 1.0 l/ha at GS49, while the standard fungicide programme for Lanark in 2011 was: bixafen + prothioconazole (Siltra$_{Xpro}$) 0.5 l/ha at the GS31 and prothioconazole 0.175 l/ha + chlorothalonil 0.5 l/ha at GS39. In 2011, additional treatment groups were: standard application of polymer in combination with reduced rate fungicide (prothioconazole (0.175 l/ha) + chlorothalonil 0.5 l/ha at GS39) and standard fungicide programme preceded by a single application of polymer at GS24.

The major disease severity assessment was carried out at GS73-74 assessing percentage infection of 10 plants per plot at random. The diseases assessed for were powdery mildew (*Blumeria graminis* f. sp. *hordei*), barley scald or leaf blotch (*Rhynchosporium secalis*) and Ramularia leaf spot (*Ramularia collo-cygni*). Green leaf area (GLA) was also assessed (expressed in percent of total leaf area). Plots were harvested when ripe and yield was measured and expressed as t/ha at 85% dry matter.

Statistical analysis of the experimental data was carried out using GenStat software version 11.1, and means from different treatment groups were compared using Least Significant Difference test (LSD).

RESULTS

2010

Mildew infection was significantly decreased in plants treated with the polymer, in comparison to the untreated control, in the field trial at Cauldshiel in 2010 (Fig.1). A greater level of disease control was provided by the standard fungicide treatment. These reductions in mildew levels were accompanied by increases in grain yield in both polymer and fungicide treatments (Fig. 1).

2011

In the 2011 trial at Lanark the polymer provided significant control of mildew and *Ramularia* when applied in combination with reduced rate fungicide (Figs 2A, 2B). Although the polymer treatment on its own had no significant effect on yield, large, significant yield increases were obtained with the polymer plus reduced rate fungicide, and the standard fungicide treatments (Fig. 2). Also, green leaf area was increased significantly in all treatments compared to the untreated control (Fig. 2D). In addition, single application of the polymer in combination with the standard fungicide programme provided the highest significant yield increase (data not shown).

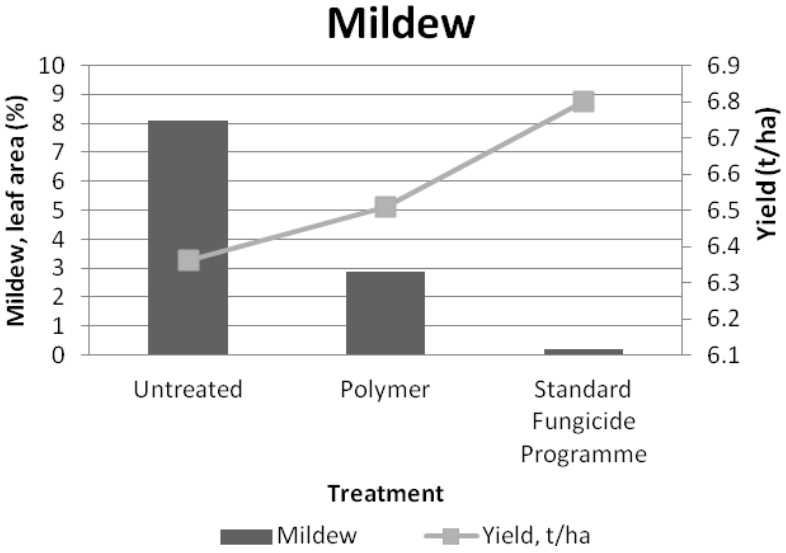
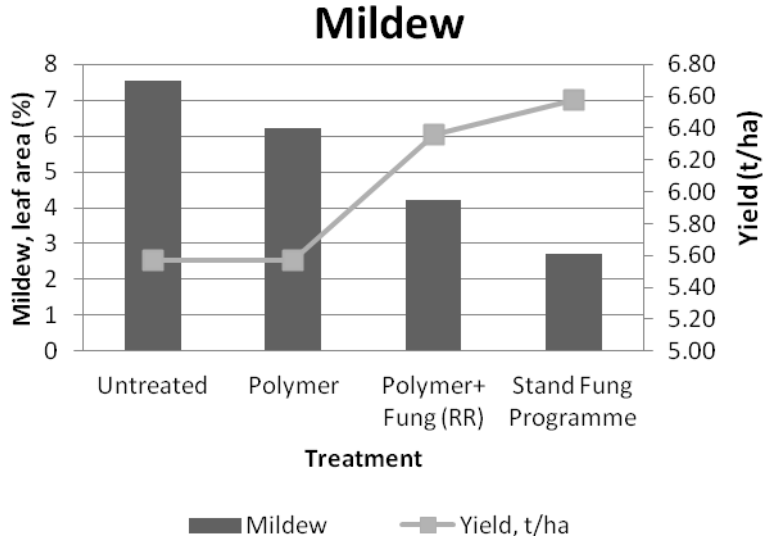
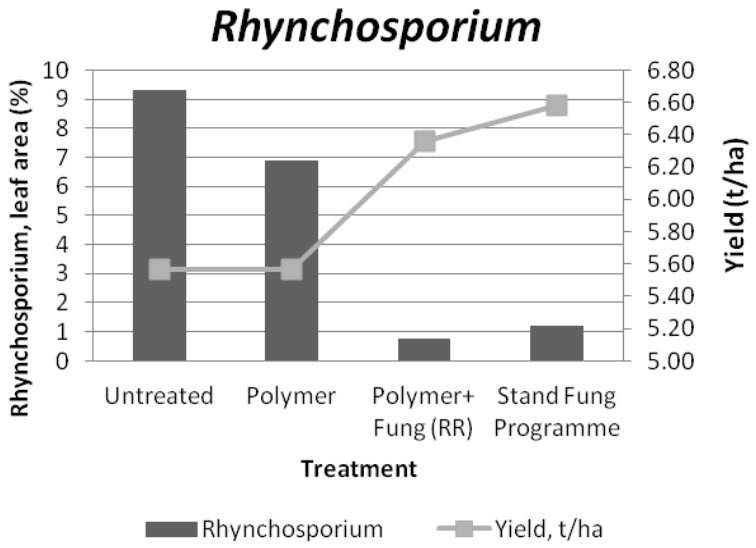


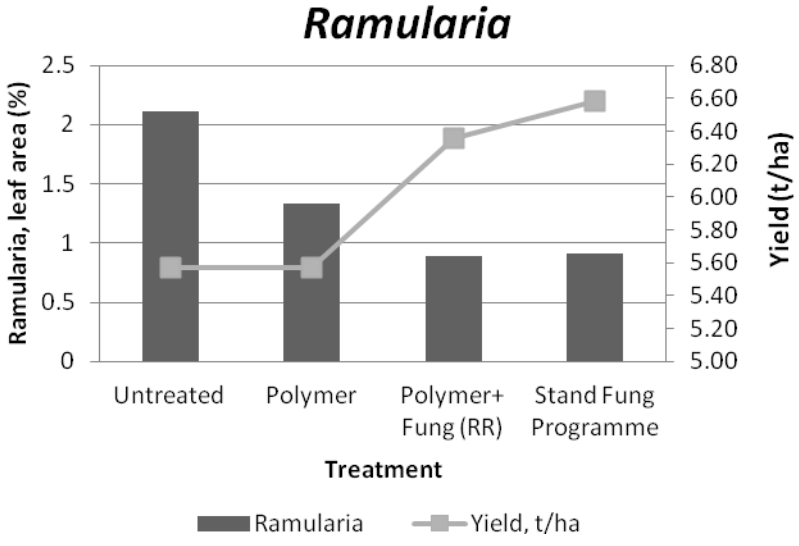
Figure 1. Effects of polymer and fungicide treatments on levels of powdery mildew and grain yield in the spring barley cultivar Optic at Cauldshiel, 2010. Differences between mildew levels are statistically different (LSD=1.674), while differences in yield are not statistically significant.



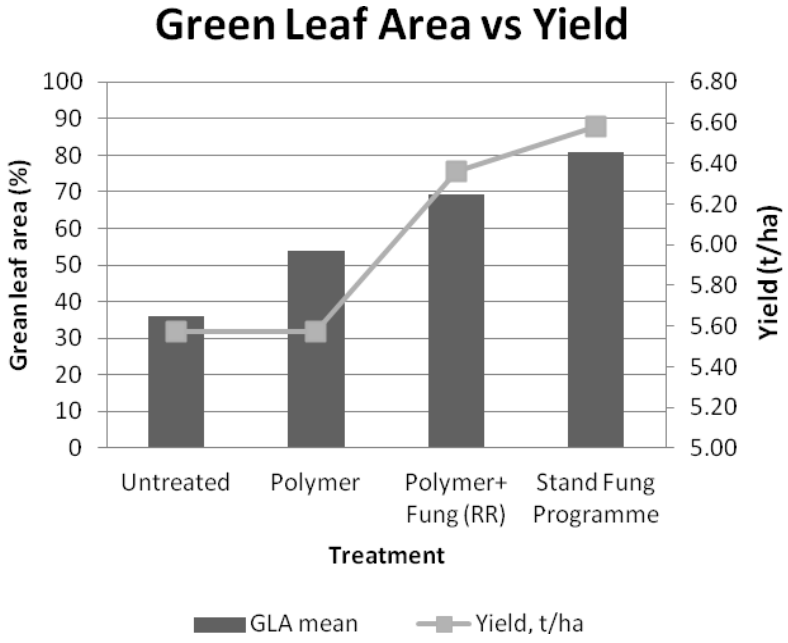
A


Rhynchosporium
Rhynchosporium, leaf area (%)
Yield (t/ha)
10
9
8
7
6
5
4
3
2
1
0
6.80
6.60
6.40
6.20
6.00
5.80
5.60
5.40
5.20
5.00
Untreated
Polymer
Polymer+ Fung (RR)
Stand Fung Programme
Treatment
Rhynchosporium
Yield, t/ha

B


Ramularia
Ramularia, leaf area (%)
Yield (t/ha)
2.5
2
1.5
1
0.5
0
6.80
6.60
6.40
6.20
6.00
5.80
5.60
5.40
5.20
5.00
Untreated
Polymer
Polymer+ Fung (RR)
Stand Fung Programme
Treatment
Ramularia
Yield, t/ha

C



D

Figure 2. Effects of polymer and fungicide treatments on disease levels, green leaf area and grain yield in the spring barley cultivar Optic at Lanark, 2011. Disease levels showed statistically significant decrease (A, LSD=2.056; B, LSD=6.467 and C, LSD=0.772) and yield and green leaf area were significantly increased (D, LSD for yield 0.530, for GLA 16.75) in plants treated with polymer and reduced rate fungicide (RR), and in plants under standard fungicide program, compared to the untreated control. Polymer on its own had significant reducing effect on *Ramularia* leaf spot (C).

DISCUSSION

The results obtained from experiments conducted over two subsequent seasons demonstrate significant disease control provided by the arabinoxylan polymer, as well as positive effects on GLA and yield. In particular, the polymer was most effective when used in combination with reduced rate fungicide treatment. Interestingly, combinations of resistance elicitors and reduced rate fungicide have been shown to provide effective disease control in spring barley under field conditions (Walters et al., 2010).

Because the polymer provides only partial coverage of the leaf surface, the effect on disease levels cannot be solely attributed to disguising the leaf surface or creating a physical barrier to penetration (Rätsep, unpublished results). Rather, it is possible that the polymer induces resistance in treated plants. This is currently under investigation using biochemical and molecular markers of induced resistance.

This work shows for the first time, that an arabinoxylan polymer from maize cell walls, applied with reduced rate fungicide, provides effective disease control on spring barley under field conditions. Such an approach could help to reduce the amount of fungicide applied to the crop, providing both cost and environmental benefits.

ACKNOWLEDGEMENTS

We would like to thank Grain Research and Development Corporation (GRDC) of Australia for funding this project.

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RHYNCHOSPORIUM COMMUNE CELL WALL PROTEINS AS POTENTIAL TARGETS FOR NOVEL FUNGICIDES

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Summary: *Rhynchosporium commune* is one of the most destructive pathogens of barley worldwide causing yield losses of up to 40%. The pathogen cell wall and especially cell wall proteins (CWPs) play a key role in the establishment of the pathogenesis. Some CWPs essential for the core biology of the pathogen during infection may represent potential targets for new environmentally friendly fungicides.

Rhynchosporium commune, formerly known as *R. secalis* (Zaffarano *et al.,* 2011), is one of the most destructive pathogens of barley worldwide. It can lead to yield losses of up to 40% and a decrease in grain quality (Shipton *et al.,* 1974). Populations of *R. commune* can change rapidly, defeating new barley resistance genes and fungicides after just a few seasons of their widespread commercial use (Newton *et al.,* 2001). New EU regulations may lead to loss of the most effective triazole fungicides, making *R. commune* control even more problematic.

In pathogenic fungi, the cell wall and especially cell wall proteins (CWPs) play a key role in the establishment of the pathogenesis. Cell walls form the outer structure protecting the fungus from the host defence mechanisms and initiating the direct contact with the host cells by adhering to their surface. Fungal cell wall also contains important antigens and other compounds modulating host immune responses (Ruiz-Herrera *et al*., 2006).

R. commune germinated conidia transcriptome sequencing generated a list of over 10 different CWPs potentially involved in pathogenicity. *R. commune* genome and interaction transcriptome sequencing provided further information about the extent of CWPs families as well as a subset of genes expressed during barley colonisation by *R. commune*.

One of the most revealing strategies to elucidate gene function is to remove its activity through mutagenesis, knockout, or gene silencing. *R. commune*, like most fungal plant pathogens, is haploid which allows the use of knockouts. Transcription profiling of *R. commune* CWPs during the development of infection helped to prioritise them for functional characterisation including their effect on pathogenicity through targeted gene knockout, complementation and biochemical studies.
Characterisation of the different types of *R. commune* CWPs will enable us to understand their role in cell wall integrity and pathogenicity. Information about composition, disposition and structure of constituent polysaccharides, glycoproteins and proteins is essential for complete

understanding of the host-pathogen interaction. Some CWPs essential for the core biology of the pathogen during infection may represent potential targets for new environmentally friendly fungicides.

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THE USE OF PLANT ELICITORS IN THE CONTROL OF BACTERIAL INFECTION IN FIELD VEGETABLES

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Summary: Plant defence elicitors are small molecules that trigger induced resistance pathways. While some are used as a standard treatment against bacterial and fungal pathogens (e.g. Probenazole), the underlying science to others is less well defined. A key question is whether elicitors can be used to control opportunistic plant bacterial pathogens that are prevalent in soil and so replace the only viable option in current use; copper oxychloride. Elicitors were tested against opportunistic plant pathogens of broccoli (*Pseudomonas fluorescens, P. marginalis, Pectobacterium carotovorum*) and onion (*Burkholderia gladioli* pv *allicola*) in field and glasshouse trials, respectively. Elicitors had a beneficial effect in reducing the likelihood of infection in onions. Although reproducible trends were evident from the broccoli trials, there were no clear significant differences in the incidence of bacterial disease.

INTRODUCTION

Bacterial disease of field vegetables incur significant financial burden on growers. For example, in the UK losses from broccoli head-rot can cost the industry over £15 million per year. Head-rot (spear rot) is the symptomatic disease caused by opportunistic plant pathogenic bacteria, mainly *Pseudomonas fluorescens, Pseudomonas marginalis* and *Pectobacterium carotovorum* (Harling and Sutton, 2002). Internal bacterial infections of onions account for approximately 2% of all marketable losses year on year (cut test results from 40-45,000 tonnes of crop, 2000-2010, Allium and Brassica Centre), although individual fields/stores have been recorded with levels of up to 40%. The main cause of bacterial rot in onions is *Burkholderia gladioli* pv. *alliicola* (*Bga*) (previously *Pseudomonas gladioli*) (Yabuuchi *et al.,* 1992). These are all soil-borne bacteria that infect plants in opportunistic manner and symptomatic disease only occurs when the environmental conditions are conducive.

Current treatment options for opportunistic bacterial pathogens are limited to microcidal chemicals, principally those containing copper oxychloride. Treatments are generally applied as a prophylactic, when the weather conditions are expected to lead to disease. However, continued use of copper is finite given its phytotoxicity and toxicity in the general environment. There are few other treatments that target the bacteria and some such as antibiotics are not permitted in the UK. Furthermore, the pseudomonads in particular are especially adaptable to sub-lethal levels of microcides and there are reports of resistance to copper oxychloride.

It is not feasible to eliminate these bacteria from the crop sites because of their ubiquity. However, plant defence response elicitors trigger induced resistance pathways and may present a viable alternative. A range of compounds can elicit defence pathways and historically elicitors are based on natural compounds, such as chitosan (Walters *et al.,* 2005). Others mimic plant hormones (*cis*-jasmone) and some have been well characterised in terms of which defensive pathway they activate. Probenazole is used as a standard treatment for rice blast in Asia (Watanabe *et al.,* 1977) and glasshouse studies have suggested that BABA and Bion can reduce head-rot symptoms in broccoli (Pajot and Silue, 2005). A group of licensed fungicides contain strobilurins, which have proven elicitor activity (Herms *et al.,* 2002); this class includes azoxystrobin (Amistar®) and pyraclostrobin (Flyer®).

The aim of the project was to test whether elicitors could limit bacterial infection and symptomatic disease in both broccoli and onions, in field and glasshouse trials, respectively.

MATERIALS AND METHODS

Experimental trials

Eight experimental field trials for broccoli were run over two years, at two sites in Scotland. 18 treatments were tested, in replicates of six, three of which were also treated with head-rot bacteria, at 10^6 cfu/ml (Trial 1) or 10^4 cfu/ml (Trials 2 - 4). Broccoli (vars. Marathon, Parthenon) were grown on a 100 m x 80 m site. Disease was assessed from 20 plants per plot. For the licensed fungicides-trial, disease was assessed from 60 plants per plot. Onions (var. Red Baron) were grown from seed for five months in compost, in glasshouses. Twenty replicate plants were assessed per treatment. Mechanically damaged onion leaves were infected with *B. gladioli*, at 10^6 or 10^4 cfu/ml.

Applications

Elicitors were applied either as the sole 'fungicide' for broccoli or incorporated into a standard fungicide programme for onion (Tables 1, 2). Three applications were made to broccoli at 10-day intervals. Four applications were made to onions at 18-day intervals, incorporated to fungicides treatments 1, 3, 5 and 7. Bacteria were applied to broccoli after the first and second elicitor applications and to onions after the treatment 3.

Laboratory validation and disease assessment

PCR amplification was used to detect *Pectobacterium* (Toth *et al.,* 1999), pseudomonads (Spasenovski *et al.,* 2009) or *B. gladioli* (Whitby *et al.,* 2000). The incidence of head-rot was scored as 'Healthy' or 'Diseased', and the extent assessed on 5-point scale of symptoms. The total *B. gladioli* load in onions was determined from microbial counts (Salles *et al.,* 2006). A Generalised Linear Model (glm) with binomial error and logit link was used to detect whether the probability of onion infection was influenced by treatment.

Table 1. Elicitors, fungicides and concentrations used in the trials.

Elicitors/Fungicides	Concentrations	Labels used in charts
BABA	1 mM	A
Bion	1 mM	B
cis-jasmone [a]	3.2 mM	C
Probenazole [a b]	0.2 mM (trial 1)	D
Probenazole [a b]	1.0 mM (trial 2)	E[c]
Yea foliar®	0.3 % (v/v)	E[c]
azoxystrobin (azoxy) (Amistar®)	1 L / ha	F
proquinazid (proq)(Justice®)	0.25 L / ha	
pyraclostrobin(pyrac) (Flyer ®)	1 L / ha	
cupric oxychloride (cup oxy)	5 kg / ha (broccoli)	
(Cuprokylt®)	2 kg / ha (onion)	
dimethomorph and mancozeb (dimeth + manc) (Invader®)		2 kg / ha
benthiavalicarb-isopropyl + mancozeb (benth + manc)(Valbon®)		1.6 kg / ha
cyprodinil and fludioxonil (cyp + flud) (Switch®)		1 kg / ha
metalaxy M and mancozeb (met + manc)(Fubol Gold®)		1.9 kg / ha
prothioconazole and fluoxastrobin (pro + fluox) (Unicur®)		1.25 L / ha

Activator-90 at 0.05 % (v/v) was added to all treatments.
a: addition of 0.01 % Tween 20
b: addition of 1 % (v/v) acetone
c: Probenazole 1.0 mM replaced Yea Foliar® in broccoli Trials 2 - 4.

Table 2. Standard Fungicide Programme for onion trial.

Application	Fungicide	Additions
1	dimeth +manc	azoxy/ pro + fluox / cup + oxy /Elicitors
2	benth+manc, Cypr +flud	cup oxy[a]
3	dimeth and manc	azoxy/ pro +fluox / cup oxy /Elicitors
4	met and manc, cyp + flud	
5	dimeth +manc	azoxy/ pro + fluox /cup oxy /Elicitors
6	benth + manc, Cyp + flud	cup oxy
7	dimeth + manc	azoxy/ pro + fluox /Elicitors
8	dimeth + manc	

[a] cup oxy was always present in combination with pro+fluox

RESULTS

Trials were set up to test the elicitors applied either singly or in multiple combinations on broccoli and the incidence and extent of bacteria head-rot assessed as the plants reached harvestable age. Although the level of disease was too low to determine statistically significant differences between the treatments, reproducible trends emerged (Fig. 1). Probenazole (labelled D) and azoxy (labelled F) and their combinations appeared to provide reproducible protection, in all the trials. In contrast the combination of BABA and Bion (AB) resulted in the highest incidence of disease in Trials 1 & 2, as well as the greatest extent of disease (data not shown). The combination of Bion and *cis*-jasmone (BC) resulted in the highest incidence of disease in Trials 3 & 4. The effect of licensed fungicides with reported elicitor function (azoxy,proq and pyr) together with Probenazole, were compared to cup oxy on a commercial site. The majority of treatments gave similar protection to cup oxy, with no significant differences (not shown). However, plots treated with proq had significantly higher numbers of diseased plants; in addition, the extent of head-rot was higher across the diseased plants. Increasing the concentration of bacterial inoculum 100-fold (to 10^6 cfu/ml) did not increase the level of disease. However, head-rot was prevalent during the fungicide trial on a commercial site, where no bacterial inoculum was added, and both diseased and healthy tissue from the trial was found to contain all three species of head-rot bacteria, as assessed by PCR. Infection of broccoli florets under laboratory conditions showed that *Pseudomonas fluorescens* was responsible for the greatest extent of disease in comparison to *Ps. marginalis* and *Pectobacterium carotovora*, although all three species were capable of causing typical head-rot symptoms on broccoli heads (not shown).

Treatment of onion plants with combinations of elicitors (Table 1) resulted in a significant reduction in the level of *B. gladioli* compared to the fungicide programmes with azoxy, pro + fluox or cup oxy(Fig 2). Furthermore, the beneficial effect was seen even at extremely low levels of bacterial inoculum (10^4 cfu/ml). Soft-rot was only visibly evident at much higher levels of incoulum (10^8 cu/ml) injected directly into the bulb tissue.

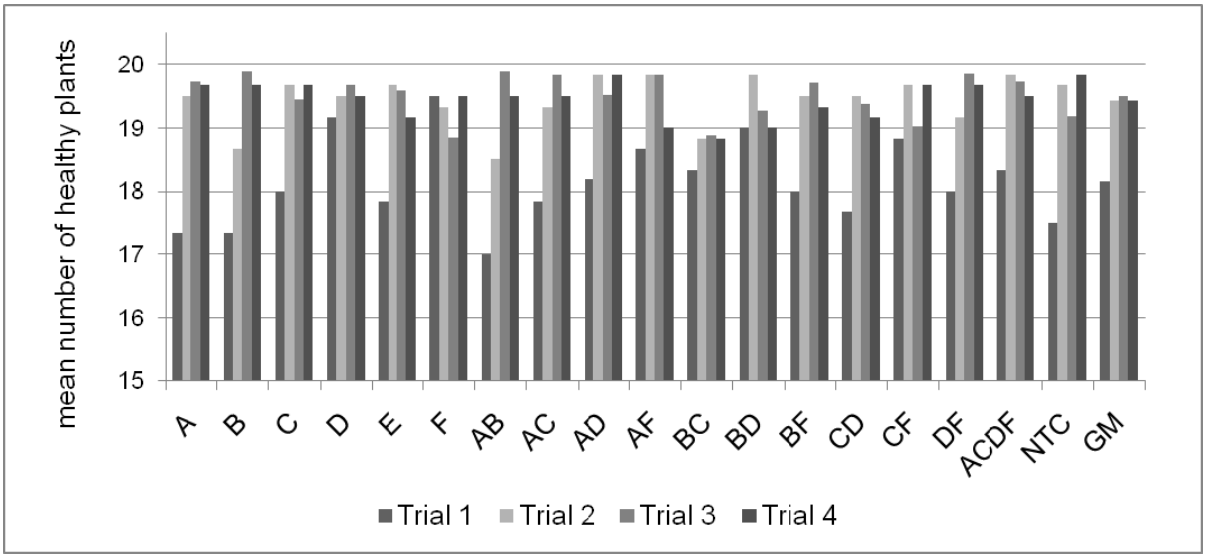

Figure 1. Average number of healthy broccoli plants (without head-rot symptoms) All four trials are clustered for each of the 17 treatments. Values are also provided for the no treatment control (NTC) and grand mean (GM).

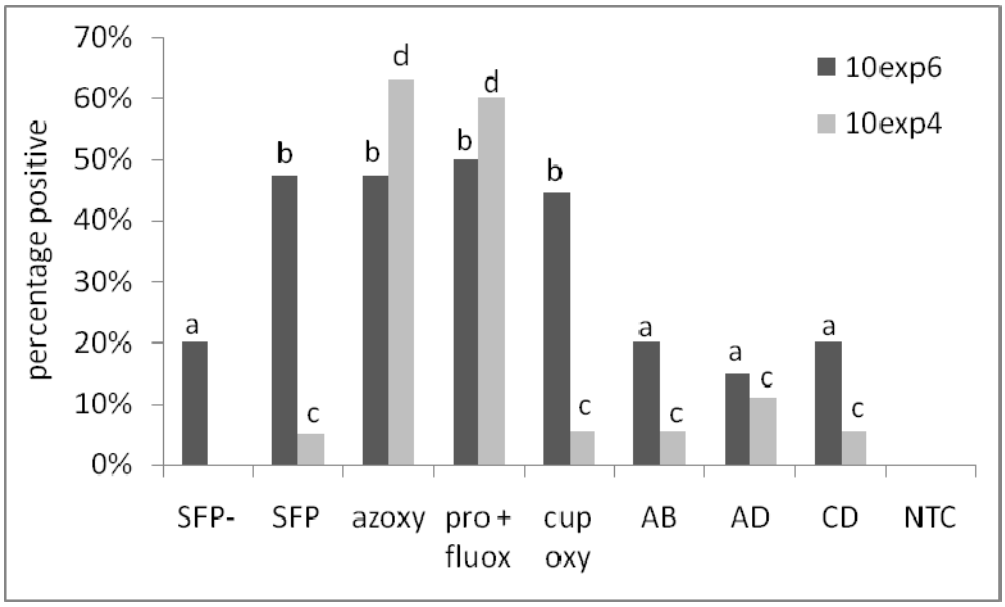

Figure 2. Presence / absence chart of treated onion samples infected with *Bga*. The percentage of infected samples is shown for both dosages. Significant differences between the treatments for each doasge are indicated by different letters. The treatments are as detailed in Table 1: SFP- refers to SFP with no bacteria; cup oxy includes pro + fluox; NTC refers to the no-treatment control.

DISCUSSION

Elicitors were tested on two different horticultural crops to determine whether they had an effect on symptomatic disease caused by opportunistic plant pathogenic bacteria. Broccoli trials were run over two years to test experimental elicitors and an additional trial was set up on a commercial site to compare fungicides, some of which have reported elicitor activity. Reproducible trends emerged from the experimental trial, although it was not possible to draw any statistically significant conclusions. In general there was a low level of disease, despite addition of a head-rot cocktail of bacteria that were shown to cause soft rot under laboratory conditions. This suggests that environmental effects strongly influence the likelihood of symptomatic disease. In the commercial trial, the elicitors resulted in similar protection against disease as cup oxy, which was significantly higher than that seen with proq. It is interesting that some treatments resulted in an increase in the disease incidence (and in some cases the extent) in broccoli, including some elicitor combinations and fungicides. It is possible that these treatments altered the native microflora associated with the plants sufficiently to allow an increase in head-rot bacteria proliferation and subsequent disease.

In the onion trials, incorporation of the elicitors into the standard fungicide programme resulted in a significant decrease in the likelihood of *Bga* infection compared to programmes that included azoxy, pro + fluox or cup oxy. The average level of bacteria in the elicitor-treated onions was marginally lower than the standard fungicide programme, although not significantly. It is possible that an alternative programme, with reduced input from standard fungicides may result in a larger difference. As far as we are aware, this is the first report of the use of plant defence elicitors in control of *Bga* on onions.

Evidence for a beneficial effect of elicitors against head-rot on broccoli has been demonstrated for BABA and Bion (Pajot and Silue, 2005). It is possible that we were unable to repeat this effect because the plants were grown outdoors, rather than under more defined conditions in a glasshouse. This may also explain, in part, why the elicitors proved effective against *Bga* in the onion trial, also run in a glasshouse. In order to be able to apply elicitors as a feasible alternative to current treatment options, further research is needed to determine the optimal timing and method of delivery, as well as provide a fundamental understanding of the pathways they trigger in the context of different plant species and the resulting effects on different pathogens.

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DEVELOPING NOVEL METHODS TO INVESTIGATE THE RELATIONSHIP BETWEEN EYESPOT DISEASE AND YIELD LOSS

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Summary: Eyespot is an important stem base disease of cereals caused by the fungi *Oculimacula acuformis* and *O. yallundae*. In this study, novel methods were used to investigate the relationship between eyespot disease and yield in winter wheat. Different fungicide treatments were applied at GS 32 or GS 39 of the crop. *Oculimacula* DNA was extracted from soil before fungicide application and at GS 51 and quantified using a Real-time PCR assay. Chlorophyll fluorescence induction kinetics were used to calculate the performance index of plants as an estimate of photosynthetic efficiency following treatment for disease control. The predominant pathogen causing eyespot disease in the crop was *O. yallundae*. There was a significant but weak relationship between visually assessed disease score at GS 73 and yield. Boscalid application at GS 32 significantly increased performance index of treated plants at GS 69 and reduced disease severity, fungal biomass of *O. acuformis* in soil and DNA of *O. yallundae* in wheat stems.

INTRODUCTION

Eyespot is a damaging stem base disease of cereals caused by the closely related fungi *Oculimacula acuformis* and *O. yallundae*. Most published studies investigate eyespot in wheat (*Triticum aestivum*), the host which is acknowledged to be most agriculturally and economically significant.

Symptoms of eyespot disease first develop as brown-bordered, eye-shaped lesions at the base of the stem. Clarkson (1981) considered slight lesions caused by the two pathogens less yield damaging than moderate and severe lesions which, when occurring at a high incidence, can cause yield loss by weakening the stem and blocking nutrient flow (Fitt *et al.*, 1988; Ray *et al*., 2006). Past studies have been unable to identify a strong relationship between visually assessed disease at early growth stages and disease symptoms or yield loss at harvest particularly when disease was caused by *O. acuformis* (Goulds & Polley, 1990; Nicholson *et al*., 1997; Burnett & Hughes, 2004).

Oculimacula species are known to survive and proliferate on infected host stubble remaining in soil from previous crops (Fitt *et al*., 1988). There is however limited knowledge of the presence or the viability of *Oculimacula* spp. in soil. Previous work has shown that *Oculimacula* species are less competitive than other wheat pathogens such as those causing take-all (*Gaeumannomyces graminis*) and root rot (*Helminthosporum sativum*, *Fusarium culmorum*) and so are unlikely to be able to survive in soil as free-living saprophytes (Macer,

1961). One study showed that the fungi were able to survive for 36 weeks on agar plugs in soil (Deacon, 1973). However it is unclear whether these conclusions are valid in relation to natural populations in field conditions.

When plants are subjected to external stressors, such as disease, absorbed light energy may be in excess of that required for CO_2 assimilation, thus photoprotective responses occur to negate damage to the reaction centres of photosystem II (PSII), considered the most vulnerable component of the photosynthetic apparatus in plants (Murchie and Niyogi, 2011). One such response involves quenching excess light energy by emission back into the environment as fluorescence. Measuring the fluorescence signal emitted by plant chlorophylls can provide useful information of the physiological status of the plant and the efficiency of photochemical processes (Chaerle *et al*., 2007). Whilst fluorescence has been used in the past to diagnose changes in PSII efficiency due to infection by biotrophs, for example *Blumeria graminis* (Swarbrick *et al*., 2006), it has not been tested to detect changes in response to infection by necrotrophic fungi such as *Oculimacula* spp. in the field or indeed to detect changes due to application of fungicide treatment for the control of disease.

The overall aim of this study was to determine the effect of eyespot disease and *Oculimacula* spp., on the yield of naturally infected winter wheat using novel techniques examining the efficiency of PSII and to determine the yield response to fungicide treatment. Individual objectives were: i) to investigate the relationship between plant disease and pathogen DNA in soil and *in planta*; ii) to elucidate the effect of fungicide application for eyespot control on wheat photochemistry using fluorescence measurements.

MATERIALS AND METHODS

Field experimental conditions

Experiments on winter wheat cv. Gallant, naturally infected by *Oculimacula* spp., were carried out at the University of Nottingham, Sutton Bonington Campus, UK in the 2010-2011 cropping season. Plots (2 x 12 m) were established as a second wheat following winter wheat cv. Oakley. Fungicide treatments were applied at GS 32 or GS 39 with the aim of establishing a range of disease levels and to evaluate the effect of timing of fungicide efficacy. The experimental design was factorial with two factors, fungicide application at GS 32 or at GS 39. The treatments included untreated control, Opus applied at 0.75 l ha^{-1} (epoxiconazole 93.75 g l^{-1}, BASF plc) or Opus 0.25 l ha^{-1} + Tracker 1 l ha^{-1} (epoxiconazole 98.25 g l^{-1}+ boscalid 233 g l^{-1}, BASF plc). Each treatment combination was replicated four times in randomised blocks.

One over-spray of Amistar Opti (100 g l^{-1} azoxystrobin + 500 g l^{-1} chlorothalonil; Syngenta UK Ltd) was applied prior to GS 32 with the aim of controlling foliar pathogens. Other agronomy was as per standard farming practice.

Soil sampling

At GS 30, before fungicide application, soil was sampled over the trial site in a ‘w’ shaped sampling path with approximately 30 g soil taken at ten sampling points. Soil samples were allowed to dry at room temperature for 48 h in open bags and fragments of crop debris larger than 0.5 cm were removed by sieving. Soil (250 g) was used for DNA extraction. Soil was

homogenised in Nalgene bottles containing ball bearings and cetyl-trimethyl ammonium bromide (CTAB) buffer and was further processed using a method developed at FERA (Sand Hutton, York, UK). DNA was eluted with a Kingfisher® mL (Thermo Electron) using MagneSil® paramagnetic particles (Promega UK Ltd.) and pathogen DNA quantification carried out per gram of soil. At GS 51 individual plots were sampled in the same manner.

Fluorescence measurements

Fluorescence was measured at GS 69 using the FluorPen FP 100-MAX (Photon Systems Instruments, Brno, Czech Republic). Twenty plants per plot were randomly selected and chlorophyll fluorescence induction kinetics measurements, including performance index measurements, were carried out on the flag leaf as per manufacturer's instructions. Performance index (Pi_Abs) combines three parameters:- density of PSII reaction centres, ability to feed electrons between PSII and PSI and trapping rate of PSII reaction centres, thus providing an overall estimate of crop photosynthetic efficiency.

Disease assessments and DNA quantification

At GS 87, whole plants were collected and secondary tillers discarded. Disease assessment was made on the main shoot using the ordinal rating scale of eyespot lesion severity, where 1 was slight, 2 was moderate and 3 was severe; according to the amount of stem girdling and softening as defined by Goulds & Polley (1990). Main shoots were then cut to 5 cm long stem bases, grouped according to their disease scores (slight, moderate or severe) and processed for DNA extraction using the method described by Ray *et al*. (2004). Total DNA in the extracts was measured using the Nanodrop 1000 (Thermo Fisher Scientific Inc., USA). Pathogen DNA was quantified using TaqMan probe quantitative PCR assay using iQ supermix (Bio-Rad Laboratories Inc., UK). PCR conditions and primers were used as described by Walsh *et al*. (2005).

Statistical analyses

All data were analysed using analysis of variance (ANOVA) or regression analysis using GenStat® Version 11 for Windows (VSN International Ltd., UK). DNA data were transformed using log10 transformation where necessary to normalise residuals before proceeding with ANOVA.

RESULTS

At GS 30, DNA of *O. acuformis* and *O. yallundae* quantified in soil was 0.144 pg $g^{-1}$and 6.012 pg g^{-1}, respectively.

At GS 51, DNA of *O. acuformis* in soil was 86% lower in plots treated with epoxiconazole + boscalid at GS 32 than in untreated plots (Table 1). There was no significant effect of fungicide application on DNA of *O. yallundae* in soil (Table 1). Less DNA of both *O. yallundae* and *O. acuformis* was quantified in stems treated with epoxiconazole + boscalid at GS 32, however reductions were only significantly different from the untreated control for DNA of *O. yallundae* (Table 1).

Table 1. Log10 transformed pathogen DNA quantified in soil and in stems, at GS 51 and at GS 87 respectively, and yield of cv. Gallant. Back-transformed means are shown in parentheses. OA = *O. acuformis*, OY = *O. yallundae*.

	Log10 Pathogen DNA				
	Soil (pg g^{-1})		Stems (pg g^{-1})		Yield
Treatment a.i. (g a.i. ha^{-1})	OA	OY	OA	OY	t ha^{-1}
untreated	-0.54 (0.29)	-0.03 (0.93)	4.43 (27.04)	5.08 (120.23)	12.20
epoxiconazole (93.75)	-1.18 (0.07)	-0.40 (0.40)	4.54 (34.51)	4.95 (88.51)	12.61
epoxiconazole (98.25) + boscalid (233)	-1.32 (0.05)	-0.26 (0.55)	4.13 (13.37)	4.83 (67.45)	12.92
P	0.05	0.50	0.117	0.032	0.31
LSD	0.661	0.658	0.4088	0.1822	0.969
CV	11.8	13.6	9.4	3.7	7.7

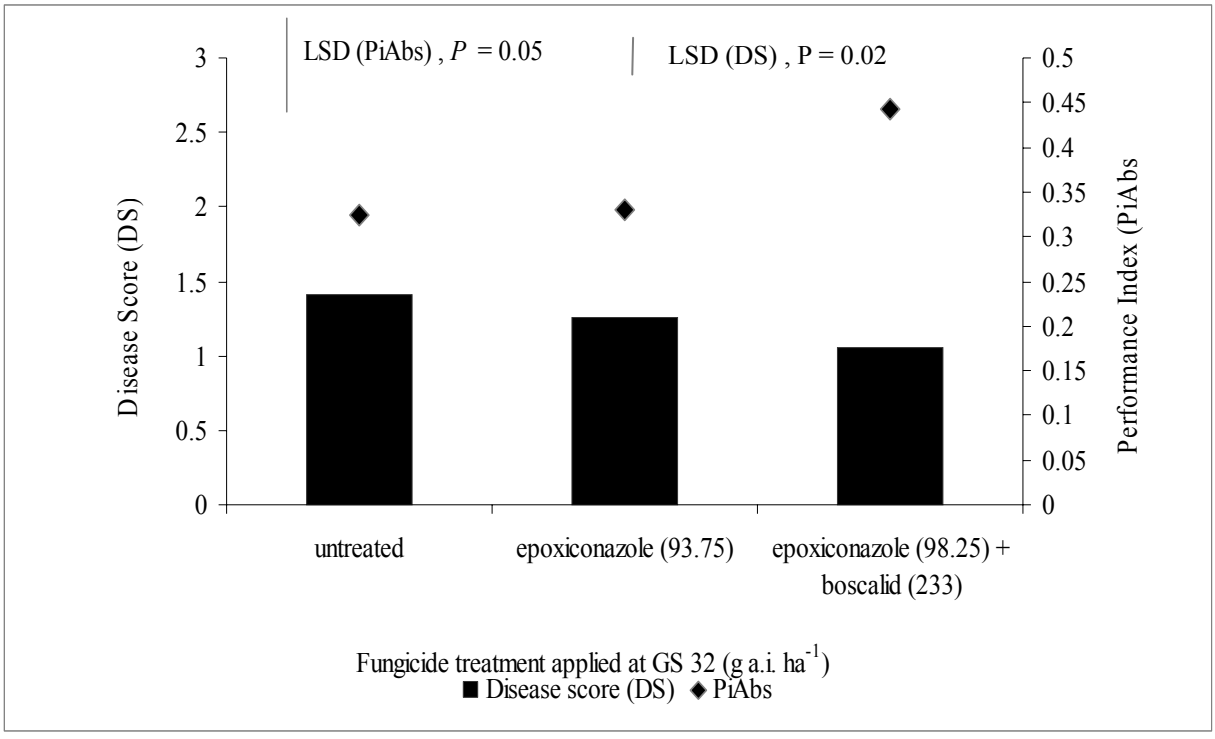

Figure 1. Disease score at GS 87 and performance index at GS 69 for winter wheat, cv. Gallant treated with different fungicides at GS32.

ANOVA revealed that there were no significant interactions between timing of fungicide application for any of the collected data. Fungicide application at GS 39 failed to reduce disease score or raise the performance index (data not shown). Fungicide application was most effective in reducing disease severity and increasing performance index at GS 32 (Figure 1). Application of epoxiconazole + boscalid at GS 32 reduced disease score by 25% and increased performance index by 27% compared to untreated (Figure 1). There were no significant differences between untreated and epoxiconazole only treatment for disease score or performance index.

There were no significant relationships between DNA quantity for individual *Oculimacula* spp. in soil at GS 51 and in stem at GS 87 (results not shown). However regression analysis showed a weak but significant relationship accounting for 14% of the variance between total *Oculimacula* DNA quantified in stems and visual disease score (Y= 0.65 (log10 total stem DNA) + 2.06, P = 0.029.that stem DNA of *Oculimacula* spp. relates to visually assessed disease score at GS 73. Log10 total stem DNA and DS

Regression analysis also revealed a significant relationship between DS at GS 87 and yield, accounting for 24% of the variance (Y= 1.82 (DS at GS 87) + 14.8, P=0.005).

DISCUSSION

This study has shown that *Oculimacula* spp. are present in quantifiable amounts in soil where second wheat is grown. Furthermore, the application of boscalid + epoxiconazole, resulted in significant reduction of fungal biomass of *O. acuformis* in soil, potentially decreasing available inoculum quantity for eyespot infection following GS 32. No such effect was observed for DNA of *O. yallundae* and further studies are needed to confirm the present results and elucidate the effects of specific fungicide treatments on soil inhabiting fungi such as *Oculimacula* species or the relationship between *Oculimacula* persistence in soil and eyespot disease development. *Oculimacula acuformis* was present at lower DNA concentrations than *O. yallundae* in soil and in stems, thus the predominant pathogen causing eyespot disease in this study was *O. yallundae*.

Regression analysis found that there were no significant relationships between DNA levels in soil at GS 51 and in stems at GS 87 indicating that fungal quantity in soil is not a reliable indicator for pathogen biomass on plant stems in this study. There were no significant interactions for timing of fungicide application and fungicide application was most effective at GS 32. Application of boscalid + epoxiconazole reduced disease severity by 25%, DNA of *O. yallundae* in stems by 5% and DNA of *O. acuformis* in stems by 7% when compared to the untreated. Although there were no significant differences in final yield between treatments, boscalid + epoxiconazole treated plots yielded more than untreated or epoxiconazole only treated plots. Application of boscalid + epoxiconazole also increased the performance index of plants significantly compared to the untreated control. The observed increase in performance index in this case may be due to direct effects of the fungicides on PSII photochemistry, or due to indirect effects via reductions of disease and/or fungal biomass in stems.

Further work will be undertaken to investigate the effect of different soil and stem pathogen quantities on disease development, yield and plant physiological responses. Novel methods are also under development for improving detection and quantification of *Oculimacula* spp. and for investigating the viability of the pathogen inoculum and its role in disease epidemiology.

ACKNOWLEDGEMENTS

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THE EFFECT OF *MICRODOCHIUM NIVALE* AND *M. MAJUS* ON THE ESTABLISHMENT OF SPRING BARLEY AND OATS: EVIDENCE OF HOST PREFERENCE

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Summary: Spring barley and oats with a range of infection levels of *Microdochium* seedling blight *(M. nivale* and/or *M. majus*), were sown on three dates during March and April 2011. There was no evidence of a correlation between barley seed infection level and seedling loss at emergence, but there was a significant correlation for oat seed ($R^2 = 0.75$). The lack of seedling loss in barley may have been due to the period of warm soil temperatures and low rainfall directly after sowing. An experiment to look at which *Microdochium* species (*M. nivale* and/or *M. majus*) were present on Scottish crops has shown that spring oat seed is almost exclusively populated by *M. nivale* whereas spring barley seed has more *M. majus* infection. There was no evidence of pathogen differences between areas in which seed was grown.

INTRODUCTION

In the UK, seedling blight, caused by *Microdochium nivale* (Fr.) Samuels & I.C. Hallett, reduces seedling emergence in winter wheat and winter oats when untreated seed is sown (Cockerell *et al*., 2009). Evidence suggests that there is a high risk for spring wheat when seed has an infection level of 30% and above but less is known about spring barley and oat seed. Cockerell *et al.* (2009) showed that although spring barley infection with *M. nivale* or *M. majus* (herewith referred to as *Microdochium*) was not usually associated with reduced crop emergence, it could occur with untreated spring barley at infections of 58% if field conditions are favourable to the disease, i.e., a cold, wet seed-bed at germination. For spring oats, pot experiments showed no losses at infection levels up to 28%. Previously, Humphreys *et al.* (1998) have found that *Microdochium* infection levels of between 5 to 61% significantly correlated with plant establishment in winter-sown oats. They also saw links with infection level and plant population density and grain yield. Haigh *et al.* (2009) showed that seedling blight symptoms developed on plants grown at 3°C but not on plants grown at 22°C. Also, those plants affected by seedling blight went on to show lower vigour indicating that *M. nivale* and *M. majus* are affecting subsequent plant growth, not just seedling emergence.

Simpson *et al.* (2000) and then Glynn *et al.* (2005) confirmed that *M. nivale* and *M. majus* are distinct species using phylogenetic evidence and biological differences. Prior to this, Lees *et al.* (1995) revealed polymorphisms among isolates which indicated the presence of a sub-group and asked the question of whether there may be differences in host, epidemiology or the geographical distribution of these two pathogenic sub-groups. Simpson *et al.* (2000) noted that there were conclusive differences in the ability of *M. nivale* and *M. majus* to grow on different

cereal hosts, and that at a temperature of 10°C only *M. nivale* caused significant disease of oats. However, in a mixed inoculation trial they found that *M. majus* showed a selective advantage on wheat and oats and *M. nivale* showed a selective advantage on rye.

This is the first year of a study undertaken to determine at what seed infection level *Microdochium* will cause a problem with emergence and seedling loss in spring barley and oats. This study will also look at the differences in distribution of *M. nivale* and *M. majus* on spring sown barley and oats and the subsequent seedling loss.

MATERIALS AND METHODS

Seed lots

Untreated spring barley and oat seed lots with a range of *Microdochium* infection levels were selected from samples submitted to the Official Seed Testing Station (OSTS), SASA for testing. Samples originated from various areas of Scotland. Details of seed lots are given in Table 1.

Table 1. Seed lot, variety and percentage *M. nivale* and *M. majus* from 2010/11

Seed lot	*Type*	*Variety*	*Area*	*% germination*	*% infection*
1	S. Barley	Waggon	Caithness	92	91
2	S. Barley	Westminster	Moray	96	49
3	S. Barley	Concerto	Aberdeen	98	6.5
4	S. Barley	Optic	Aberdeen	96	17
5	S. Barley	Belgravia	Perth	94	10
6	S. Barley	Waggon	Fife	95	40
7	S. Barley	Waggon	Aberdeen	84	41
8	S. Barley	Concerto	Forfar	98	4
9	S. Oats	Atego	Lanark	85	23.5
10	S. Oats	Firth	Perth	90	12
11	S. Oats	Firth	Fife	97	5.5
12	S. Oats	Firth	Banff	81	42.5
13	S. Oats	Firth	Perth	95	2

Agar plate tests

Agar plate tests to ascertain the level of *Microdochium* on the seed lots were conducted in accordance with the International Seed testing Association (ISTA) method 7-022.

Germination test

Germination tests were conducted in accordance with ISTA Rules 2011, using the rolled-paper towel method.

Field experiment

A field experiment was conducted using all the seed lots. Ten metre square plots were sown, with a target rate of 400 seeds/m^2. A randomised block design was selected for the trial, with four blocks sown over three dates. Sowing dates were 2 weeks apart. Seed lots were sown both untreated, and treated with fludioxonil + tefluthrin (Austral® Plus) at the manufacturers recommended rate. Only oats were sown at the 3rd sowing date. Percentage seedling loss due to sowing untreated seed with *Microdochium* infection was calculated as a percentage of treated seed plant populations. The first sowing was made on the 23rd March 2011.

Colony identification of *M. nivale* or *M. majus*

Colonies of *M. nivale* or *M. majus* were identified following a simple DNA extraction in TrisEDTA buffer. They were tested by polymerase chain reaction (PCR) to discriminate between these two species. The primers used were described by Glynn *et al.* (2005), EFMaj-F/EFMic-R for *M. majus* and EFniv-F/EF-Mic-R for *M. nivale* identification. Results were viewed after electrophoresis on 1% agarose gel stained with Gel-Red (Biotium) at 0.01%.

RESULTS

Field experiment

For the spring barley, no differences were observed between sowing one and two and no significant correlation found between increased seed infection and seedling loss S1 $R^2 = 0.037$, S2 $R^2 = 0.010$.

For the spring oats, there was a significant increase in seedling loss with increasing seed infection for sowings two and three ($R^2 = 0.753$ and $R^2 = 0.751$ respectively), the greatest differences are observed with the seed lots having 23.5% and 42.5% infection levels. There was also a good correlation with increasing infection level and the percentage germination (Figure 1). Differences can be observed between sowing dates for the oats with sowing three having the least seedling loss.

Microdochium species identification

The identification of *Microdochium* species from the colonies isolated from agar plates showed that there is a greater proportion of *M. majus* on barley, except seed lot 8 which has equal infection of *M. majus* and *M. nivale* (Figure 3). However, for this seed lot it represents only four colonies in total. On oats, *M. nivale* was predominant with only a small percentage of *M. majus* occurring.

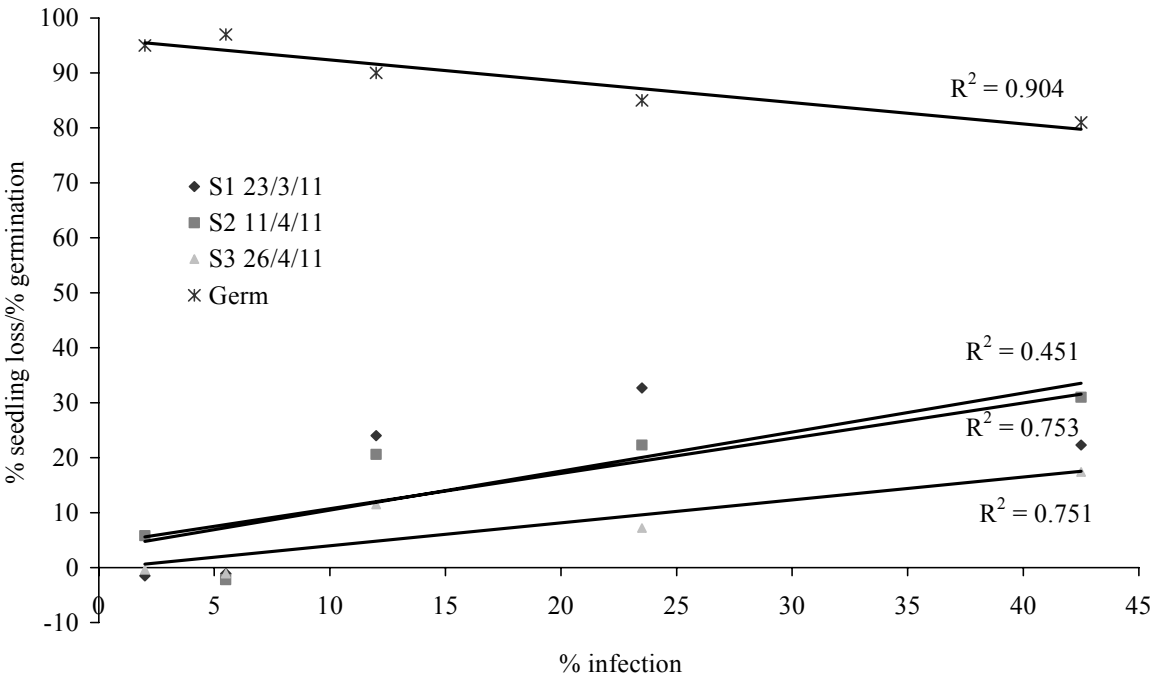

Figure 1. Percentage untreated germination and mean seedling loss against percentage infection for three sowings of spring oats sown March to April 2011.

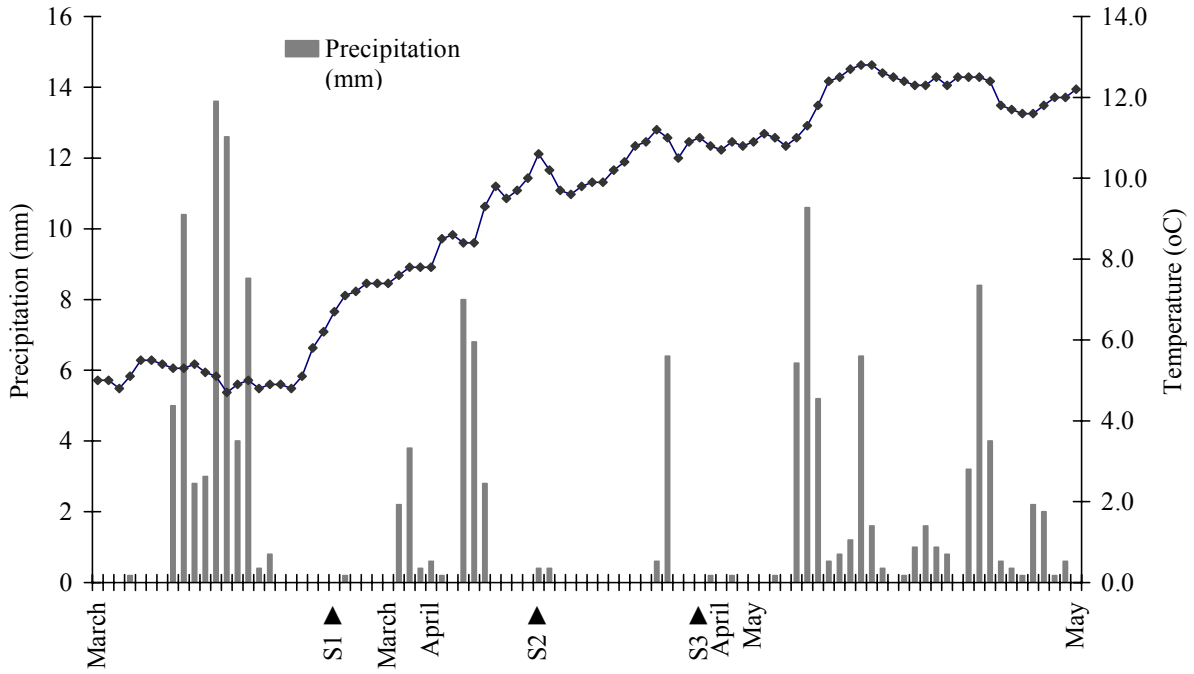

Figure 2. Precipitation and soil temperatures during March to May 2011, sowing dates are indicated by S1, S2 and S3.

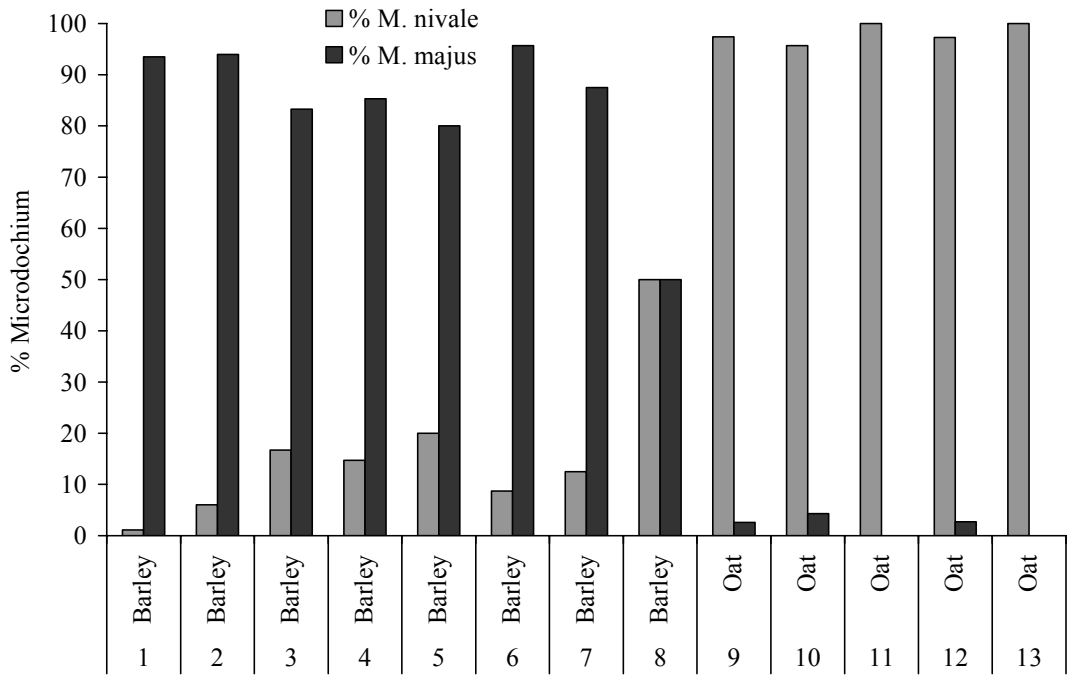

Figure 3. Percentage of each *Microdochium* species present on seed lots sown.

DISCUSSION

A field sowing in 2008 showed that a high seed infection level of 58% *Microdochium* infected barley caused significant seedling losses where untreated seed was sown in early April. This seedling loss was exacerbated by a week of adversely cold weather immediately after sowing. In this experiment no significant losses were observed in the untreated spring barley despite obtaining samples with seed infection levels as high as 91%. However, significant losses were observed for two sowings in the spring oats where the highest infection level was 42.5% (Figure 1). It is thought that the unusually good spell of weather immediately after the first sowing on 23rd March 2011, where soil temperatures rose rapidly and rainfall was mostly low, has reduced the effect infection levels may have had on the spring barley and on sowing one of the oats (Figure 2).

Identification of *Microdochium* species isolates from the agar plate test has shown a clear difference in host preference of each species. Almost all the colonies identified from the oat seed lots were *M. nivale*, whereas there was usually an 80:20 split in the colonies isolated from the barley seed lots with the majority of colonies being of the *majus* type (Figure 3). So, contrary to the findings of Simpson *et al*. (2000), in this experiment it has been *M. nivale* which has shown to have a selective advantage on oats. Differences in host defence mechanisms, seed bed environment or pathogenicity differences between *M. majus* and *M. nivale* could be responsible for this observation.

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THE POTATO COUNCIL'S PLANT HEALTH STRATEGY

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Summary: In 2011, Potato Council reframed its commitment to plant health research and communication. The purpose was simply to present to industry clearly and concisely a set of well defined interests and activities. This is required so that all stakeholders, from individual growers to supply chains and advisors could take stock of their own roles and responsibilities and actively participate in preserving a valuable and enviable status. The context for this exercise was a predictable cycle of new disease reports brought to a head by recent *Dickeya* findings. The reframing now allows further debate with industry so that next time the GB Potato industry are collectively challenged by a new threat all stakeholders understand that they all have a part to play if we are to maintain value within the potato sector.

INTRODUCTION

The plant health agenda is far from new to Potato Council (PCL). Since 1997 PCL has invested in over 80 plant health related projects with a total value greater than £14.55m and involvement was determined through strategic reviews within our R&D and KT functions. In 2010 the potato sector, through consultation, agreed that we should review our commitment to plant health activities and develop a strategy so that roles and responsibilities across a broad range of stakeholders and levy payers could be determined. This "setting out our stall" was considered a vital step if all sectors of the industry were to work collectively in response to new threats and challenges. A number of political, commercial and evidential drivers listed below influenced our need for the strategic review.

- A relatively predictable cycle of new pest and disease reports within GB (Dehnen-Schmutz *et al*., 2010)

- A thriving seed export trade that for the first time exceeded 100,000 t shipped based in part on our reputation for a high status of plant health

- Evidence from overseas that emerging threats could become costly realities (e.g. zebra-chip in New Zealand) and that preparedness of all industry players is vital in mitigating against losses. Notably the financial consequences of the New Zealand scenario are estimated at $120m since initial discovery in 2006 (Ron Gall, pers. comm.)

- Anecdotal evidence (during discussions about prevention of *Dickeya* and *Epitrix*) that our producer-base and supply chain has a variable understanding of, and appetite for, risk.

- A complex cycle for review of plant health related legislation from EU Plant Health Directive 2000/29/EC to Potatoes Originating in Egypt (England) Regulations 2004.

- A continuing need to ensure industry is well placed to respond to outcomes of ongoing EC directives relating to brown rot (98/57/EC), ring rot (93/85/EEC as amended), PCN (2007/33/EC) and EU Thematic Strategy for Pesticides.

- A new context around "Big Society" introduced by the coalition government with associated discussions around cost sharing, roles and responsibilities, industry ownership etc.

- New opportunities within AHDB to share expertise on crop protection issues.

Five pillars (or work-streams) were developed for plant health as outlined in Table 1, which collectively provide the basis for our plant health strategy. Subsequent sections describe work areas, plans and progress for each pillar.

Table 1. Potato Council's five pillars of plant health

Pillar	Title	Owner
I	Taking care of domestics	Head of Communications
II	Providing a platform	Head of Seed and Export
III	Learning through collaboration	Head of R&D
IV	Accounting for new threats	Head of R&D
V	Changing awareness and behaviour	Head of Comms. & Head of Seed and Export

Pillar I: Taking care of domestics

In Pillar I we recognise that research and knowledge transfer relating to "domestic" plant health concerns (e.g. blight, aphid and virus, PCN) remain vital and that threat status may be elevated by changes in availability of crop protection products, introduction of new strains of pest and pathogens, selection of aggressive, virulent or pesticide-resistant strains or adaptation of current populations to climate change. Our current research effort concentrates on priority subjects with R&D funding committed to understanding the impact of haulm destruction techniques on blackleg/pit rot development, virulence factors associated with *Erwinia*, the impact of mineral oils on transmission of virus, resistance of aphids to neonicotinoids, integrated control of PCN, powdery scab and late blight and the control of free living nematodes and tobacco rattle virus.

Full titles and affiliations for the current program are available at www.potato.org.uk

Our KT effort continues to concentrate on mainstay decision support and intelligence tools around blight, aphid mapping and PCN control.

The Fight against Blight campaign is now into its 8th year, scouts continue to report and subsample outbreaks and their effort provides local intelligence and an isolate collection for further analysis of the population. Blight maps continue to be viewed frequently by industry, achieving 12 000-16 000 visits depending on the season, and continue to provide the hook for delivering new best practice. Most recently this relates to control of growth on potato dumps following the withdrawal of PDQ and dichlobenil as control options. Potato Council's response has been to instigate the application for a SOLA for use of Diquat and to ensure rules relating to application of glyphosate are understood by industry.

Potato Council's Potato Cyst Nematode (PCN) management tool initially brought together the outcomes of research into the impacts of resistance, tolerance and rotation on *Globodera pallida* populations and was distributed as an educational tool for use in the industry. In response to new requirements for growers to demonstrate effective PCN management as require by the EU PCN Control Directive the tool has been re-built in an on-line format. This rebuild includes new data on resistance and tolerance characteristics for recently-introduced varieties and a separate module for those managing *Globodera rostochiensis* populations. The tool will be launched in spring of 2012.

Aphid control remains fundamental to production of high health status seed and PCL's aphid intelligence website continues to provide timely intelligence that dovetails with statutory monitoring networks and provides a platform from which to launch new communications; for example outcome of ongoing PCL research into the role of mineral oils for virus prevention.

Pillar II: Providing a platform

In Pillar II the Potato Council are seeking to understand and clarify the role of the Safe Haven Scheme as a potential platform for mitigating existing and new plant health risks. The scheme was developed in 2003 following British Potato Council-led industry discussions that in turn followed three separate outbreaks of ring rot in England & Wales. What was clear from discussions at the time was that producers had been content to rely on standard certification and official import inspection and diagnostic tests to assess risks and such measures were considered disproportionately small given the potential consequences of the disease establishing a footing in GB. The scheme established a seed potato crop assurance inspection process for farm hygiene, seed origin and flow of crop and provided greater confidence that members' crop was free from ring rot than was the case previously. The majority of GB's seed area (60%) is now within the scheme.

Current activity relating to the scheme has two objectives. The first is to review pros and cons of the scheme, establish whether it affords protection against diseases other than Ring Rot and decide whether amendments to the scheme would broaden its scope. This is being done systematically by hypothetically testing the scheme against seed-borne threats identified through Pillars III and IV. Initially this has been done for *Dickeya* with the assistance of John Elphinstone at FERA. This exercise has concluded that the existing scheme contributes to a reduced risk of *Dickeya* spread and development but does not address particular possible transmission pathways from irrigation water. We have not considered it necessary to seek amendments to the scheme at this stage but have invited scheme members to fine-tune their irrigation practices and have published best-practice guidelines. Further pathogens / diseases will undergo the hypothetical test once identified.

The second objective relates to broader promotion of the scheme and potential benefits to the ware sector. Initial steps include an assessment of current attitudes and awareness to the scheme as part of a national seed-customer satisfaction survey later in 2011.

Pillar III: Learning through Collaboration

In this pillar we recognise that GB's first line of defence against new plant health threats is the domain of Government, devolved administrations and related agencies and is delivered through a range of quarantine and import inspection activities. We welcome new approaches to consultation being adopted where industry can interact with policy teams more directly prior to formal consultations. We also recognise that on occasion, sporadic ingress of new pests and pathogens can lead to established GB populations which require concerted and collaborative industry activity to maintain production at realistic costs. Our objective is to develop a knowledge base of global plant-health related responses so that when required, we can assist in initiation of effective and cost-efficient industry measures. Sub-objectives relate to establishing and maintaining global and domestic communication networks and to cataloguing practices and approaches that have been effective elsewhere.

Communication networks

Regular contact is made with numerous organisations globally and domestically to improve PCL's knowledge of responses to plant health issues, measures and priorities. Examples are shown in Table 2.

Where appropriate Potato Council attempt to deliver some of the key individuals to GB events where it is believed there is potential benefit in sharing best practice. Recent examples have included Ian Toth from the Hutton Institute speaking at Sutton Bridge on the risks of *Dickeya* for the 2011 PCL winter workshop.

Sharing information on pest and disease prior to establishment is crucial for risk assessment e.g. with PSTV and *Tuta absoluta* (South American tomato moth) where potential control control methods are being examined and grower guidelines had been developed by HDC and are being shared with Potato Council.

Table 2. Regular partners in Potato Council's plant health dialogue

Group	Organisation
Domestic – AHDB	HDC HGCA Chief Scientist
Domestic – govt and research	DEFRA FERA Scottish Government SASA SAC James Hutton Institute Natural Resources Institute NFU's England & Wales and Scotland Potato Processors' Association
Stakeholders	Fresh Potato Suppliers Association Pre-Basic Growers Association
Overseas – levy organisations	Ausveg Potatoes South Africa Potatoes NZ Canadian Hortuculture Council United States Potato Board
Overseas - other	European and Mediterranean Plant Protection Organisation (EPPO) International Potato Centre (CIP) Arvalis (France) Wageningen University (Netherlands) Horticulture Australia (HAL) Horticulture NZ NJF Network (Scandinavia)

Separately, AHDB has recognised efficiency gains by making best use of its expertise through the development of a Crop Protection Group chaired by Mike Storey. As a consequence, areas of expertise and primary contacts have been established as illustrated in Table 3.

Table 3. Crop protection interests: lead and shared roles assigned within AHDB

Crop Protection area	PCL	HDC	HGCA
Diagnostics and soil sampling	X	X	
Pesticide performance		X	X
Integrated Pest Management	X		
Pesticide stewardship	X	X	X
Resistance management			X
Biopesticides		X	
Herbicides in rotation			X
Plant health	X		
Free living nematodes	X	X	X
Composts	X	X	X
Slugs			X

Review of approaches

Currently many approaches to quantifying possible impact and industry resource requirements to tackle new disease threats should a threat turn into reality have been considered. PCL are now developing a hybrid of approaches used elsewhere and establishing links with EU's Pest Risk Analysis process to ensure preparedness.

Pillar IV: Accounting for new threats

In pillar IV Potato Council use the evidence collected through pillar III to establish a portfolio of potential threats for which a high level "watching brief" is maintained. PCL has well established mechanisms for reviewing priorities, allocating R&D funding and deciding on communication priorities. Industry is involved in decision making via the Research & Knowledge Transfer committee who are responsible for making recommendations relating to priorities. Pillar IV provides the vital link whereby PCL executive provides clear and concise accounts of potential threats as part of the priorities-review process.

Since initiation of the plant health strategy, risk based reviews have been developed for *Dickeya*, the South American Tuber Moth (*Tuta absoluta*), Stolbur phytoplasma, Flea Beetle (*Epitrix*), Potato Spindle Tuber Viroid and the psyllid/zebra chip complex. So far, none of these reviews have triggered a change in our allocation of research funding except for *Dickeya* where new R&D was funded jointly with Scottish Government; although the Psyllid/Zebra Chip complex will now become the subject of further industry discussion and scenario planning. Reviews for the others will be continually modified as new information from the global network becomes available.

Pillar IV will also allow us to explore triggers for releasing reserves from PCL's accounts in times of crisis.

Climate change will result in overall temperature rises, with drier summers and wetter winters and greater volatility in weather events. There will be consequences too on established pests and diseases and for the incidence and emergence of new threats. The dynamics of changing aphid /virus interactions and on populations of free-living, root knot and cyst nematodes are potential risks for the industry and PCL support for strategic research in these areas to build GB preparedness/response.

Pillar V: Changing awareness and behaviour

This pillar represents the levy-payer / industry facing part of PCL plant health strategy. Levy payers, stakeholders and wider industry will look for Potato Council to set the agenda for plant health. This agenda must firstly be to stimulate debate on the priorities both within the plant health sphere, and in relation to other challenges. Protecting long term plant health will often come in conflict with shorter term needs of the market and business survival. This conflicting pressure and divergent perspectives on a risk/reward equation is one that requires industry debate. The role of Potato Council is to help the industry to identify the issues and make informed decisions.

In order to set realistic objectives relating to levy-payers' understanding of and attitudes towards risks and threats from new pests and pathogens it is necessary to undertake baseline surveys. This activity is currently in chain and surveys have been designed following consultation with SASA, FERA and NFU to ensure meaningful outcomes can influence future resource allocation. Stratified surveys will run through September 2011 and will inform an industry stakeholder group who will be invited to assist PCL in shaping the deliverables relating to the strategy. Typically, this type of "shaping" entails revisions to all PCL Knowledge Transfer strategies and communication plans and ensures a relevant plant health or risk related message or theme is incorporated into all regular outputs (newsletters, e-bulletins, workshops, field events. Preparation of a "toolkit" to underpin a larger plant health campaign is already underway with revisions to pest and disease manuals, disease guides and resistance rating summaries scheduled for the current year.

PCL's vision is for an industry that, armed with reliable, timely, independent and relevant information can take account of plant health threats and risks and for individuals to understand their own roles and responsibilities to allow the industry to maintain its enviably high plant health status.

Stakeholder engagement

Engagement is vital to agreeing roles and responsibilities relating to plant health. Further PCL hosted workshops and forums are planned and will continue to inform the strategy; these will supplement existing scheduled meetings with the Potato Processors' Association, Fresh Potato Suppliers' Association and National Farming Unions.

Plant health workshops with FERA, who are taking more proactive role in interpretation of EU legislation and requirements for industry, provide PCL with the opportunity to feed in industry views prior to legislative change.

The current Levy payer questionnaire on 'Have Your Say on R&D' identifies established pests and diseases, rather than the emerging threats, being of key concern to respondents when

questioned on the sustainability of their businesses and in particular they identified PCN, Late Blight and Blackleg/soft rots.

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DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR THE DETECTION OF *'DICKEYA SOLANI'*

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Summary: '*Dickeya solani*' is a recently emerged bacterial pathogen of potato that has had a great impact on the potato industry of Israel and many European countries and poses a significant threat to GB production. '*D. solani'* is highly aggressive and results in symptoms and disease similar to that of blackleg. Current diagnostic methods are time-consuming and expensive. Work is being carried out to reduce the time taken to make a diagnosis and to develop a single, specific test for '*D. solani*'. A real-time PCR assay, based on the *fusA* gene has been developed and evaluated against a panel of 110 representative strains from the genera *Dickeya* and *Pectobacterium*, alongside two other real-time PCR assays, developed independently and jointly by JHI and Fera. All assays performed equally well and were shown to positively identify '*D. solani*' with only a small number of false positives and only one false negative. Further evaluation of these assays is underway to ensure they are suitable for routine use.

INTRODUCTION

Members of the genus *Dickeya* (formerly *Erwinia chrysanthemi*) cause diseases on numerous crop and ornamental plants World-wide. The first disease reports of *Dickeya* spp. in European potatoes were probably attributable to *D. dianthicola*; originally detected in the Netherlands in the 1970s then spreading to other countries. Since 2004-5 a new, highly aggressive pathogen, when compared against *D. dianthicola* and the more established European blackleg pathogens, *Pectobacterium atrosepticum* and *P. carotovorum* subsp. *carotovorum*, has been spreading across Europe and to Israel *via* trade in seed potatoes and is causing increasing economic losses (Toth *et al*., 2011). Once established, '*D. solani*' can rapidly displace these other established pathogens to become the principal cause of wilting and blackleg symptoms in potato. Although disease symptoms caused by '*D. solani*' are often indistinguishable from typical blackleg it is evident that '*D. solani*' can produce symptoms at lower inoculum levels and is considerably more aggressive than *D. dianthicola* and *Pectobacterium* spp.. '*D. solani*' can cause disease over a wide temperature range and also has a higher optimal temperature for disease development, which may explain, in part, the domination of this pathogen in certain European countries (van der Wolf *et al.*, 2007). The aggressiveness of *'D. solani*' is thought to increase with increasing ambient temperature and it also benefits from increased rainfall as the flooding of fields allows for the bacteria to spread between plants; spread also occurs vascularly from

mother to daughter tubers (Czajkowski *et al.*, 2010). In 2007 *'D. solani'* was responsible for a loss of 20% of potato stocks during certification in the Netherlands either as a consequence of downgrading or rejection at a cost of €25million (Toth *et al.*, 2011).

Scotland and Northern Ireland are currently the only countries in Europe to enforce a 'nil tolerance' for *Dickeya* spp., in an attempt to keep their potato production free from this pathogen. Irrespective whether other countries adopt a similar approach or rely on existing control measures enshrined in their seed certification schemes, reliable diagnosis of the pathogen is the first step towards effective control. Because symptoms alone are unreliable as a means of determining the presence of '*D. solani*' some form of diagnostic test is therefore essential. The polymerase chain reaction (PCR) is increasingly used for the specific detection and identification of bacterial plant pathogens. Currently diagnostic protocols for *Dickeya* spp., based at least in part on PCR, are expensive, time-consuming and it can take up to two weeks to perform a reliable diagnosis. The most widely used procedure at present for identifying *Dickeya* spp. involves initial enrichment and/or selective isolation steps prior to PCR using the ADE primers (ADE1/ADE2) from the pectate lyase (*pel*) gene (Nassar *et al.*, 1996) to detect all known members of the genus. Species identification to '*D. solani*' can only be achieved by sequencing either the *recA* (Parkinson et al., 2009) or *dnaX* genes (Sławiak et al., 2009), then comparing against database entries or by sequencing alongside known reference strains. The aim of this study, therefore, is to develop a diagnostic protocol specific for '*D. solani'* which must be cost-effective, fast and accurate and which can be used to test direct from potato plants, tubers and environmental samples. A previously designed multi-locus sequence typing (MLST) system (Kowalewska *et al*., 2010), developed to characterise *D. dianthicola* strains but which also included a number of '*D. solani*' strains was utilized to design the real-time PCR assay.

MATERIALS AND METHODS

Design of *fusA* primers

The *fusA* gene was selected as a target for further study after visual inspection of phylogenetic trees, generated previously (Kowalewska *et al*., 2010), showed good separation between '*D. solani'* isolates and known reference strains from the genus *Dickeya* and *Pectobacterium*. Using the ClustalW method of the MegAlign program in Lasergene v7.0.0 (DNASTAR Inc.) the *fusA* sequences of 62 *Dickeya* and *Pectobacterium* strains and 20 '*D. solani'* isolates were aligned. From these data a TaqMan® '*D. solani'* specific probe was designed, in addition to flanking primer sequences. The assay was evaluated using the protocol described below. In addition, this assay was compared against two other real-time assays developed by JHI and Fera, jointly and independently of this study, by exploiting draft genome assemblies from 16 *Dickeya* isolates and reference strains (Pritchard *et al*., unpublished data). The accuracy, effectiveness and efficiency of the three assays were analysed and the results are described here.

Real-time PCR

The *fusA* real-time PCR assay, based on the probes and primers described (Table 1), was evaluated against two previously designed assays (SOL-C & SOL-D; Pritchard et al., unpublished data), using a collection of 110 *Dickeya* and *Pectobacterium* strains. These strains

had been stored on freezer beads at -80°C. Strains were streaked onto crystal-violet pectate medium (CVPM) plates and incubated for 48 hours at 36°C. Cultures were then re-streaked onto Nutrient Agar and incubated overnight at 36 °C. A loopful of colonies was suspended in 500µl of sterile water (Sigma) and boiled at 100 °C for 5 minutes. The sample was then ready to be used in the PCR reaction or stored at -20 °C until needed.

Table 1. Primers and probes used in *fusA* real-time assay.

Assay	Forward primer	Reverse primer	Probe
fusA	GGTGTCGTTGACCTG GTGAAA	ATAGGTGAAGGTCAC ACCCTCATC	TGAAAGCC ATCAACTG GAATGATT C

Master mix (24 µl; Table 2) was added to each well of a 96-well plate (MicroAmp optical well plate with barcode) with an electronic pipette (Autorep) in a flow hood for each of the three tested. Boiled cells (1µl) were added to each well (1 µl of sterile H_2O (Sigma) for negative controls). The plate was sealed with PCR optical film.

Table 2. PCR reaction mix.

1x Reaction	
Taqman master mix	12.5 µl
Forward primer (5 pmol)	1.5 µl
Reverse primer (5 pmol)	1.5 µl
Probe (5 pmol)	0.5 µl
Template	1 µl
Sterile H_2O (Sigma)	8 µl
TOTAL REACTION AMOUNT	25µl

Table 3. Real-time PCR cycle.

Temperature	Time	Number of cycles
95°C	10 mins	x 1 cycle
95°C	15 s	x 40 cycles
60°C*	1 mins	

The cycle was run on Applied Biosystems 7900HT real time PCR machine in standard mode, detecting FAM/TAMRA and using ROX as the passive reference. Data was taken at the extension (*) step only.

RESULTS

Initial screening and comparison of the three real-time assays was carried out against 110 samples of known *Dickeya* and *Pectobacterium* spp., the results of which are shown in Table 4. Real-time, or quantitative, PCR is typically used to measure the amount of DNA expressed in the reaction, based on the critical threshold (Ct) value. The Ct value is the cycle at which the fluorescence becomes detectable and is inversely proportional to the logarithm of the initial number of target cells; however, in this case the Ct value is used qualitatively to indicate either positive or negative identification of '*D. solani*.' Ct values less than or equal to 25 signified the sample to be positive as '*D. solani*' and Ct values greater than 25 or "undetermined" were denoted as being negative and therefore, not '*D. solani*.' In this study 97% of the strains received as '*D. solani*' were correctly identified using all three assays and only 5-7.5% of non-'*D.solani*' strains were incorrectly identified (false positives). Both the fusA and SOL-C assays gave identical results, with SOL_D performing marginally better through a smaller number of false positives.

Table 4. Results from initial screening of three real-time assays against *Dickeya* and *Pectobacterium* spp, with Ct values <25 recorded as positive and Ct>25 or "undetermined" denoted as negative.

Species Tested	*fusA* Positive	Negative	SOL-C Positive	Negative	SOL-D Positive	Negative
D. solani	29	1	29	1	29	1
D. dianthicola	2	11	2	11	1	12
D. zeae	2	14	2	14	1	15
D. dadantii	0	5	0	5	0	5
Other *Dickeya* and *Pectobacterium* spp.	2	44	2	44	2	44
Total	35	75	35	75	33	77

All three real-time assays produce similar results. Each correctly identified the majority of '*Dickeya solani'* strains, however, one strain, a river water isolate, was not detected by any of the assays. The majority of other *Dickeya* and *Pectobacterium* strains were correctly identified as negative with the exception of four strains: two *D. dianthicola* and two *D. zeae*. All *D. dadantii* strains, the species considered to be the most closely related to *D. solani*, were detected as negative.

DISCUSSION

The 110 strains which represented a range of *Dickeya* and *Pectobacterium* species were tested with the three assays: *fusA,* SOL-C and SOL-D. Results from all three showed little variation as similar results were obtained from each assay. All but one of the '*D. solani*' isolates were correctly detected as positive with a Ct value of less than 25, however, there were four false positives. *'Dickeya solani'* had only emerged within the past five years and knowledge of the

species is limited. The samples used had been gathered prior to this study from a variety of sources and it is possible that some of them were originally misidentified. Incorrect labelling of the strains may account for the improper identification as positive or negative. Overall the assays seem promising in testing specifically for '*D. solani'*. Further testing and improvement of the techniques will be carried out to reduce the false positives and false negatives.

In addition to a lack of diagnostics specific for '*D. solani*' the sampling preparation methods are also time-consuming and work is being done to reduce the time it takes to prepare a sample for PCR. Currently tubers must be cored, pathogen extracted and enriched, streaked onto selective media, further cultured on nutrient agar. From here samples go through conventional PCR which is only specific to the level of *Dickeya* sp. Sequencing based on *recA* then identifies the strain of *Dickeya*. Further work will focus on improving the sample preparation and the real-time assay, in order to reduce the time taken for a diagnosis in addition to making the test more specific for '*D. solani.'*

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SAFEGUARDING THE HEALTH OF POTATOES IN SCOTLAND

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Summary: An annual programme of testing and surveillance is undertaken by The Scottish Government in order to safeguard potato production in Scotland from quarantine and non-indigenous pests. This includes potato quarantine testing and pathogen testing of nuclear stock, specific surveillance for viroid, bacterial, nematode and insect pests, inspection of seed potatoes and varietal susceptibility testing for potato wart disease (*Synchytrium endobioticum*) and potato cyst nematodes (*Globodera* spp.).

INTRODUCTION

The Scottish Government (SG) undertakes measures to protect Scottish potato production from serious pests and diseases. Many are required in plant health legislation. Other measures are part of the Scottish Seed Potato Classification Scheme administered by the SG, such as the requirement for pathogen tested nuclear stock to be the starting point of seed potato production and official inspections of growing crops and tubers. These official controls are augmented by the actions of growers, industry and the public, for example the implementation of the Safe Haven Scheme, grower co-operation with the certifying authority and notifications by the public of the rare findings of *Leptinotarsa decemlineata* (Colorado beetle) or other suspect symptoms. This paper aims to describe some of the measures undertaken by SG to safeguard potato production in Scotland since 2004. Data from earlier years was presented in Scientific Reviews of SASA (1992-1997, 1997-2000 and 2000-2003; www.sasa.gov.uk).

LEGISLATIVE REQUIREMENTS

A number of potato pests are regulated in the EU as quarantine pests in the plant health directive (Council directive 2000/29/EC, PHD). These are either not present in the EU (listed in Annex IAI or Annex IIAI) or are under official control (listed in Annex IAII or IIAII). Potatoes for planting are prohibited from entering the EU (Annex III), unless they have been subjected to post-entry quarantine testing for quarantine organisms (Commission directive 2008/61/EC). Potatoes moving in trade within the EU must meet the specific requirements in the PHD for all the quarantine pests (Annex IV).

The UK has protected zone status for some organisms e.g. *L. decemlineata* (Annex IB of the PHD), which means that other member states must ensure that potatoes and other materials are free from Colorado beetles when destined for the UK. The UK is required to undertake surveys to demonstrate the continued absence of the pest. In addition, specific measures for four potato quarantine pests which occur in the EU are contained in control directives (*Clavibacter michiganensis* ssp. *sepedonicus* (potato ring rot) (Council directive 93/85/EEC as amended);

Globodera pallida and *G. rostochiensis* (potato cyst nematodes, PCN) (Council directive 2007/33/EC); *Ralstonia solanacearum* (brown rot) (Council directive 98/57/EC as amended); and *Synchytrium endobioticum* (wart disease) (Council directive 69/464/EEC)). These measures have to be applied in all Member States and all, apart from the wart disease directive, require surveillance to be undertaken. Finally, the EU passed emergency legislation for the potato pest, *Potato spindle tuber viroid* (PSTVd, Commission directive 2007/410/EC) and this includes a requirement to undertake surveillance.

As well as plant health requirements, Scottish seed potato regulations are more stringent than EU requirements. Scottish potato production is derived from pathogen-tested nuclear stock, which involves testing for common potato pests and also includes tests for some quarantine pests and tests that would detect many non-indigenous pests. In 2010 the SG introduced a nil tolerance for the damaging pests *Dickeya* spp. in The Seed Potatoes (Scotland) Amendment Regulations 2010 and The Plant Health (Potatoes) (Scotland) Amendment Order 2010. In order to justify this tolerance, annual surveillance for *Dickeya* spp. is carried out.

TESTED POTATO MATERIAL - PREVENTING THE INTRODUCTION OF PESTS

Potatoes for planting from non-EU countries are tested in post-entry quarantine by the UK Potato Quarantine Unit (UKPQU) and all starting material for the seed potato classification scheme (nuclear stock) is pathogen-tested by the Nuclear Stock Initiation Unit (NSIU) at SASA. These procedures ensure that potato production in Scotland is based on material that has been tested for freedom from quarantine pests and pathogens specified in the seed potato classification scheme. The Scottish seed potato certification system is a "flush through" system, which is generation limited and new tested material is produced annually to replace the material leaving the system. In addition to testing of potatoes for planting, official inspection, sampling and testing for the presence of pests is undertaken at ports of entry on ware potatoes from outside the EU. As most imports are via English ports, this work is largely done by The Food and Environment Research Agency (Fera) staff. Such testing is primarily for ring rot and brown rot, but inspections are also done for other non-indigenous pests, such as *Epitrix* spp.

Potato quarantine testing

In potato quarantine testing at SASA, specific tests are done for PSTVd, viruses (*Andean potato latent virus* (APLV), *Andean potato mottle virus* (APMoV), Arracacha virus B-oca strain (AVB-O), *Potato black ringspot virus* (PBRV), *Potato latent virus* (PotLV), *Potato leafroll virus* (PLRV), *Potato mop-top virus* (PMTV), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus P* (PVP = Potato rough dwarf virus), *Potato virus S* (PVS), *Potato virus T* (PVT), *Potato virus V* (PVV), *Potato virus X* (PVX), *Potato virus Y* (PVY), *Potato yellow vein virus* (PYVV), *Potato yellowing virus* (PYV), *Tobacco rattle virus* (TRV), *Tomato black ring virus* (TBRV), *Tomato spotted wilt virus* (TSWV)) and bacteria (*Candidatus* Liberibacter solanacearum, *C. michiganensis* ssp. *sepedonicus*, *Dickeya* spp., *Pectobacterium* spp., *R. solanacearum*). Sap from the potato material is inoculated onto test plants that will detect most of the listed pests and unknown pathogens and the potatoes are also grown for a complete vegetative cycle and observed for symptoms of pests (EPPO, 2004). Table 1 provides a summary of the number of lines submitted for quarantine testing since July 2003 and interceptions. Of particular note was the interception of PSTVd in one line from Korea in 2007-8. Infected lines are destroyed, or subjected to virus elimination techniques and retesting to verify freedom.

Table 1. Summary of numbers of potato lines tested by the UKPQU and interceptions (data from 1 July to 30 June annually)

	2003 -4	**2004 -5**	**2005 -6**	**2006 -7**	**2007 -8**	**2008 -9**	**2009 -10**	**2010-11**
No. lines; (Countries)[1]	1 (IL) 2 (NL)[2, 3] 12 (NZ) 12 (US)[4]	2 (CA) 3 (IN)	3 (CA) 1 (IT)[3] 2 (NZ) 7 (US)	2 (CA) 28 (US)	1 (CA) 1 (IN)[2] 15 (KR) 3 (NO) 2 (UA) 17 (US)	1 (BE)[2, 3] 2 (CA) 3 (UA) 26 (US)	3 (BY) 1 (CA) 1 (RU) 17 (US)	1 (PE) 6 (US)
Total	**27**	**5**	**13**	**30**	**39**	**32**	**22**	**7**
Pests intercepted	PVY (NL)			PVS (US)	PSTVd (KR) PVM (UA)	PVS (BE) PVY (BE)	PVS (BY) PVS (BY)	

[1] ISO country codes; [2] tubers; [3] lines which could not be issued with a plant passport; [4] seed and *in vitro* lines.

In addition to imported material, testing by the UKPQU is done on accessions from the Commonwealth Potato Collection (CPC), one of the World's major potato gene banks held at The James Hutton Institute (JHI), Dundee (Jeffries *et al.,* 1993) (Table 2).

Table 2. Testing of accessions from the Commonwealth Potato Collection

	2004	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**
No. accessions			-		58	59	77	73
No. fully tested plants	587	3052	-	625	984	995	1181	1239

This collection was tested for freedom from PSTVd in the 1970s (Jeffries, 2001). However, in order to ensure that the collection is also free from seed transmitted quarantine viruses (APLV, AVB-O, PBRV, PVT and PYV), testing has been undertaken annually on accessions as they have been regenerated and retesting for freedom from PSTVd has also been done. In addition, testing of the "Hawkes (Birmingham) Collection" was done when it was integrated into the CPC (Jeffries, 1991). Testing for the same pests has also been done on breeding material used for the generation of new potato cultivars at the JHI. This ensures that new cultivars meet the requirements for freedom from these quarantine pests and can be issued with a plant passport.

Nuclear stock production

Pathogen testing at the NSIU includes specific testing for PSTVd, PLRV, PMTV, PotLV, PVA, PVM, PVS, PVV, PVX, PVY, TBRV, *C. michiganensis* ssp. *sepedonicus, Dickeya* spp., *Pectobacterium* spp. and *R. solanacearum* and inoculation of potato sap to indicator plants *Capsicum annuum, Chenopodium amaranticolor, C. murale, C. quinoa, Datura metel, Nicotiana bethamiana, N. bigelovii, N. clevelandii, N. debneyi, N. occidentalis*- P1, *N. tabacum* cv. White Burley will also detect most pests of potato. Potatoes are also grown for a vegetative cycle and observed for symptoms of pests. The numbers of clones tested since 2003 are given in Table 3. Every year a number of common pests have been detected. Infected plants are usually discarded; occasionally virus elimination is done.

Table 3. Number of clones tested annually by the NSIU

	2004	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
No. clones	156	157	147	139	153	132	185

SURVEILLANCE FOR QUARANTINE PESTS AND NON-INDIGENOUS ORGANISMS

Specific surveys are done for a number of quarantine and non-indigenous pests. In addition, official inspections are undertaken during the growing season and on harvested tubers before marketing. These inspections are required for all seed potato crops in the seed potato classification scheme, but they also underpin all plant health activities, providing evidence of freedom from pests. The SG employs more than 100 inspectors operating out of seven area offices to undertake inspections. The SG also audits micropropagation and minituber production systems and monitors the health of minitubers, with *ad hoc* surveillance and follows up where necessary. A proportion of ware crops are also inspected to ensure that the high health of potato production in Scotland has been maintained. These inspections are particularly important for verifying the absence of Colorado beetle and symptoms of wart disease, ring rot and brown rot. Samples of plants with suspect symptoms are sent to SASA for investigation.

Potato ring rot and brown rot

Annual surveys for the presence of *C. michiganensis* ssp. *sepedonicus* and *R. solanacearum* have been undertaken since the relevant EU control directives came into force and using the methods specified in the directives. The sampling strategy is based on risk and involves sampling of tubers, including samples from: each seed grower; potatoes from other parts of the EU (and UK) prior to planting and from the daughter crop; irrigated crops; and a proportion of targeted ware crops (Table 4). Samples from Scottish watercourses are also tested for *R. solanacearum*. *C. michiganensis* ssp. *sepedonicus* and *R. solanacearum* have never been detected in Scottish potatoes and the large numbers of samples tested provide confidence in the health of Scottish potato production.

Table 4. Laboratory samples (tubers and water) tested for quarantine bacteria.

	2004	2005	2006	2007	2008	2009	2010
Ring rot							
Domestic	1187	1219	1277	1330	1256	1261	1254
Imports[1]	97	11	5	26	10	50	12
Brown rot							
Domestic	1187	1243	1332	1437	1284	1261	1282
Water	476	594	585	375	303	423	471

[1] Tested for both ring rot and brown rot. Most imports are for trials purposes.

R. solanacearum was found in river water in 2000 (Wood *et al.,* 2002) and eradication of the alternative host *Solanum dulcamara* was undertaken (Saddler *et al.*, 2008). No *R. solanacearum* has been detected in Scottish river water since 2001.

***Dickeya* spp.**

Tuber samples and stems submitted during growing crop inspections were tested for *Dickeya* spp. from 2006. In 2010 Scottish legislation was introduced. Numbers of samples from 2006-2010 were 291, 123, 133, 511 and 949 respectively. Watercourses were also sampled and tested. There have been no findings in potatoes of Scottish origin.

Potato spindle tuber viroid

From 2007, EU Member States were required to survey host plants for PSTVd. Prior to this, samples had been taken from any tomato crops showing symptoms of yellowing and from potato material from non-UK sources received for trials purposes at SASA. Numbers of plants tested in Scotland using a DIG probe method (Jeffries & James, 2005) since 2004 are given in Table 5.

Table 5. Number of plants tested annually for PSTVd (apart from testing of quarantine, nuclear stock and gene bank material)

Host	2004	2005	2006	2007	2008	2009	2010
Ornamentals[1]	-	-	-	2578	1130	1090	2760
Tomato[2]	24	7	2	770	244	540	571
Potato	-	1676	1036	960	1320	1090	260
Other (*S. dulcamara*)			19				

[1] *Petunia* and *Solanum* spp.; [2] testing of leaves with symptoms until 2007

In 2007, PSTVd was detected in *Solanum jasminoides* plants moving in trade in Scotland, and a related pospiviroid, *Tomato chlorotic dwarf viroid* (TCDVd), was found in *Petunia* cuttings (James *et al.,* 2007). All infected material was destroyed under official control and related material was tested. TCDVd was also found in 2010. This viroid has never been found naturally occurring in potato, but under experimental conditions it can cause severe symptoms in potato and has been found naturally in tomato crops in some parts of the world. Publicity material on risks associated with pospiviroids has been distributed to minituber producers.

Colorado beetle

Surveillance for Colorado beetle involves official growing season inspections of seed potato crops (two inspections) and a proportion of ware crops. In addition, targeted inspections are undertaken of vegetables at wholesale markets and the public is required to notify any findings. The SG runs regular publicity campaigns, and posters alert the public to the risks associated with this pest. No beetles have been found in potato crops. Occasional findings are made in leafy vegetables and by the public (Table 6).

Table 6. Surveillance for Colorado beetle from 2004-10 (findings in brackets)

	2004	2005	2006	2007	2008	2009	2010
No. potato crops	4606	4410	4407	4540	4777	4831	13681*
	(0)	(0)	(0)	(0)	(0)	(0)	(0)
No. inspected consignments	41	396	51	39	22	77	11
	(1)	(2)	(0)	(1)	(0)	(0)	(0)
No. findings by public	-	-	-	-	-	2	-

* Hectares

Potato cyst nematodes

The measures for control of PCN are explained in Pickup *et al*. (this volume). All land for seed potato production is required to be tested for the presence of PCN. Land where PCN is found is recorded in an official register. Seed potatoes, including farm saved seed, are not permitted to be grown in the land, and ware potato production is only permitted under an official control programme. Figure 1 shows the incidence of the two species of PCN in soil samples taken in Scotland in accordance with both the old and new EU PCN directives (respectively 69/465/EEC and 2007/33/EC) for the last 25 years. Directive 2007/33/EC also requires an annual survey of ware land, but with data only available for one year, the quantity of results from this survey is currently too small for any meaningful interpretation.

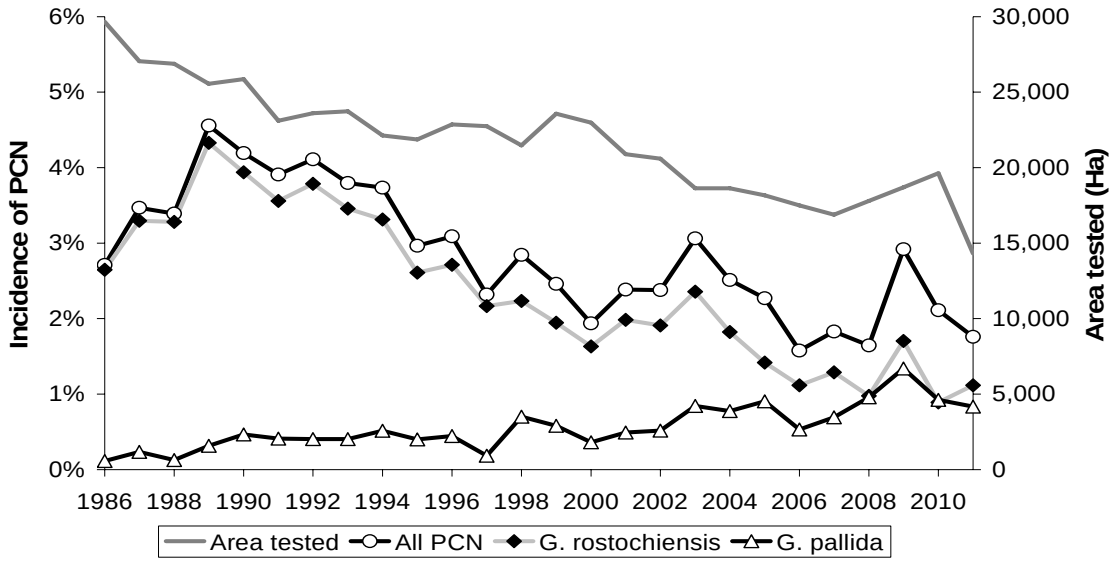

Figure 1. Incidence of PCN (*G. pallida* and *G. rostochiensis*) (left axis) and the area of land tested prior to cropping (right axis).

OTHER MEASURES TO PROTECT POTATO PRODUCTION

Wart diseases susceptibility testing

The EU control directive (69/464/EEC) requires new potato cultivars to be tested for their susceptibility to *S. endobioticum*. SASA tests cultivars for the UK using a modified Glynne-Lemmerzahl method (Browning & Darling, 1995) (Table 7). The directive specifies the measures to be taken in cases of outbreaks of potato wart disease and in the event of an outbreak SASA would test for the wart pathotype using a protocol based on the use of differential cultivars.

Table 7. Number of lines tested for wart and PCN susceptibility

	2004	2005	2006	2007	2008	2009	2010	2011
Lines	7	6	8	8	14	11	9	10

Susceptibility testing for potato cyst nematodes

As with wart disease, directive 2007/33/EC requires Member States to test new cultivars for susceptibility to pathotypes of PCN (Table 7). SASA undertakes this testing for the UK using the method in the directive. Knowledge of susceptibility to different pathotypes is important for management of any PCN outbreaks.

Training and quality assurance

The specific surveillance and monitoring by official inspectors is underpinned by comprehensive annual training in pest and symptom recognition at both growing season and tuber inspections. SG scientists are trained in diagnostic methods and operate at least to ISO 9001 (ELISA, bioassay and PSTVd methods used by UKPQU are accredited to ISO 17025). Where required, methods in the relevant EU directives are used and EPPO diagnostic protocols are also used as the basis for tests. The SG assesses new pest risks based on records of pest occurrences in the scientific literature, EPPO Alert lists and via contacts with colleagues World-wide.

CONCLUSIONS

The SG in partnership with industry stakeholders works hard to ensure the health of potatoes in Scotland. Scotland's high health status has been maintained, despite the liberalisation of the marketing requirements with the introduction of the single market in 1993. This is due to the good collaboration between government, industry bodies and growers.

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FACTORS AFFECTING THE INFECTION OF POTATO BY *RHIZOCTONIA SOLANI*

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Summary: The fungus *Rhizoctonia solani* is an important pathogen of potatoes causing stem canker and black scurf. Isolates of the fungus can be assigned to different anastomosis groups (AGs) on the basis of hyphal compatibility. The aggressiveness of different isolates of *Rhizoctonia solani* representative of four different AGs, plus one binucleate *Rhizoctonia* species, was assessed on potatoes in a controlled environment study. Several isolates caused disease but an AG3-PT isolate was the most aggressive to potato stems causing stem death and the development of sclerotia on mother tubers. The effects of seed and soil inoculum levels were investigated using an isolate of AG3-PT. Stem and tuber symptoms increased with increasing black scurf levels on seed tubers. However, disease severity was independent of soil inoculum level within the 10-fold dilution range used.

INTRODUCTION

Rhizoctonia solani is an important pathogen of potatoes. It is the causal agent of stem and stolon canker which result in quantitative losses. The pathogen also causes qualitative losses through the development of sclerotia on progeny tubers, known as black scurf. When sclerotia are present on seed tubers, they can initiate disease in subsequent crops, although infection can also occur through soil-borne inoculum. In addition to these symptoms, *Rhizoctonia* has also been implicated in causing irregular, misshapen tubers and a range of other tuber blemish symptoms such as elephant hides. Marketable yield losses of up to 30% have been attributed to the disease in some instances (Woodhall *et al*., 2008).

Isolates of *Rhizoctonia solani* can be assigned to one of 13 known anastomosis groups (AG) on the bases of hyphal interaction. Several anastomosis groups of *Rhizoctonia solani* are able to cause disease on potato. In the UK, AG3-PT is considered to be the predominant AG in potato crops, with AG2-1 and AG5 also present in a small number of instances (Woodhall *et al*., 2007). More recently, an isolate determined to be a previously undescribed binucleate *Rhizoctonia* (BNR) species was found causing tuber blemish symptoms in the UK, suggesting the population could be even more diverse.

Knowledge of the AG present is important as it could be considered one of the most important factors determining the development and severity of Rhizoctonia potato disease. For example, some AGs are limited in their ability to infect different parts of the potato plant; Hide & Firmager (1990) observed that AG8 was only capable of infecting potato roots. The AG could also influence disease management, certain AGs have different host ranges; AG2-1 is known to have a wide host range, whilst isolates belonging to the potato sub-group of AG3 are

considered relatively host specific. This could effect survival through crop rotations. Also, some fungicides are known to be selective to certain AGs (Kataria & Gisi, 1999). The fungicide pencycuron is an example of such a selective chemical, with high activity against isolates of AG2-1 and AG3, with little or no activity against isolates of AG5 and AG8.

Other factors influencing disease development is the source of inoculum initiating disease. The relative importance of seed and soil borne inoculum is still yet to be fully determined. Initially, seed-tuber borne inoculum of *R. solani* was often considered to be of primary importance compared to soil borne inoculum (Frank & Leach, 1980). Proximity of the seed-borne sclerotia to the emerging sprouts is thought to lead to consistently severe infections. However, more recent work has highlighted the importance of soil-borne infection (Kyritsis & Wale, 2004). Disease development may be affected by inoculum source, with seed-borne sources associated with severe stem cankers and soil-borne inoculum associated with severe infection of secondary stems, stolons and the development of black scurf. In addition, the relative importance of level of inoculum, for both seed borne inoculum and soil born inoculum has yet to be investigated. Little published work is available to determine the inoculum threshold for disease development to occur.

The aim of this study was to investigate the importance of AG, inoculum source (seed v soil) and inoculum level in causing Rhizoctonia potato disease in a two controlled environment experiments. One experiment compared 12 different *R. solani* isolates representative of AG2-1, AG3 (all three subgroups), AG4 (all subgroups) and AG5 plus an isolate of BNR isolated from a UK potato tuber. In another experiment, the source of inoculum and level of AG3-PT was investigated. Four different levels of soil borne inoculum were tested, along with three different levels of seed born inoculum (asymptomatic, low, high).

MATERIAL AND METHODS

Isolates

Isolates of *R. solani* were maintained on potato dextrose agar (PDA) at 20°C in the dark. Isolates were selected for glasshouse and field experiments on the basis of AG, subgroup, availability and representative pathogenicity. AG was confirmed for each isolate prior to use by real-time PCR and/or DNA sequencing.

Controlled environment experiments

Inoculum for Experiment 1 (to compare isolate aggressiveness) consisted of four 18 mm fully colonised PDA plugs taken from a culture of the appropriate isolate (Table 1). This inoculum was placed on top of mini-tuber (cv. Santé) in a 2 litre pot. Tubers were planted at approximately 15 cm depth.

Inoculum for Experiment 2 (to investigate inoculum level and source) consisted of either infested seed or soil. Soil borne inoculum consisted of pure sclerotia of AG3-PT (isolate Rs08) mixed with compost using a cement mixer to give sclerotia at the following levels (w/w) 0.005%, 0.01%, 0.02% and 0.05%. Sclerotia were prepared previously by growing on PDA for 3-4 weeks and removing with a scalpel and drying overnight. Sclerotia were then chopped into fragments of between 1-3 mm in diameter. Seed-borne inoculum was prepared the previous

year. Healthy tubers (cv. Desiree) were inoculated with isolate Rs08 and grown in controlled environment conditions. The harvested seed was washed, then separated into three batches: asymptomatic seed, infested seed with visual black scurf levels approximately 5% ('low' seed inoculum) and infested seed levels at approximately 15% ('high' seed inoculum). Asymptomatic seed consisted of seed displaying no black scurf or any blemishes associated with *Rhizoctonia*. Presence of *Rhizoctonia* in the asymptomatic seed was confirmed through real-time PCR for AG3 on DNA extracted from tuber peel. Clean seed was also prepared at the same time in un-inoculated soil and used in the control and soil inoculum treatments accordingly. Pots (height 22 cm, diameter 25 cm) were used in the inoculum level/source experiment and each contained 3.75 kg of compost. Tubers for this experiment were in the size range of 35-45 cm.

Table 1. Code, source, anastomosis group, original host, origin and year of isolation for the reference isolates of *Rhizoctonia solani* used in this study

Isolate code	Other codes	AG & subgroup	Original host	Origin
cc2023		2-1	Cauliflower	UK
Y3		2-1	Potato	UK
cc2314		2-1	Cauliflower	UK
rs09B		2-1	Potato	UK
rs08		3-PT	Potato	UK
rs09C		3-PT	Potato	UK
GH3		3-TB	Tobacco	USA
Tom19a		3-TOM	Tomato	USA
cc1903	R25,	4 HG-I	Bean	UK
cc2317		4 HG-II	Cauliflower	UK
44Rs	ATCC 14007	4 HG-III	Sugar beet	Japan
Rs09A		5	Potato	UK
cc43		BNR	Potato	UK

All controlled environment experiments were conducted at 18°C with light for 16 h a day. All plants were watered as required and each treatment consisted of four replicate plants in separate pots containing John Innes No. 3 compost as the growing medium. All plants were harvested after fours weeks and were assessed using the disease keys for stems from Carling & Leiner (1990) The relative surface area of black scurf on the seed tuber was also recorded using the black scurf severity key from Woodhall *et al*. (2008).

Statistical analysis

All statistical analyses were carried out using Genstat release 13 (VSN International Ltd). Analysis of the data from the controlled environment experiment was performed by ANOVA. Where data were skewed, an arcsine transformation was performed. The contrasts directive within Genstat was used to compare treatment levels where many comparisons were possible.

RESULTS

Experiment 1, comparing isolate aggressiveness

Results of the controlled environment trial comparing different isolates representative of several different AGs are given in Table 2. Eight of the isolates tested were able to infect potato stems and were successfully re-isolated from at least one inoculated plant. Two of the four AG2-1 isolates infected potato stems producing slight stem canker lesions, similar to the BNR isolate. Of the AG4 isolates tested, the AG4 HG-II isolate did not appear to infect potatoes whilst the other isolates, representative of the other subgroups did infect potatoes. The AG5 isolate caused relatively severe lesions, second only to the AG3-PT isolate, Rs08, which was the most aggressive isolate tested. This was the only isolate to cause stem death. In addition, sclerotia only developed on the mother tubers of plants inoculated with isolates of AG3-PT. No disease was observed in the non-inoculated control plants.

Table 2. Effect of isolate from different AGs/subgroups on severity of stem canker, stem death, root disease and development of black scurf of mother tuber

Isolate code	AG & subgroup	Stem disease key(0-4)[a]	Stems killed (%)	Mother tuber black scurf severity key (0-5)[c]
cc2023	2-1	0.0 (0.0)[b]	0	0.0 (0.0)
Y3	2-1	0.0 (0.0)	0	0.0 (0.0)
cc2314	2-1	1.1 (6.1)	0	0.0 (0.0)
rs09B	2-1	0.4 (2.4)	0	0.0 (0.0)
rs08	3-Potato	2.9 (9.8)	54.2	2.0 (8.1)
rs09C	3-Potato	0.6 (3.7)	0	1.5 (6.1)
GH3	3-Tobacco	0.0 (0.0)	0	0.0 (0.0)
Tom19a	3-Tomato	0.0 (0.0)	0	0.0 (0.0)
cc1903	4 HG-I	0.4 (2.4)	0	0.0 (0.0)
cc2317	4 HG-II	0.0 (0.0)	0	0.0 (0.0)
44Rs	4 HG-III	1.4 (6.8)	0	0.0 (0.0)
Rs09A	5	2.0 (8.1)	0	0.0 (0.0)
cc43	BNR	1.0 (5.7)	0	0.0 (0.0)
Control		0.0 (0.0)	0	0.0 (0.0)
LSD (P<0.05; df 55)		(1.99)	15.93	(1.55)

[a]Key from of Carling & Leiner (1990).
[b]Arcsine transformed values for non-normal data given in parenthesis.
[C]Key from Woodhall *et al.* (2008).

Experiment 2, investigating inoculum level and source

Disease assessments for plants inoculated with different levels of soil-borne or tuber inocula are given in Table 3. No disease was present in the non-infested soil or asymptomatic seed treatments. Stem infection and stem death were observed in all other treatments. No significant difference (P>0.05) can be seen between plants inoculated at different levels of soil inoculum. The number of stems killed was highest in the plants with high seed inoculum treatment and this was significantly higher than the low seed inoculum plants (P=0.45). In almost all the

AG3-PT inoculated plants, sclerotia developed on the mother tubers irrespective of whether the inoculum originated from seed or soil. The surface area of black scurf on mother tubers was higher where the seed inoculum level was high, compared with the low seed inoculum treatment (P=0.045).

Table 3. Effect of inoculum source and inoculum level on severity of stem canker, stem death, root disease and development of black scurf of mother tuber

Soil inoculum (w/w)	Seed inoculum	Stem disease key[a]	% Stems killed	Mother tuber black scurf severity key (0-5)[c]
0.005	0	3.0	33.3 (31.3)[b]	2.0
0.01	0	2.9	27.1 (27.6)	2.0
0.02	0	2.2	27.1 (27.6)	2.0
0.05	0	2.8	39.2 (38.7)	1.5
0	Asymptomatic	0.0	0.0 (0.0)	0.0
0	Low	1.1	16.7 (17.6)	2.0
0	High	2.6	52.0 (50.1)	4.0
0	0	0.0	0.0 (0.0)	0.0
LSD (P<0.05; df 31)		1.44	(28.11)	0.52

[a]Key from of Carling & Leiner (1990).
[b]Arcsine transformed values for non-normal data given in parenthesis.
[C]Key from Woodhall *et al.* (2008).

DISCUSSION

This study confirms that isolates of AG3-PT are amongst the most aggressive to potato stems. However, other AGs also caused severe stem disease, including isolates belonging to AG2-1, AG4 and AG5. However, only the AG3-PT isolates caused sclerotia to develop on the mother tuber. The development of such sclerotia on progeny tubers could facilitate its survival and dispersal in seed, perhaps at least partially explaining why AG3-PT is the predominant AG in UK potato crops. Isolates from tomato and tobacco subgroups of AG3 failed to cause disease on potato stems, suggesting that these subgroups may be host specific.

Some differences in pathogenicity occurred with the three AG4 subgroups tested. Isolates of AG4 HG-I and AG4 HG-III both caused disease whilst AG4 HG-II did not. Isolates from these subgroups have previously been isolated from solanaceous weeds in Brazil and shown to have pathogenicty to potato (Silva-Barreto *et al*., 2010). Although AG4 HG-II is widespread in the UK (Budge *et al*., 2009) it has never been reported in UK potatoes, suggesting that this subgroup of AG4 is of little concern when considering potato diseases in the UK. Amongst AG2-1 isolates, a wide range of pathogenicity existed. AG2-1 is a genetically diverse group and also one with a wide host range. A large variation in pathogenicity to potato has been observed previously (Woodhall *et al*., 2008) which was why four isolates of AG2-1 were tested in this experiment. Further work to investigate the determinants of pathogenicty and diversity amongst AG2-1 is required.

In this study, infected soil from as little as 0.005% (w/w) and sclerotia infested seed both caused significant levels of stem lesions and stem death. Considerable disease developed at the lowest soil borne inoculum level (0.005%) suggesting the inoculum threshold for disease to occur is somewhat lower. Further work is required to determine this threshold and if it is affected by environmental conditions such as moisture and temperature. Asymptomatic tubers which were PCR-positive failed to cause stem disease. Therefore the presence of a positive PCR from tuber DNA may not always be an accurate indicator of disease.

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THE EFFECT OF *SPONGOSPORA SUBTERRANEA* SOIL INOCULUM LEVEL ON POWDERY SCAB

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Summary: Field trials were carried out over 2 years to ascertain whether a link between initial levels of soil-borne *S. subterranea* inoculum and powdery scab on progeny tubers could be established. In each year, four inoculum levels were created, including an un-inoculated control. These inoculum levels were within the range previously observed in commercial potato fields in Britain. Two cultivars were compared, the relatively powdery scab susceptible cv. Agria, and the more resistant cv. Nicola. *In situ* monitoring of soil temperature and moisture content enabled disease to be related to environmental conditions. In both years, disease incidence and severity was found to be significantly greater in plots with more dried potato peel incorporated, and cv. Nicola had significantly less incidence and severity of disease than cv. Agria. These experiments illustrate that quantifying initial levels of soil-borne *S. subterranea* inoculum can be used to assist in the prediction of disease risk. In addition the use of resistant cultivars has been confirmed as a robust method of reducing disease risk where inoculum is present.

INTRODUCTION

The relationship between soil inoculum level of *S. subterranea* and the incidence and severity of powdery scab is not fully understood. The biphasic nature of the life cycle ensures that the pathogen is both persistent, with the production of resting spores, but also capable of very rapid multiplication through the formation of secondary zoospores given suitable environmental conditions. A high initial inoculum level offers the pathogen a greater opportunity to infect and cause disease, thereby establishing a link between initial inoculum level and disease. However, due to the complex interactions between environmental conditions, root infection, primary and secondary infection cycles and host resistance, disease development may be high, irrespective of initial inoculum levels. This may explain why it has not been clear whether a relationship between initial soil inoculum levels and disease incidence and severity exists given the contradictory findings of a number of studies. Parker (1984), Christ (1989) and van de Graaf *et al.* (2005) found no relationship between inoculum level and disease whereas Qu *et al.* (2006) and Nakayama *et al.* (2007) did note a relationship.

The aim of these experiments was to establish the relationship between increasing initial inoculum levels in the soil and the development of powdery scab on progeny tubers in a field trial repeated over two years. Further work will ascertain whether environmental conditions at key developmental stages, which lead to higher levels of disease than predicted by initial inoculum levels can be identified. Results from this research will enable diagnostic soil tests

for powdery scab to be better interpreted in terms of the associated disease risk and will aid disease management decisions.

MATERIALS AND METHODS

In 2009 and 2010 field trials were set up to investigate the effect of soil inoculum level on powdery scab development at final harvest. The powdery scab susceptible potato cultivar Agria and the moderately resistant cultivar Nicola were compared. All seed was kept at 4°C in the dark until required. For each seed stock 50 tubers were assessed visually for powdery scab symptoms.

In each of the two years four main plots were created, each was amended with different amounts of dried tuber peel taken from tubers heavily infected with powdery scab (Levels 1-4 where Level 1 received no additional inoculum and levels 2 to 4 received increasingly greater amounts). After the inoculum had been incorporated into the drills, soil samples (25 x 10g samples bulked) were taken from each main plot for assessment of *S. subterranea* level (spore balls / g soil) as determined by real-time PCR. Soil DNA extractions were carried out according to the methods of Brierley *et al.* (2009) and the amount of *S. subterranea* DNA detected using the assay of van der Graaf *et al.* (2003) was expressed as spore balls /g soil.

Each main plot consisted of 4 rows of 12 plants, surrounded by guard rows and separated by at least 2 rows. Two reps of each cultivar were planted in each main plot. Trials were irrigated. *In situ* monitors (Delta-T devices) recorded soil temperature and moisture content during each season. At the time of final harvest, all progeny tubers from each plot were assessed for powdery scab using a score of 0 to 6, depending upon the percentage coverage of symptoms http://www.spongospora.ethz.ch/LaFretaz/scoringtable.htm. Results were expressed as the mean incidence and severity score of powdery scab per plot. For illustrative purposes, symptom coverage of tubers with a score of 1 and 4 are shown in Figure 2.

RESULTS

Seed and soil

Visual assessment of seed stocks revealed that both cultivars had low levels of powdery scab contamination (cv. Agria 10 and 14 %; cv. Nicola 14 and 44 % incidence in 2009 and 2010 respectively). Mean severity did not exceed 0.9 for either cultivar in either year.

The range of inoculum detected using real-time PCR in the main-plots ranged from close to zero to 57 sporeballs / g soil (Table 1), which is within the range found in fields used in commercial potato production, 0 to 148 sporeballs / g soil (Brierley *et al.*, in prep). The amount of inoculum detected in Level 4 using real-time PCR was lower in 2010 than 2009. The amount of inoculum detected in Level 3 (which received only one tenth of the dried potato peel added to Level 4), was approximately 10 fold lower than that detected in Level 4. However as inoculum levels below 1 sporeball / g soil may be detectable, but not accurately quantified, there was no discernable difference between detectable inoculum levels in Levels 1 and 2 in 2009, and between Levels 1, 2 and 3 in 2010, as all these soils had less than 1 sporeball/ g soil.

Table 1. *Spongospora subterranea* inoculum (sporeballs/ g soil) quantified in main-plots inoculated with varying levels of sporeballs.

	Soil inoculum (spore balls / g soil)			
	Level 1 (no added inoculum)	Level 2 (1:50 dil of high)	Level 3 (1:10 dil of high)	Level 4 (high)
2009	1.2	0.1	3.7	57.2
2010	0.6	0.7	0.6	5.7

Effect of inoculum level

In both 2009 and 2010, there was a significant difference (ANOVA: $p<0.01$) between both the incidence and severity of disease in both cultivars in plots with more dried potato peel added (Figure 1). However, the differences were more marked in 2009 than 2010. Progeny tubers from plots to which no inoculum had been added (Level 1) had a mean disease severity score of approximately 1, in contrast to progeny tubers from Level 4 plots which had a mean disease severity score of 4 in 2009 and 3 in 2010. The difference in symptom coverage between tubers with a score of 1 and 4 are shown in Figure 2.

Cultivar resistance

Maximum disease incidence in cultivar Nicola (moderately resistant) was 72% and cv. Agria (susceptible) 99%, with disease severity scores of 1 and 3.9 respectively. In both years disease incidence and severity scores were significantly greater in cv. Agria compared with cv. Nicola (ANOVA: $p<0.01$).

Environmental conditions

Conditions around tuber initiation, approximately 49 days after planting (DAP), were relatively cool (15-18 °C) in both years, but the soil was wetter in 2010 than 2009 as indicated by a lower soil moisture deficit (Figure 3).

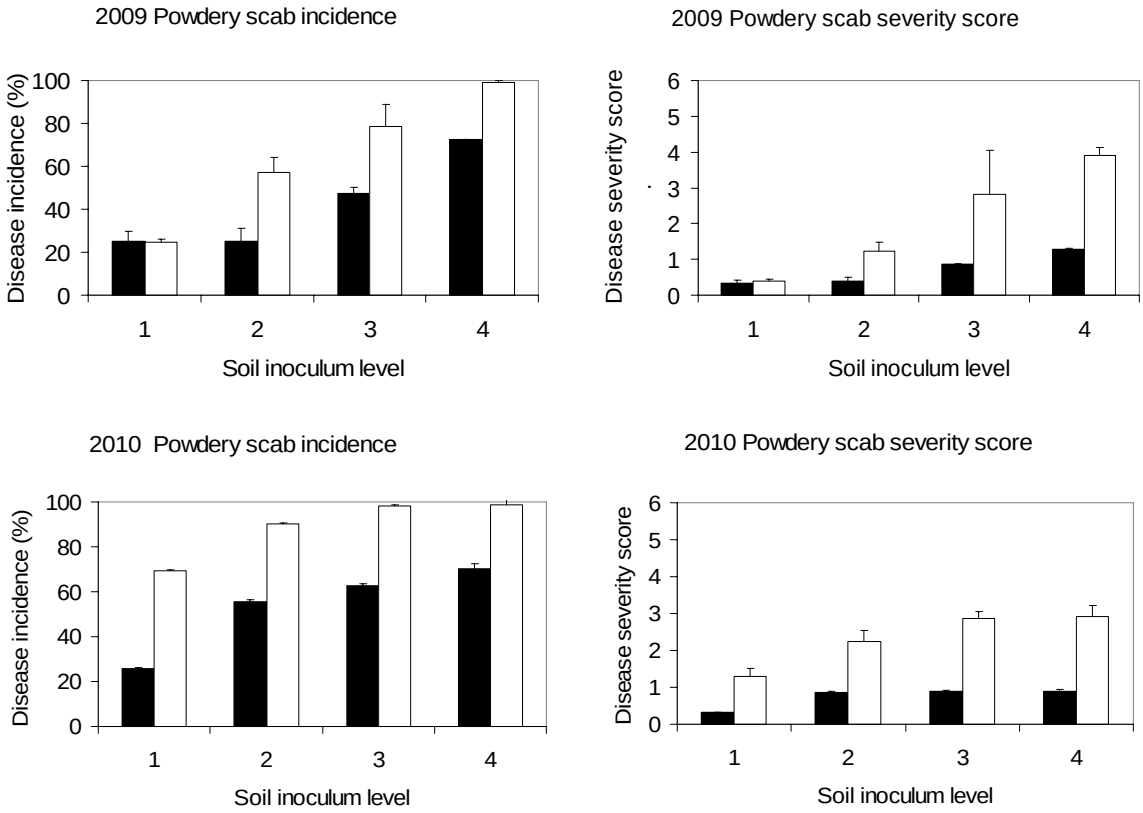
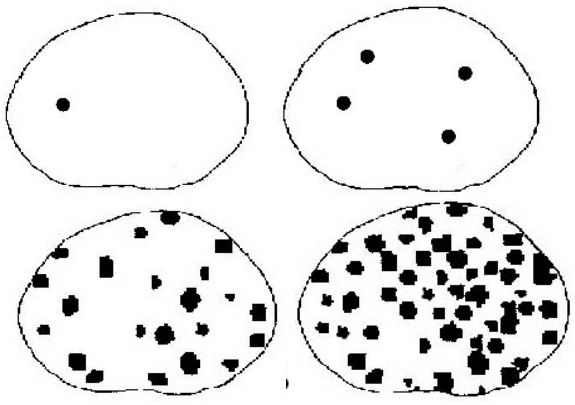


Figure 1. The effect of soil inoculum level on powdery scab incidence and severity in two cultivars, Nicola (black) and Agria (white), in the 2009 and 2010 field trials. Mean of 2 replicate plots + SE.



Score 1: Symptoms covering up to 2 % surface area of tuber

Score 4: Symptoms covering 10 to 25 % surface area of tuber

Figure 2. Range of disease coverage on tubers with a score of 1 and 4.

DISCUSSION

Different amounts of dried potato tuber peel incorporated into plots to give increasing levels of initial soil inoculum (Levels 1-4) significantly affected the amount of powdery scab on progeny tubers. Brierley *et al.* (in prep) found that of 113 soils used in commercial potato production in Britain which were tested for *Spongospora subterranea* spore ball contamination, 20 had undetectable levels and 73 had detectable levels of less than 10 spore balls / g soil. It is difficult to make direct comparisons with the majority of other studies regarding levels of sporeballs, as different methodologies have been used in their quantification. However, Nakayama *et al*. (2007) found a relationship between natural soil borne inoculum (sporeballs /g soil) and disease in commercial potato fields, but recorded a number of incidences where disease developed without any inoculum detected in the soil. They used conventional PCR techniques to quantify soil inoculum, and it may be that with the increased sensitivity of the real-time PCR assay, inoculum would have been detected, albeit at low levels in a number of these soils. However, other studies investigating the affect of soil inoculum on disease may have used higher inoculum levels than those used in the study described here, thereby masking the relationship between inoculum level and disease seen when soil inoculum is at a level of 10 spore balls / g soil or less. For example, van de Graaf *et al.* (2005) found no difference in the amount of disease observed when between 5 and 50 sporeballs / g soil were added. With the development of a reliable method for the extraction of DNA from soil (Brierley *et al.*, 2009) followed by quantification of *Spongospora subterranea* using real-time PCR (van de Graaf *et al.*, 2003) we have successfully determined levels of inoculum found in field soils in Britain and established a relationship between initial inoculum level and disease (Brierley *et al.*, in prep). The experiments described here add to this body of evidence that links soil inoculum levels of *S. subterranea* to an increasing risk of disease.

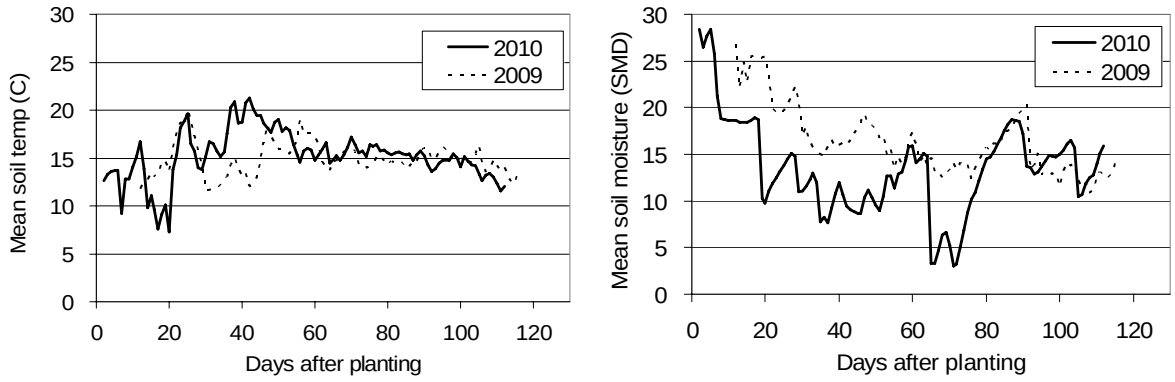


Figure 3. Soil temperature and moisture in field trials in 2009 and 2010.

The occurrence of approaching 100 % incidence of disease in the more susceptible cultivar Agria is an indication that conditions were favourable for disease development in both years. This is supported by the *in situ* monitoring data, which shows that the soils were both cool and damp throughout both the 2009 and 2010 growing seasons. In general, more disease was found in the main plots which had low initial inoculum levels (Levels 1 and 2) in 2010 than the corresponding plots in 2009. Conditions around tuber initiation, approximately 49 days after planting (DAP) in both years, were relatively cool (15-18 °C), but the soil was wetter in 2010 than 2009, as indicated by a lower soil moisture deficit. These conditions may have resulted in a greater degree of secondary infection in 2010 than 2009, thereby increasing the level of

disease above that predicted from initially low inoculum levels. However, the mean maximum disease severity score of cv. Agria in Level 4 was lower in 2010 than in 2009. If relatively high levels of disease arising from low initial inoculum levels (Levels 1 to 3) can be attributed to soil wetness, why was disease in the highest inoculum level (4) not greater? The interactions between soil moisture and inoculum level on root infection and disease development are being investigated further.

In conclusion, these experiments add to an increasing body of evidence demonstrating that quantifying levels of soil-borne inoculum can assist in the prediction of disease risk, with the caveat that given particular environmental conditions, low levels of inoculum may cause severe disease. The use of resistant cultivars is a robust method of reducing disease risk where inoculum is present.

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POTATO CYST NEMATODES: INITIAL IMPRESSIONS OF THE IMPACT OF THE NEW EU DIRECTIVE IN SCOTLAND

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Summary: Since 1 July 2010 when Directive 2007/33/EC on the control of PCN came into force, implementation of the new statutory measures by the Scottish Government has resulted in an increase in the rate of pre-crop soil sampling of 114%. SASA has increased its capacity for soil testing by introducing new methodologies: automated cyst extraction, PCR diagnostics and a bespoke data management system. Growers now receive their results sooner and increases in the costs of soil testing have been minimised. For 2011, there was a 27% decrease in the area of land submitted for PCN testing (c.f. 2010), however following simplification of SPCS rules, the land tested and unconditionally cleared for seed potato production increased to 94.7% from 88.7% in 2010. The incidence of PCN in samples was 1.8%, similar to 2010, whilst the area recorded as infested increased from 1.8% to 5.3%. The proportion of the two species of PCN found in 2011 was consistent with the proportions recorded in recent years. There was an overall increase of 494 ha in land recorded as infested and it is likely that the 2007 Directive will result in further increases in the amount of land recorded as infested in future years.

IMPLEMENTATION OF DIRECTIVE 2007/33/EC

Directive 2007/33/EC on the control of PCN came into force on 1 July 2010. This new Directive replaced the previous Directive 69/465/EEC, laying out a legislative framework appropriate for the control of a quarantine pest across the European Union (Pickup, 2008). Whilst many provisions of the 2007 Directive are obligatory, such as a harmonised methodology for soil sampling, EU Members States are allowed flexibility for implementation of other provisions, such as the definition of a field and the movement of farm saved seed grown on untested land. The implementation of the 2007 Directive by the Scottish Government followed a lengthy consultation with a range of stakeholders representing the potato industry.

Harmonized soil sampling

A pre-planting soil test result clear of PCN remains a requirement for seed potato production and the 2007 Directive harmonizes the sampling rates used throughout the EU. A 'standard' rate of 1500 ml/ha is introduced, although a 'reduced' rate of 400 ml/ha can be used where the previous history of testing and cropping of the land indicates a low probability of finding PCN. The 'reduced' rate may be used if the land has not grown potatoes in the previous six years, or if records of tests show a history of PCN freedom. As more soil is taken from larger fields, the chance of detecting PCN increases with field size, assuming similar underlying levels of infestation. In recognition, lower sampling rates are applied if growers wish to have their fields tested as larger units: the 'standard' rate is reduced from 1500 ml soil/ha to 400 ml/ha once the field size exceeds a threshold of 8 ha; and the 'reduced' rate is further reduced from 400 ml/ha to 200 ml/ha for fields over a 4 ha threshold. The disincentive for the grower is that the whole of the sampled unit is recorded as infested if any PCN are found.

Charging

In Scotland a rate of 600 ml of soil drawn from a unit of up to 4 ha had been used to meet the soil sampling commitments under the 1969 Directive. For the 2007 Directive the number of samples to be drawn and tested was expected to increase three-fold. This would require extra staff and resources for field sampling and laboratory processing. After further consultation, a fee was set at £13.81 per 400 ml sample to cover the additional costs introduced by the increased level of soil sampling in the 2007 Directive. It was hoped that by introducing charges for PCN tests, growers would only submit land intended for seed potato production land for testing. In the past the area of seed potatoes entered for classifications has typically amounted to about 61% of the area tested for PCN.

Farm-saved seed

Unlike the 1969 Directive, the 2007 Directive does not discriminate between seed potatoes "intended for marketing" and other potatoes to be planted; i.e., all are now viewed as "seed potatoes" presenting similar risks of disseminating PCN. The benefits of imposing controls on classified seed are undermined if farm saved seed may be moved without similar controls. Therefore, all seed potatoes must now be produced on land which has been found clear of PCN, unless they are to be planted "within the same place of production". There is scope for local interpretation of the area within which farm saved seed grown on untested land can be used. For Scotland, the place of production is defined as the same holding on which the potatoes were produced. Holdings are identified by farm code and where they have been amalgamated with holdings under separate farm codes, land is only to be considered as "on the same holding" if it is in the same parish, or contiguous across a parish boundary.

Control programme for ware production on infested fields

The 2007 Directive allows ware potatoes to be grown in fields recorded as infested, provided the field is subject to "an official control programme aiming at least at the suppression of potato cyst nematodes". A suitable programme is likely to include the use of resistant varieties and recognised control measures such as nematicide applications, combined with suitable rotation periods. The PCN model developed for the Potato Council is used by Inspectors of the Scottish Government: Rural Payments & Inspections Directorate (RPID) to evaluate the

detailed control programmes submitted by growers. As there are few commercial varieties currently available that have significant levels of resistance to *G. pallida*, control programmes suitable for land infested with this species are likely to rely more on rotational or nematicide control.

Legislation and Guidance

Following consultation with representatives of the potato industry, the 2007 Directive was implemented in Scotland by the Plant Health (Scotland) Amendment Order 2010, laid before the Scottish Parliament on 21 May, 2010. Fees were also introduced for PCN testing under the Plant Health Fees (Scotland) Amendment Regulation 2010. The new system, which applies to all crops planted from 2011 onwards for the production of classified seed potatoes or farm saved seed, and the fees applicable, is set out in guidance notes (SASA, 2010a). Growers who want to grow ware potatoes on land where PCN has been found (including land which is "scheduled" under the old system) need to agree an Official Control Programme to suppress the PCN populations with staff from their local RPID office (SASA, 2010b).

Under the 1969 Directive, seed production was prohibited on infested land and ware production limited to fully resistant varieties, unless crops were either harvested prior to cyst production or planted following disinfestation of the field. The rules of the Scottish Seed Potato Classification Scheme (SPCS) made maximum use of the information provided by the soil test in the interpretation of soil testing results. As dead cysts at a pre-crop test provide evidence of prior infestation, land was retested where they were recorded and then, if no live cysts were found, certificates were issued giving restricted clearance for seed production of varieties resistant to *G. rostochiensis*. Similar restrictions were also applied to units adjoining infested land or units awaiting retests. Under the 2007 Directive, such additional control measures are not a requirement and are omitted from the SPCS rules. As the sampling rate adopted in Scotland to implement the 1969 Directive had been one of the lowest rates within the EU, the introduction of a harmonized sampling rate with the 2007 Directive, removed the justification for such additional control measures.

NEW PCN TESTING PROCEDURES

Data and business management - SPUDS

From 2010, all PCN tests carried out under the 2007 Directive are managed under SPUDS (Seed Potato Universal Data System); a project implemented by SASA to revise all the business processes involved in seed potato classification, including the provision of a new IT system. The sketch map of the field to be tested is submitted by the grower to SPCS administration at SASA where it is translated onto an Ordnance Survey map and a sampling pattern is generated by SPUDS. A receipt showing the sampling pattern and stating costs is sent to the grower for confirmation. If the grower is content, an RPID inspector will sample fields according to the map and pattern set out on the receipt, and attach bar-coded labels generated by SPUDS to the samples. These 400 ml samples are returned to the RPID area offices, crated and collected by SASA. The transfer of the mapping work to SASA releases RPID field staff from drawing maps and hand labelling sampling bags. In addition growers have the opportunity to review the interpretation of their sketch maps before the land has been tested.

Cyst extraction and diagnostics

After arrival in the Nematology Laboratory at SASA, the soil samples are dried, and any cyst nematodes present in the soil are extracted using a carousel developed specifically for SASA by MEKU Pollähne, Wennigsen, Germany. The carousel automates the process of cyst extraction, based on the same principles of sieving and flotation used previously at SASA (Fenwick, 1940). Trials using soil samples seeded with a known number of PCN cysts have demonstrated a cyst recovery rate for the carousel of 93%. This figure compares well with the minimum detection efficiency of 60% recommended in a recent report covering proficiency tests carried out across a series of EU PCN testing laboratories (Ladeveze & Anthoine, 2010). The automation of the carousel enables one person to process 25 samples per hour, compared with 7.5 samples per hour using the Fenwick Can process. It also has an environmental benefit of using less water per 400 ml sample (21 litres c.f. 90 litres).

Although samples from a small minority of fields are analysed by visual examination where it is important to know whether dead cysts are present, most extracts progress directly to an automated high throughput polymerase chain reaction (PCR) diagnostic technique developed at SASA (Reid *et al*., 2010). This is a novel PCR method which uses the entire 'float' (debris) extracted from the carousel and eliminates the need to visually extract PCN cysts from the float. A first PCR assay is completed to confirm the presence of *Globodera* spp. and a second assay provides confirmation and diagnosis to species. Using the automated PCR, one person can carry out diagnoses on up to 300 samples per week, compared with the visual examination of a maximum of 65 samples per week. The PCR diagnostic is also used to support visual examinations. Once a single PCN cyst has been confirmed, the diagnosis on the float can be completed by PCR. Using bar-coded samples, SPUDS tracks sample progress, records diagnostic information and provides letters notifying test the growers of their test results.

PCN TESTING FOR 2011 GROWING SEASON

Area tested and overall sampling rate

As a result of applications for PCN testing of 1656 fields in advance of potato planting for 2011; 14,150 samples of 400 ml were drawn from land covering a total area of 14,320 ha. This equates to 395 ml of soil/ha. In comparison to testing for the 2010 season under the 1969 Directive; 6059 first samples of 600 ml were drawn from 19,627 ha which equates to 185 ml of soil/ha. Based on the data from these two years, the introduction of the 2007 Directive has increased the sampling rate by 114%. The total area tested for 2011 is down by 27% c.f. 2010. A decrease was expected as the results of tests over the previous three years remained valid for the 2011 crop and the introduction of charging encouraged growers to take advantage of this 'banking' of previous years' test results. Data for testing for the 2012 season will be required to provide a clear picture of the impact of the 2007 Directive on the area of land tested. Only 410 ha of land was submitted for testing at the standard rate (1500 ml/ha), therefore the majority of the land tested (97%) was sampled at the lower rate (400 ml/ha). Of this 410 ha, 267 ha (65%) was sampled for the purpose of derecording.

Comparing the proportion of samples taken from different geographical areas between 2010 and 2011, testing decreased from 35% to 28% in Grampian (area covered by RPID offices in

Elgin and Inverurie), but increased elsewhere. The additional controls on farm saved seed may contribute towards the proportionate increase in soil sampling in the areas outside Grampian.

Table 1. Summary of RPID/SASA PCN testing 2010 & 2011

RPID Area Office	2010 Samples	2011 Samples	2010 Samples with PCN	2011 Samples with PCN	2011 Area with PCN
Perth	3245 (54%)	8254 (58%)	84 (2.6%)	207 (2.5%)	7.5%
Inverurie	1785 (29%)	3406 (24%)	19 (1.1%)	27 (0.8%)	3.4%
Inverness	351 (6%)	900 (6%)	1 (0.3%)	0 (0%)	0.0%
Galashiels	305 (5%)	884 (6%)	0 (0%)	3 (0.3%)	1.6%
Elgin	306 (5%)	502 (4%)	3 (1.0%)	3 (0.6%)	2.3%
Other areas	59 (1%)	204 (1%)	1 (1.7%)	9 (4.4%)	6.8%
Total	6051	14150	108 (1.8%)	249 (1.8%)	5.3%

Incidence of positive PCN test results

Table 1 shows an increase in samples failing for PCN for both 2010 and 2011 in proportion to the increase in the total samples. The overall incidence of positive diagnoses for PCN was similar for the two years at 1.8%. As the sample size decreased from 600 ml to 400 ml, detection efficiency can be interpreted as having increased. However, the comparable figure for 2009 was 2.9% so it is unlikely that a statistically significant change in the detection efficiency will be demonstrated until several years of testing data are available. Following implementation of the 2007 Directive, several samples are usually taken from each sampling unit, so the area of land testing positive for PCN has increased from 1.8% to 5.3%. Simplifying the SPCS rules has limited the implications of a positive PCN test to just the sampled unit of land and therefore the remaining 94.7% of the land tested receives unconditional clearance for seed production. For 2010, only 88.7% of land tested received such unconditional clearance as a result of either the presence of dead cysts or the proximity to infested areas.

Incidence of *Globodera pallida* and *G. rostochiensis*

Of the 249 samples containing viable PCN, *G. pallida* was present in 118 (47%) and *G. rostochiensis* was present in 158 (67%) (27 samples contained both species of PCN). In 2010, viable PCN were found in 108 samples, with *G. pallida* present in 54 (50%) and *G. rostochiensis* present in 56 (52%) (2 samples contained both species). Although *G. pallida* has been present in an increasing proportion of PCN samples in recent years, the 2011 data are consistent with this pattern and the levels of year-to-year variation.

Comparison of analysis methods

Of 14,150 samples, 5503 (39%) were examined using the visual method and 8647 (61%) by PCR on the entire float. The incidence of PCN in the visually examined samples was marginally higher (1.8%) compared with those diagnosed by PCR (1.7%). Care should be taken in drawing conclusions on the comparability of the two methods from these results as there is bias in the selection of the two data sets. The method of analysis was determined by

the previous history of PCN testing for the sampled unit. Samples examined visually originated from land entered for derecording and land for which no previous test results were available, whereas the samples diagnosed by PCR would have been taken from land recently tested (i.e. since 2000) and found free from PCN.

Growers submitted 72 soil test applications requesting sampling for the derecording (descheduling) of 267 ha of land under quarantine due to PCN infestation. PCN cysts (viable and non-viable) were found in 205 ha (77%) of land submitted for derecording – up from 49% in 2010. Viable PCN were found in 89 ha or 33% of the land tested (up from 14% in 2010). The remaining 178 ha were therefore derecorded. As the standard sampling rate (1500 ml/ha) is used for derecording, PCN are more likely to be detected when compared with either sampling at the lower rate or at the rate used under the 1969 Directive. Therefore, an increase in PCN detection in land submitted for derecording would be expected.

Overall Change in Infested Land

As viable PCN were found on 761 ha (including derecording samples) and 267 ha were entered for descheduling, the net change in the area of scheduled land following the 2011 pre-crop PCN testing programme, was an increase of 494 ha. The implementation of the new PCN Directive is likely to lead to an increase in the total area of land recorded as infested with PCN which had remained relatively unchanged since 1996.

Laboratory efficiency

Considerable efficiency savings have been achieved through the redesign of the laboratory methodology and the SPUDS data management system as described above. During autumn 2010, the nematology laboratory reported test results on an average of 790 ha of land per week for 2011. In comparison to the previous year (using the old method and the lower sampling rate), an average of 375 ha per week was reported on over the same period. Consequently, following the introduction of the new methodology, 10,000 ha had been tested and reported by 20 December in advance of the 2011 season. For the previous season, it took until 1 February to complete the testing of a similar amount of land.

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THE GENOME SEQUENCE OF THE POTATO CYST NEMATODE *GLOBODERA PALLIDA*: AN AID TO THE DEVELOPMENT OF CONTROL STRATEGIES

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Summary: A genome sequencing project for the potato cyst nematode *Globodera pallida* is nearing completion. The current assembly of the genome is thought to contain over 85% of the genes likely to be present in the nematode. One of the uses of the genome sequence is identification of the full suite of nematode genes that encode proteins with important roles in the host-parasite interaction - effectors. We have developed assays that allow the function of nematode effectors in various important biological processes to be determined. Application of these assays has allowed us to identify nematode proteins that target the plant nucleus and that suppress the defence response of plants. These proteins represent potential control targets and may be recognised by host resistance genes. Ways in which the *G. pallida* genome sequence can be used to help develop control strategies are discussed.

INTRODUCTION

Globodera pallida, the white potato cyst nematode (PCN), is the most economically important plant parasitic nematode in the United Kingdom and also causes problems for growers in many other parts of the world. Potato cultivars containing the H1 resistance gene provide complete control of the *G. rostochiensis* pathotypes present in the UK and repeated use of these cultivars is thought to have led to selection of *G. pallida.* One survey in England and Wales has found that *G. pallida* is present in 65% of fields used for growing potatoes (Minnis *et al*., 2002). The absence of major gene resistance against *G. pallida,* coupled with the recent withdrawal of many effective nematicides as a result of changes to EU legislation, has led to problems in controlling this nematode. New strategies for control of *G. pallida,* including new tools for breeding resistance, are therefore required. The problems caused by *G. pallida,* and recognition of the need for a fresh approach in the way that control strategies are developed, convinced BBSRC to fund a collaborative project aimed at generating a genome sequence for this nematode.

G. pallida is a biotrophic pathogen. It induces the formation of a feeding site in the roots of its hosts on which it depends for all the nutrients required for development. Like other biotrophic pathogens it needs to suppress host defences in order to keep the feeding site alive and

complete its life cycle. Nematode effectors, proteins and other factors that induce the formation of the feeding site and suppress host defences, are produced in three gland cells (two subventral and one dorsal) and secreted into the host from a hollow, protrusible stylet.

Plant defences are thought to operate in a multi-layered system described by the Zig-Zag model (Jones & Dangl, 2006). Most microorganisms are detected by pattern recognition receptors that detect highly conserved pathogen molecules (Pathogen Associated Molecular Patterns – PAMPs) and induce PAMP Triggered Immunity (PTI). Successful biotrophic pathogens introduce effectors into their hosts that suppress PTI. In a second layer of plant defences these effectors, or their biological activity, are detected by host resistance proteins. Understanding the function and targets of pathogen effectors may therefore provide information about the operation of natural resistance in the host.

Although significant progress has been made in recent years in identifying and characterising effectors from *G. pallida* and other cyst nematodes (*e.g.* Jones *et al*., 2011), details of their function and how they interact with host targets are lacking. One of the major areas of research to be developed from the *G. pallida* genome sequence at The James Hutton Institute (JHI) has therefore been further analysis of effectors.

MATERIALS AND METHODS

The genome sequence for *G. pallida* was obtained using 454 and Illumina sequencing of DNA extracted from adult female nematodes. Assembly and gene prediction was carried out using a range of standard software packages including Roche Newbler gsAssembler (Margulies *et al.*, 2008), Velvet (Zerbino and Birney, 2008) and IMAGE (Tsai *et al.*, 2010). In addition, RNA was extracted from several life stages of *G. pallida* (eggs, J2, parasitic nematodes 7 days post infection (dpi), 14 dpi, 21dpi and 28 dpi and adult males) and used for Illumina transcriptome sequencing. These RNAseq reads were mapped to the *G. pallida* genome sequence using TopHat (Trapnell *et al.*, 2009) in order to aid gene finding and to allow quantitative assessment of gene expression levels across the life stages. Novel effectors were identified using scripts to identify secreted proteins upregulated in parasitic or invasive stages followed by *in situ* hybridisation to confirm gland cell expression. Homologues of effectors from other plant parasitic nematodes were identified using BLAST searching of the assembled *G. pallida* sequence. Effectors that suppress PTI or Effector Tirggered Immunity (ETI) were identified using previously reported assays (Gilroy *et al.,* 2011; Oh *et al.,* 2010).

RESULTS

The current assembly of the *G. pallida* genome, which is being used for gene finding, is 132.4 Mb long and is composed of 9,196 supercontigs. The average supercontig length is slightly below 14,400 bp with the longest just over 600Kb. The size of this assembly is probably an overestimate as some bacterial contaminating sequence still needs to be removed. Although the assembly is still fragmented compared to other published plant parasitic nematode genomes, it is anticipated that the number of fragments will decrease as information from further sequence runs and optical mapping is incorporated. Removal of bacterial contigs will also reduce fragmentation levels. A search of the genome assembly with a dataset of 248 highly conserved single copy eukaryotic genes from other organisms (CEGMA analysis)

showed that 85.9% of these sequences had matches in the current *G. pallida* assembly. Identification of the full complement of genes present in the sequence is currently in progress. However, preliminary data suggest that approximately 22,000 genes may be present. This is a higher number of genes than is present in the genomes published for other plant parasitic nematodes.

Duplicate RNAseq runs have been obtained for all life stages examined. These are being used for gene finding by mapping reads to the assembled sequence in order to identify transcribed regions (Figure 1). Comparing the depth of RNA sequence coverage in different life stages is also allowing quantitative analysis of gene expression (see below).

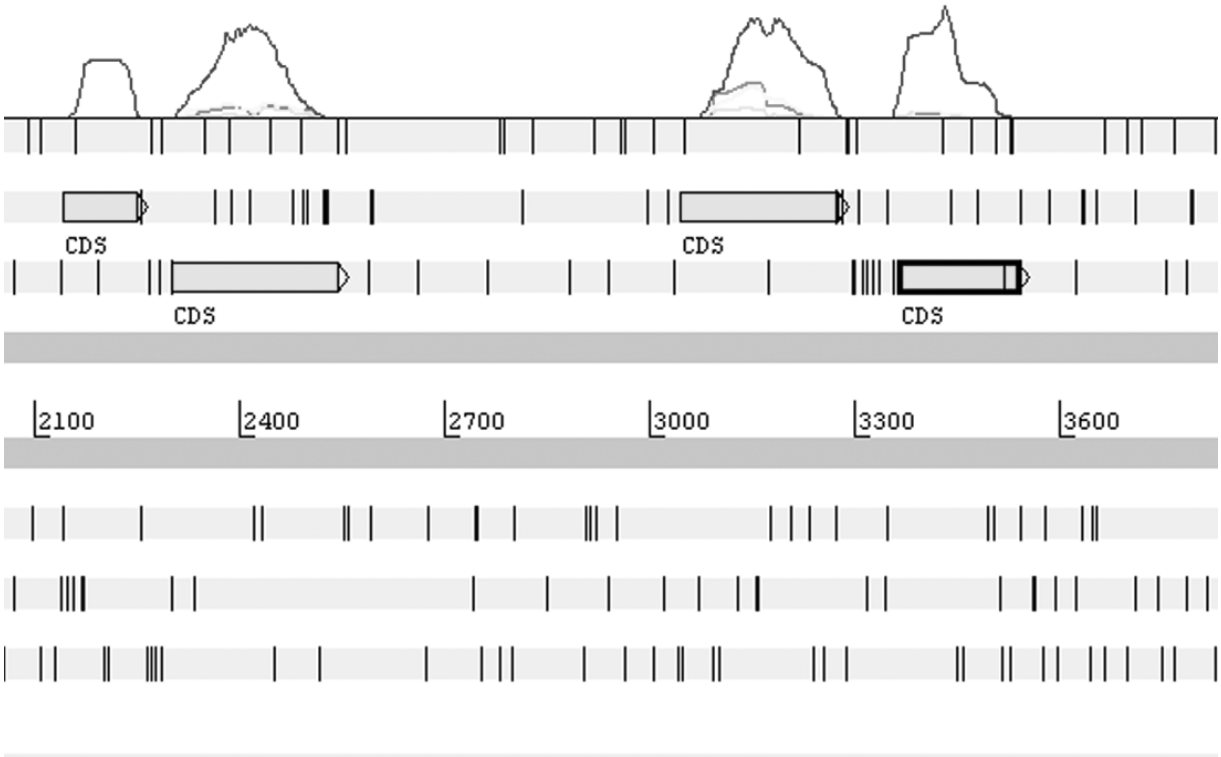

Figure 1. Mapping of RNA sequence reads from *G. pallida* to the assembled genome sequence allows identification of transcribed regions. The graph (top track) plots RNA sequence read depth at a given position for different life stages (each line representing one life stage). Regions of no coverage between graph peaks often represent introns.

A variety of bioinformatic approaches have been used to identify candidate effectors from the *G. pallida* genome sequence (Jones *et al.,* 2009). One interesting finding from this work is the observation that there is almost no overlap between the effectors present in cyst nematodes and root knot nematodes. Despite their close phylogenetic relationship and the superficial similarities in the feeding structures that they induce in their hosts, root knot nematodes and cyst nematodes are thought to have evolved the ability to maintain biotrophic interactions with

plants separately and the differences in their effector complements most likely reflect this fact. Most *G. pallida* effectors are present in small gene families but one gene family, the SPRYSECs, contains over 300 members. RNAseq analysis has shown that all SPRYSECs examined to date are expressed solely in the invasive stage juvenile, with no expression detected at later parasitic stages. By contrast, other effectors show greatly elevated expression at parasitic stages with no transcripts detected in the invasive stage. This data allows effectors with functional roles at different phases of the feeding site induction and maintenance process to be identified. We are currently focusing on further functional analysis of a panel of 30 *G. pallida* effectors, including a selection of SPRYSECs. Effectors that suppress both PTI and ETI have been identified in this panel.

DISCUSSION

We have generated a draft genome sequence for *G. pallida* and have also generated transcriptome sequence from a wide range of life cycle stages of this nematode. This data will improve the genome sequence by providing a measure of expression levels of every gene in the genome at each stage of the life cycle. The availability of a genome sequence for *G. pallida* offers the prospect of a step change in the way that we investigate the biology of this organism. For example, identification of new genes, such as nematode specific neuronal genes or important components of essential pathways will provide new targets for novel nematicides. Knowledge of how *G. pallida* interacts with antagonistic organisms in the rhizosphere will aid identification and the development of effective biocontrol agents.

As part of the genome project we have also generated transcriptome data from several other pathotypes/populations of *G. pallida* that show differing virulence against several important resistance sources. Effectors are often the pathogen molecules that are recognised by resistance genes. By analysing differences in the sequences of effectors in the various nematode populations we therefore aim to understand the molecular basis of virulence in *G. pallida* and to use this information to help characterise resistance against this pathogen.

In our current work we have identified effectors that suppress host defences. This process is of key importance for PCN; if the feeding site is detected by the host and defence responses are successfully activated this would be fatal for the nematode. Understanding the mechanisms underlying suppression of host defences is a key area of research for plant pathologists working with oomycete, fungal and bacterial plant pathogens. There may therefore be much to be gained by collaboration with these researchers in order to identify shared mechanisms.

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USING THE MITOCHONDRIAL GENOME OF POTATO CYST NEMATODES TO DISTINGUISH INTRODUCTIONS

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Summary: The potato cyst nematodes (PCN; *Globodera rostochiensis* and *G. pallida*) were introduced into Europe from South America. They are now present in ware potato production land in much of the UK. Several pathotypes of PCN have been recognised in the UK (Ro1, Pa1, Pa2/3) based on their reproduction on a set of differential *Solanum* genotypes and they probably represent distinct introductions. The aim of this work was to determine if a molecular marker based on the mitochondrial DNA (mtDNA) of PCN could be used to distinguish these pathotypes and to identify other possible introductions. The assay is being tested with PCN populations in the James Hutton Institute (JHI) collection and with cysts collected recently from the field. The aim is to develop a high through-put semi-quantitative assay for use with samples collected for routine testing.

INTRODUCTION

The potato cyst nematodes (PCN; *Globodera rostochiensis* and *G. pallida*) coevolved with their hosts in South America and these pests were likely introduced inadvertently into Europe around 1850 (Jones, 1977) during collection of wild potato material used for the resistance against *Phytophtora infestans* (Hawkes, 1958). They are economically important pests which are now found in many potato producing regions around the world. In the EU they are regulated as quarantine pests and in the UK, if found in land used for ware production, the land in which they are present is subjected to official control programmes. It has been estimated that yield losses due to PCN costs the potato industry £43 million (Haydock & Evans, 1998). There are various options for the management of PCN including chemical control, lengthy rotations and the use of host resistance. The use of nematicides has been restricted by the EU (Regulation 1107/2009/EC), lengthy rotations have disadvantages to the grower and the numbers of agronomically attractive cultivars with appropriate resistance, particularly to *G. pallida,* are limited.

Management of *G. rostochiensis* in the UK using cultivars with the single major resistance gene *H1* derived from *Solanum tuberosum* subsp. *andigena* has been very successful and only the avirulent Ro1 pathotype is present in the UK. However, the widespread cultivation of cultivars such as Maris Piper that have *H1* has been attributed with the selection for *G. pallida,*

which are not controlled by *H1*. In a survey conducted in England and Wales (Minnis *et al.,* 2002), *G. pallida* was found the more prevalent species, present in 92% of PCN infestations. The results of this survey show how the use of resistance to control an economically important pest can have both positive benefits and adverse ramifications and also demonstrates the importance of understanding and anticipating the consequences of employing a particular control strategy. The development of effective and economically viable control strategies can take many years and a considerable investment of resources. To maximize their durability, it is important to evaluate how their utilization will impact on other pests or pathogens which could engender new management problems once the target of the control strategy is successfully suppressed.

The survey of Minnis *et al.* (2002) used a molecular diagnostic based on rDNA to distinguish the two species. The distribution of pathotypes or intraspecific variants within the species was not determined. In the past, bioassays have been used to distinguish pathotypes and to assess the number of possible introductions of PCN (Phillips & Trudgill, 1998). Bioassays require the availability of sufficient inoculum for replicated tests, several months to perform, suitable facilities and nematological expertise. Molecular methods have also been used to assess intraspecific diversity within UK PCN populations (Blok *et al.,* 1998, Bendezu & Evans, 2001, Bendezu *et al.,* 1998, Subbotin *et al.,* 2000). Both biological and molecular assays broadly agree that in the UK there is only one pathotype of *G. rostochiensis* (Ro1) and 2 pathotypes of *G. pallida* (Pa1 and Pa2/3), however both biological and molecular assays also indicate that there could be additional unusual populations such as Luffness from East Lothian Scotland (Blok *et al.,* 1997, Phillips & Trudgill, 1998). The Luffness population has high levels of virulence to potato genotypes with partial resistance derived from *Solanum vernei* and also has genetic characteristics which distinguish it from other *G. pallida* Pa2/3 populations. The potential for unusual populations to outcompete, spread and eventually create new management problems highlights the need to develop methods to identify these populations at an early stage so that appropriate control can be taken. Also, the co-localisation of different pathotypes which have previously been geographically separated is important as these are places where novel hybrids could be generated, which may have unexpected and undesirable phenotypes.

The new PCN Directive (2007/33/EC), which came into effect on July 1 2010, aims to prevent the spread of PCN within Europe and suppress current infestations. Various measures have been agreed including the requirement of statutory authorities to conduct an annual random survey of 0.5% of ware land according to agreed sampling procedures. In Scotland, Science and Advice for Scottish Agriculture (SASA) has developed with JHI, a DNA based assay for the detection and identification of PCN species (Kenyon *et al.,* 2010, Reid *et al.,* 2010). This assay is now used at SASA for soil samples from both seed and ware land. Application of an additional diagnostic test could provide information on the distribution of the Ro1, Pa1 and Pa2/3 pathotypes and unusual populations in Scotland. The JHI and SASA are collaborating on the development of this test.

Mitochondrial DNA (mtDNA) has been widely used in phylogenetic studies to investigate relationships within species and to distinguish introductions of non-indigenous species. The maternal inheritance of mtDNA and the faster rate of mtDNA evolution compared to typical nuclear markers are generally accepted as valid assumptions concerning the use of mtDNA for phylogenetic studies with relevance to diagnostics. The mitochondrial genome of *G. pallida* is unusual in that it consists of multiple small circular mitochondrial DNA (scmtDNA) molecules (Armstrong *et al*., 2000) in contrast to the typical metazoan mtDNA which comprises a single

circular mtDNA molecule that encodes the 12 or 13 proteins involved in electron transport and oxidative phosphorylation, ribosomal RNAs and tRNAs. The presence of this multipartite scmtDNA structure in diverse populations of PCN (Armstrong *et al.,* 2000, 2007) suggests that this evolved early in the speciation of PCN. An explanation for this unusual mtDNA structure and how normal mitochondrial functions are achieved is elusive. Some of the mtDNA genes which are presumed to be essential are absent, present as pseudogenes, or duplicated between scmtDNAs and each of the scmtDNAs has substantial noncoding regions. However, despite this unusual structure, both coding and noncoding regions have provided useful molecular targets for investigating intraspecific relationships with *G. pallida*. For example, the mtDNA cytochrome *b* (cyt-*b*) gene was sequenced from both European and South American populations of *G. pallida* and this revealed both the likely geographic origin of the European populations of this species and the intraspecific relationships between populations in Peru (Picard & Plantard, 2006, Picard *et al.,* 2004, 2007, Plantard *et al.,* 2008). In addition, 3 subgroups were distinguished within European *G. pallida* populations based on cyt-*b* sequence polymophisms. Sequence diversity within cyt-*b*, however, is much lower than in the noncoding region of scmtIV (Armstrong *et al.,* 2007, Hoolahan unpublished).

We have been investigating whether the sequence diversity in the noncoding regions could be useful for a high throughput and sensitive molecular assay to distinguish populations of *G. pallida*. The noncoding region of scmtDNAIV had been sequenced from several *G. pallida* populations (Lindley, Gourdie, Luffness, Pa1, P4A, P5A) (Armstrong *et al.,* 2002, 2007, Hoolahan & Blok unpublished). These sequences were aligned and internal primers were designed within conserved regions to generate PCR products of ~1000bp that encompass a region of considerable sequence polymorphism for these six populations. The concordance of restriction fragment length polymorphisms (RFLPs) patterns that are produced following digestion of the PCR products from the different sequence types and from different *G pallida* populations is being investigated.

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A LONG TERM GENOTYPIC ANALYSIS OF SCOTTISH PEACH-POTATO APHIDS: WHAT HAS HAPPENED TO THE BRAVEHEART CLONE?

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Summary: The population structure of peach-potato aphid *Myzus persicae* (Sulzer), in Scotland is composed of a limited number of genotypes some of which have increased on a vast scale. The recent antecedent population (1995-2001) was dominated by two insecticide sensitive clones (I and J (Braveheart)) and one clone with some insecticide resistance (C). In 2001 the first aphids with resistance to dimethyl-carbamates, *via* a mechanism known as MACE, were detected in Scotland. Most of these clones had short term success disappearing from the population after one to two years. In 2007 one of these MACE clones (O) expanded rapidly. Unlike its predecessors this clone did not disappear and at the same time the numbers of clones C, I and J declined rapidly. Since 2007 two new MACE clones, P and Q and a new non-MACE clone R have appeared.

INTRODUCTION

The peach-potato aphid *Myzus persicae* is one of the most widespread and well studied aphids. It is a major problem for agriculture because it spreads plant viruses amongst crops. It is extremely polyphagous and has the ability to reproduce both sexually and asexually. Sexual forms are produced in autumn, these mate and lay eggs which overwinter on peach, *Prunus persica* (van Emden *et al*. 1969). Asexual forms are produced for the remainder of the year and in some areas with few peach trees such as the UK, asexual forms predominate (Blackman 1971, Kasprowicz *et al*. 2008a, b), this is particularly true for Scotland, where the climate is unsuitable. The sexual stages provide the opportunity to generate novel gene combinations and the asexual stages provide the opportunity for rapid amplification of successful gene combinations under selection (clonal expansion).

One of the main factors contributing to the success of *M. persicae* is the rapid evolution and spread of insecticide resistance genes. The species has evolved several different types of insecticide resistance including resistance to organophosphates by the overproduction of carboxylesterases, resistance to the dimethyl-carbamate pirimicarb *via* a mechanism known as MACE (Modified Acetyl Cholinesterase), resistance to pyrethroid insecticides by two closely related target site mechanisms known as kdr and super-kdr and more recently resistance to neonicotinoids (Puinean *et al*. 2010, Bass *et al.* 2011). While carrying an insecticide resistance gene is likely to give a clone a selective advantage in the population, it is thought that these restriction mechanisms may also carry a fitness cost.

The *M. persicae* population in the Scotland has been well-characterized and three clones have been highly successful over the past 20-30 years (including one clone designated as 'Braveheart') (Fenton *et al*. 1998). Other clones have had varying success with some occurring for short periods (1-2 years) and disappearing after that (Fenton *et al*. 2005; Kasprowicz *et al*. 2008 a,b). Successful clones do not always carry any of the known insecticide resistance genes and the factors influencing their success are not fully understood. In this paper we show the current genotypic analysis of the Scottish populations and discuss what happened to the Braveheart clone.

MATERIALS AND METHODS

The data set consists of thousands of individual aphids sampled over Scotland in a 16 year period (1995-2011) collected from crop hosts potato (*Solanum tuberosum*) and oil seed rape (*Brassica napus*) or from 12.2m high aphid suction traps. DNA was extracted and microsatellite loci amplification analysis and visualisation were carried out (see Malloch *et al*. 2006). Six microsatellite loci, M35, M40, M49, M63, M86 and myz9 (Sloane *et al*. 2001) were chosen on the basis of their resolution. Eighteen aphid lineages (microsatellite multilocus genotypes or MLGs) were designated clones A-R.

RESULTS

The *M. persicae* population in Scotland is composed of an extremely limited number of MLGs and some of these have increased dramatically, relative to other clones (Fig. 1). For the last decade, fewer than 30 MLGs have been found and of these, only 18 have been found on more than one occasion. The recent antecedent population (1995-2001) was composed of two insecticide-sensitive genotypes (I and J (Braveheart)) and a clone carrying some target-site insecticide resistance to pyrethroids (C). These clones are entirely asexual and can no longer produce sexual forms (Pozarowska, 1987; Kasprowicz *et al*. 2008b).

In the last decade the use of insecticides such as dimethyl carbamates (e.g. pirimicarb) has increased and clones resistant to this insecticide have entered the UK (A, B, H, M, N, O and P). Most of these clones expanded rapidly but their numbers subsequently collapsed. This appearance and disappearance of MACE genotypes occurred from 2001-2007 and has been described as clonal turnover (Kasprowicz *et al*. 2008a). In 2007 a new MACE genotype (O) appeared in large numbers but unlike the previous MACE clones it did not decrease and by 2008 it dominated both field and flying populations. In the spring of that year, large numbers of this clone were found in areas that had never been treated with insecticides. 2009 saw the first appearance of clone P (MACE) and in 2010 new clones Q (kdr, skdr, MACE) and R (non-kdr, non skdr, non-MACE) were detected. Clone P has increased in number and current populations (summer 2010) are composed of 40% clone P 30% clone O 10% clone Q, 15% clone R and 5% clone A (R3, kdr MACE). Continued genotypic analysis will allow us to monitor the success of these new UK lineages.

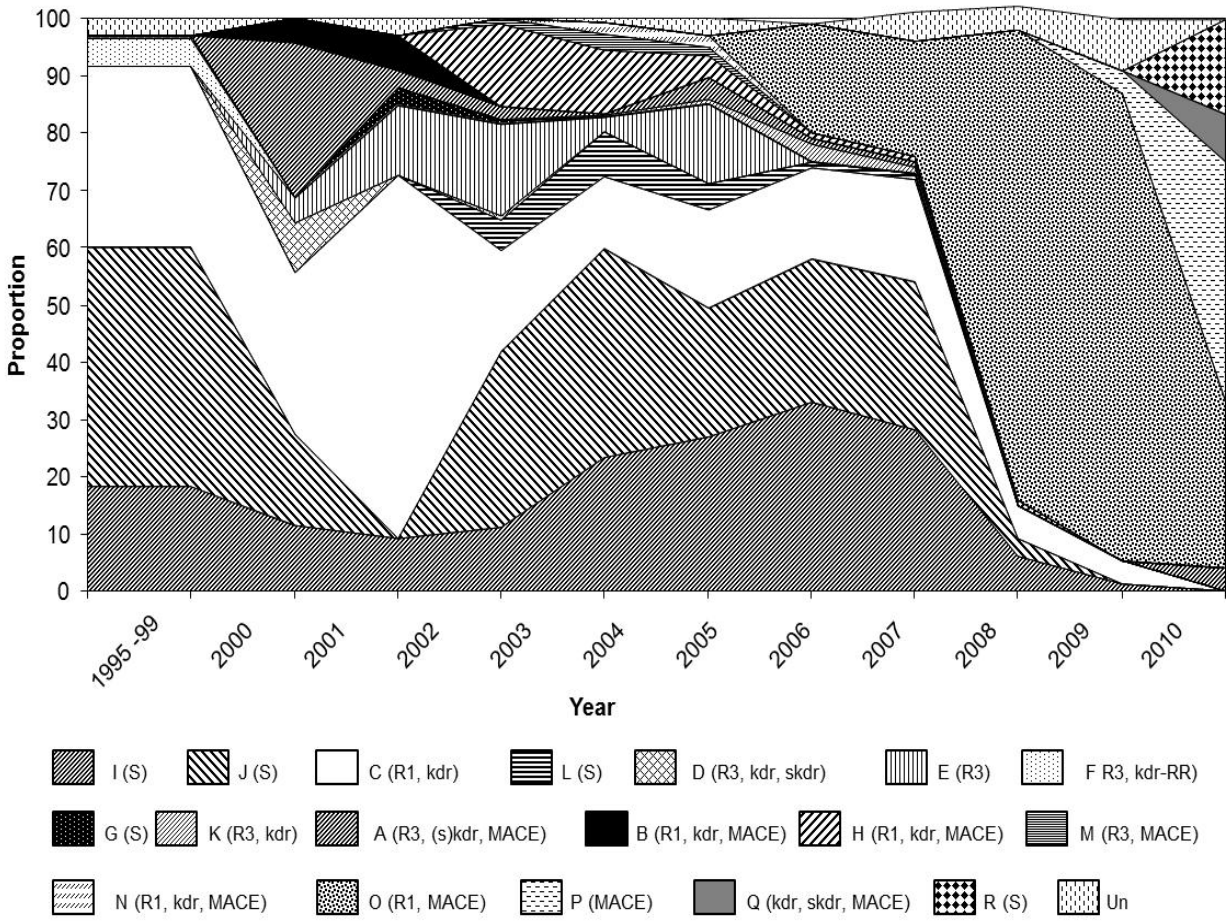

Figure 1. The figure shows the proportion of clones in Scotland *M. persicae* population for the years 1995 – 2010 as detected by genotyping. [For most seasons, between 100 and 300 *M. persicae* individuals are analysed.]

DISCUSSION

Genotypic analysis of the Scottish *M. persicae* population has revealed a limited but dynamic population structure. Determining the factors which contribute to the long term success or failure of any clone will aid the development of sustainable control measures in the future. The dominance of two insecticide sensitive clones (I and J) and one resistant clone (C, non-MACE) from 1995 - 2001 was followed by a period of clonal turnover of MACE genotypes (2001 - 2007). This is thought to be linked to the increased use of dimethyl carbamates and the subsequent collapse of these genotypes may be due to possible fitness costs suffered by aphids carrying MACE and other insecticide resistance mechanisms. Such genotypes may also have increased vulnerability to the activity of parasitoids (Fenton *et al*. 2010a) or an inability to adapt to winter conditions. For example, in their first season in the UK resistant genotypes from an obligate sexual population would produce sexual forms in autumn. In the absence of peach trees these would be a reproductive dead end.

Clonal Turnover and Evolutionary Fitness

Aphid pests evolve through heritable genetic variation, selection and adaptation (Fenton *et al*. 2010a). Figure 1 shows the difference in temporal dynamics of clones which clearly demonstrate that there are selective forces acting on the Scottish population. Evolution can occur rapidly in an agricultural environment through strong selective pressures imposed by man-made activity including exposure to insecticides (Fenton *et al.* 2010a). Surprisingly from 1995 (when this study began) until 2001, the insecticide spraying regimes appear to have had little effect on the genotypic composition of the Scottish *M. persicae* population with 2 insecticide sensitive clones (I and J) and one clone with some insecticide resistance (clone C kdr) continuing to survive and dominate. Many variables can influence the ecology of aphid populations including host plant availability, natural enemies, geography, climate and temperature. Until 2001 these or some other factors appear to have had a greater influence on the genotypic composition than agricultural insecticide control strategies. The first aphids carrying MACE were detected in England in small numbers in 1995. By 2001, Scotland experienced a major breakdown of insecticide control due to MACE carrying aphids (clones A and B) (Fenton *et al*. 2005). Since then there has been a period of clonal turnover with MACE clones H, M, N, O and P replacing A and B, with each successive colonizer appearing to be more successful. Although most of these clones expanded rapidly, their numbers subsequently collapsed and the key to a clone's long term survival is still unclear. It is not solely due to its resistance mechanisms but may also be due to an ability to counteract the negative fitness affects of carrying resistance genes plus other genetic qualities making the clone more suited to the UK ecosystems (Kasprowicz *et al.* 2008a). Clone O may be the first UK MACE colonizer which possesses all the qualities required for long term survival.

The Success of Clone O

It has been suggested that insecticide resistance has an effect on reproductive performance (Eggers-Schmacher, 1983; Foster *et al.* 2000, 2003). The reproductive performance of the clones has been examined (Fenton *et al.* 2010b) and to some extent the MACE clones that appear later tend to have better growth rates than their predecessor and this would support the hypothesis that over time resistance mechanisms recombine into better genetic backgrounds (Fenton *et al.* 2010a). Many of the earlier MACE genotypes also carried high levels of esterase and/or the kdr mechanism which may have made them suffer more from fitness costs and parasitoid attack. This is thought to be a consequence of their behavioural response to the alarm pheromone (E)-B- farnesene. Studies have shown that insecticide-resistant aphids have an increased vulnerability to parasitoid attack and this is associated with the possession of two insecticide resistance mechanisms, esterase and kdr (Foster *et al*. 2005, 2007). The current insecticide regime in Scotland and the rest of the UK appears to have favoured clone O which only carries MACE and therefore would not be subject to the fitness costs associated with other resistance mechanisms (although it may carry unknown forms of resistance). In addition, it is likely that clone O must have some physiological advantages over the previous clones and is better adapted to local conditions. The exceptionally cold winters in 2009 and 2010 appear to have had little impact on its survival. Clone P, like clone O, only appears to carry MACE and its numbers have increased to 40% of the total population analysed in 2010. Both clone O and P have all the potential properties of aphid super-clones i.e. a genotype that can multiply to very large numbers over many seasons and occur over a large geographical area. If this does occur, the use of insecticides based on dimethyl carbamates will be ineffective and other control measures will need to be employed in order to limit both aphid numbers and the

numerous plant viruses that the species is known to transmit. This long term study has revealed the dynamic nature of the Scottish populations of *M. persicae* and the ever changing challenges for agricultural production which they present. Continuing ecological studies of *M. persicae* clones and careful monitoring of the Scottish and UK populations will allow preparation of appropriate control strategies at the start of a season and should help reduce the possibility of control breakdown of this important aphid pest.

What Has Happened To The Braveheart Clone?

The predominant Scottish population from before 1995 to 2001 was composed of two insecticide-sensitive genotypes, I and J (Braveheart) and a clone carrying some non-MACE insecticide resistance (C). These three genotypes dominated the population until 2001 when large numbers of the first MACE aphids were detected. Significant numbers of C, I and J were still found until 2008 when the population consisted of almost all genotype O, even in areas that were not treated with insecticides. No individuals of genotype C, I or J have been found in Scotland since 2008. Whilst we have identified possible factors that have favoured the expansion of genotypes O and P, the reasons for the sudden decline of C, I and J are unclear. However, this time period does coincide with a rapid rise in the acreage of brassicas that receive seed treatments with imidacloprid and winter OSR in particular which would have been suitable for overwintering by these genotypes may no longer fulfil this role.

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POTATO MOP-TOP VIRUS IN FIELD TRIALS IN NORWAY

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Summary: *Potato mop-top virus* (PMTV) causes spraing (unsightly brown arcs and rings in tubers) and yellow chevrons or shortened internodes (mopping) in the leaves and stems of infected plants. Economic losses are due to poor tuber flesh quality leading to whole crop rejection. PMTV is prohibited in seed potatoes exported to some countries and detection of spraing in tubers will lead to whole consignments being rejected by authorities. The disease is prevalent in cool and damp environments. Some potato cultivars are particularly sensitive and PMTV-infected plants produce tubers with severe spraing symptoms. The cultivar Saturna is widely used in the Scandinavian potato processing industry and is particularly sensitive. Confirming other reports, some varieties appear to be systemically infected without exhibiting extreme symptoms. Results of the field trials examining infection rates within 3 sites in Norway are discussed.

INTRODUCTION

Potato mop-top virus (PMTV) causes spraing symptoms (unsightly necrotic brown arcs and rings in tubers of susceptible cultivars, sometimes referred to as corky ring spot) and yellow chevron markings or shortened internodes in the leaves and stems of infected plants. Economic losses can be considerable through poor internal tuber quality, particularly in processing and salad potato crops. Also PMTV affects trade as it is prohibited in seed potatoes exported to some countries. The virus is transmitted in nature by a soil-borne plasmodiophorid (*Spongospora subterranea*) that itself causes the disease powdery scab on tubers. Both diseases are prevalent in cool, damp conditions. The resting spores of *S. subterranea* remain infective in soil for years. When the spores hatch they release zoospores that infect nearby plant roots or developing tubers and introduce PMTV to the plant (Jones & Harrison, 1969; Arif *et al.*, 1995; Kirk, 2008). The resting spores maintain PMTV capable of infecting plants for a much longer time than is feasible to control by crop rotation (Jones & Harrison, 1969; 1972), which makes eradication of PMTV from infested fields very difficult.

PMTV is an important virus affecting potato industries (ware and seed potato) through much of Northern Europe and elsewhere. The probable origin of PMTV is the Andean region of South America (Salazar & Jones, 1975). The virus has been reported in Costa Rica, in North and South America, Asia and in Japan (reviewed in Santala *et al*., 2011). In Europe, PMTV was first detected in Ireland and the UK (Calvert & Harrison, 1966) and the Netherlands (van Hoof

& Rozendaal, 1969) but has not been reported there since. PMTV also occurs in the Czech Republic (Novak *et al*., 1983) and Switzerland (Schwärzel, 2002).

The Nordic countries (Norway, Sweden, Denmark and Finland) have been affected by PMTV for the last thirty to forty years (reviewed in Santala *et al*., 2011). Spraing symptoms constitute a severe quality problem in the potato industry in these countries.

The present report summarises data from part of a large project examining the effects of PMTV on a range of potato cultivars in field trials in Norway.

MATERIALS AND METHODS

Resistance trials

The field trials were carried out in 2007 in three different regions in Norway. The trial sites were at Farmen in Vestfold County (59°N, 10°E), Sola in Rogaland County (58°N, 5°E) and at Hegra in Nord Trøndelag County (63°N, 11°E). All locations were previously tested positive for *S. subterranea* and PMTV.

Fourteen different cultivars were grown in the trials, including Berle, Bintje, Bruse, Fakse, Gladiator, Jupiter, Kerrs Pink, Mozart, N-93-7-6, N-97-21-18, N-98-19-12, Oleva, Saturna and Tivoli. Cultivar Saturna was used as a control as it is known to develop symptoms when infected with PMTV and cv. Gladiator was included because it is known to be very resistant to powdery scab. The remaining cultivars have shown none or only a few rings and arcs when previously grown in PMTV infested soil.

All trials were grown in a randomized block design with five plants per plot in six replicates. One plant of cv. Saturna was added at both ends of the plot to check the distribution of PMTV in the trial site. All trials were planted by hand, grown as a normal potato crop regarding cultivation, pest control and haulm killing. Plants were harvested by hand to ensure separation of tubers from each plant.

Tuber evaluation

At harvest all tubers from each plant were collected and stored for 2 months and then exposed to fluctuating temperatures (from 4°C to room temperature) to provoke development of symptoms. The five biggest tubers from each plant were washed and evaluated for symptoms of powdery scab before testing for presence of PMTV with DAS-ELISA. Powdery scab was scored in accordance to an evaluation scheme of % cover of tuber: 0= no blisters; 1=1-5%; 2=5-10; 3=10-25%; 4=25-50; 5=50-100%. Finally tubers were cut into slices of 5-7 mm to look for presence of brown arcs and spots in the flesh. Severity of the symptoms was divided into 5 categories: 0= no spraing; 1= one or few spots; 2= many spots; 3= one or a couple of arcs or rings; 4= many arcs or rings in the flesh.

Detection of PMTV

Five thin slices from the stolon end were cut with a potato peeler. The slices were mashed using a Pollähne sap press (Erich Pollähne GMbH, Wennigsen, Germany) and the sap collected

in a 1.2 ml plastic tube containing 600 µl extraction buffer for 'bulbs and tubers' (Bioreba AG, Switzerland). From the mixture, 100 µl was transferred to one well of a Nunc microtitre plate (non-sterile), pre-coated with PMTV IgG (Bioreba). Plates were incubated at 4°C overnight. After washing the plates three times with washing buffer, 100 µl of PMTV conjugated IgG was added to each well and the plates were stored at 4°C overnight. During incubation the plates were kept in small plastic bags to avoid evaporation. On the third day the plates were washed three times with washing buffer and 100 µl of substrate were added to each well. Plates were stored in the dark at room temperature for two hours before measuring the absorbance at 405 nm wavelength. A sample was regarded positive when the value of the absorbance was more than two times greater than the the negative control. Meristem tubers were used as healthy negative controls and cv. Saturna tubers with symptoms were included as positive controls.

RESULTS & DISCUSSION

The field results are presented in Table 1 for tuber spraing symptoms (0.0 being none and 1.0 having spraing symptoms in 100% of tubers) and Table 2 the proportion of tubers with virus (0.0 being none, 1.0 being 100% infected). Following analyses of variance, data were submitted to Fisher's protected least significant difference test and grouped.
From the results in Table 1 it is evident that there was a good infection level within all three sites during 2007 with infection levels ranging from 8.0% to a high of 68.0%. A number of cultivars exhibited a lot of symptoms, including cv. Saturna (spraing susceptible control) and N-98-19-12 at three sites and also cvs Oleva, Jupiter and N-97-21-18 at two of the sites. The cultivars Tivoli and Bintje exhibited few symptoms at all three sites while cvs Rustique and Gladiator exhibited few symptoms at 2 sites. None of the cultivars however were completely free of symptoms.

The mean levels of virus in tubers are presented in Table 2, with virus being found throughout the trials at all three sites. The mean proportions of tubers in which the virus was detected range from 7.0% for cv. Kerrs Pink at Trøndelag to a high of 87.0% for cv. Bintje at the Vestfold site. The cultivar Bintje appears to have high infection levels, while cvs Saturna, Fakse and Jupiter have comparatively low virus infection levels.

When comparing the results in Table 1 (spraing symptoms) with the results in Table 2 (presence of virus in tubers) it would appear that those cultivars exhibiting many spraing symptoms tend to have fewer tubers infected. This would indicate that cultivars such as Saturna, Jupiter, Oleva, and N-97-21-18, if infected, express spraing symptoms and that other cultivars, such as Tivoli, Bintje, Rustique and Gladiator in which it is relatively common to find virus, exhibit few symptoms and therefore have a high proportion of symptomless tubers carrying the virus. These results highlight awareness that PMTV-infected but symptomless tubers of a number of cultivars may play a role in the transfer of the virus to new sites along with its vector *S. subterranea.* It is also evident from these results that breeding for resistance to PMTV and also the inspection of seed potatoes cannot reliably be based on visual indexing symptoms alone.

Table 1. Mean proportion of tubers exhibiting spraing symptoms harvested from three sites in 2007.

	Jæren	Vestfold	Trøndelag
Berle	0.23* abc†	0.28 bc	0.13 ab
Bintje	0.14 ab	0.06 a	0.08 a
Bruse	0.27 bc	0.28 bc	0.10 ab
Fakse	0.24 abc	0.22 abc	0.06 a
Gladiator	0.20 abc	0.12 ab	0.09 a
Jupiter	0.53 de	0.16 abc	0.41 c
Kerrs Pink	0.13 ab	0.19 abc	0.23 b
Mozart	0.25 abc	0.14 abc	0.13 ab
N-98-19-12	0.35 cd	0.58 ef	0.38 c
N-97-21-18	0.50 de	0.47 de	0.09 a
Oleva	0.30 bc	0.65 f	0.13 ab
Rustique	0.07 a	0.30 cd	0.04 a
Saturna	0.68 e	0.65 f	0.58 d
Tivoli	0.13 ab	0.08 a	0.04 a

*, 0.0 – none, 1.0 – 100% tubers with spraing symptoms.
†, Letters beside figures are derived from Fisher's protected least significant difference test, allowing pairwise comparisons to identify which cultivars were significantly different from one another.

Table 2. Mean proportion of tubers containing PMTV virus harvested from three sites in 2007.

	Jæren	Vestfold	Trøndelag
Berle	0.53* cdef†	0.54 b	0.23 abcde
Bintje	0.67 ef	0.87 d	0.40 efg
Bruse	0.66 def	0.60 bc	0.36 def
Fakse	0.37 abc	0.26 a	0.17 abcd
Gladiator	0.52 cdef	0.59 bc	0.44 fg
Jupiter	0.28 ab	0.76 cd	0.14 ab
Kerrs Pink	0.72 f	0.62 bc	0.07 a
Mozart	0.49 bcde	0.67 bc	0.54 g
N-98-19-12	0.53 cdef	0.23 a	0.12 ab
N-97-21-18	0.38 abc	0.32 a	0.27 bcdef
Oleva	0.43 abcd	0.16 a	0.54 g
Rustique	0.57 cdef	0.27 a	0.33 cdef
Saturna	0.21 a	0.13 a	0.17 abc
Tivoli	0.70 ef	0.58 bc	0.27 bcdef

*, 0.0 – none, 1.0 – 100% tubers infected.
†, Letters beside figures are derived from Fisher's protected least significant difference test, allowing pairwise comparisons to identify which cultivars were significantly different from one another.

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THE BIODIVERSITY AND EPIDEMIOLOGY OF *POTATO VIRUS Y* (PVY) IN SCOTLAND

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Summary: *Potato virus Y* (PVY) is the most prevalent potato virus in Scotland where the PVY^{N} strain has increased significantly over the past decade. A survey was undertaken to assess the diversity of PVY^{N} present in Scottish seed crops. Partial genome sequencing revealed that 81% of PVY^{N} isolates belong to the recombinant European (EU)-NTN group, suggesting a relative homogeneity of PVY field isolates. The timing of transmission and spread of representative PVY virus isolates (PVY^{O}, $PVY^{EU\text{-}NTN}$, $PVY^{NA\text{-}NTN}$) was also assessed. Preliminary results suggest that these isolates are likely to be transmitted in a comparable fashion by similar aphid species. Contrastingly, frequency of tubers infection was found to differ significantly between isolates, as $PVY^{EU\text{-}NTN}$ was detected in 66% of infected potato plants. This suggests that $PVY^{EU\text{-}NTN}$ might out-compete PVY^{O} and $PVY^{NA\text{-}NTN}$ resulting in a higher translocation to tubers, potentially explaining the prevalence of $PVY^{EU\text{-}NTN}$ in the surveyed crops.

INTRODUCTION

Potato virus Y (PVY) is the type member of the genus Potyvirus, family *Potyviridae*. It is a serious virus pathogen that affects commercial potato crops worldwide. PVY is estimated to be responsible for 45% of all disease related yield losses in potato crops in the UK at an estimated annual cost of £30 - £40 million (Valkonen, 2007). PVY is transmitted non-persistently by aphid vectors, whereby the aphid acquires the virus on its stylet when probing the leaf surface and transmission occurs when the aphid probes subsequent plants. Virus acquisition is rapid and, although the virus may only be retained by the aphid for a short time, aphids can quickly transmit virus without colonising the crop. Consequently, to be effective at limiting transmission, aphicides need to reduce the chance of an aphid probing.

PVY exists as a complex of strains which can be distinguished on the basis of their biology (symptoms they elicit on plants), serology and genome analysis. PVY strains have been divided into three major groups: the ordinary or common strain (PVY^{O}), the stipple streak strain (PVY^{C} leaf drop of potato) and PVY^{N} (vein necrosis on tobacco) (Singh *et al.,* 2008). More recently, genome characterization has identified recombinant types such as PVY^{NTN} (N-Tuber Necrosis) and $PVY^{N\text{-}Wilga}$ within the necrotic PVY^{N} group. Recombinant PVY^{N} strains such as PVY^{NTN} can cause potato tuber necrotic ringspot disease (PTNRD). PTNRD has lead to large economic losses across Europe due to the reduction in marketability of affected crops. Surveys of virus populations within seed potato crops in Scotland have identified a recent drift in the PVY

population structure from PVY^O and PVY^C towards PVY^N, a trend observed worldwide. The characterisation of the population structure of PVY^N in Scottish crops is important to evaluate the risks PVY^N variants may pose to the industry.

The PVY genome consists of a single stranded positive sense RNA molecule of about 9.7kb in length. Recombination junctions (i.e. the region where the sequence is likely to have switched from one sequence type strain to another) have been identified across the PVY genome (Schubert *et al.*, 2007). Sequence analysis of these recombinant junctions (termed R1 and R2) should provide important information on their molecular nature and in characterizing PVY strain types found in Scottish crops.

MATERIALS AND METHODS.

Sampling of PVY^N field isolates.

All PVY^N ELISA-positive samples intercepted by SGRPID crop inspectors during the 2009 crop inspection season were propagated into tobacco plants *Nicotiana tabacum* cv. White Burley and *Nicotiana benthamiana*. Leaves of these infected plants were freeze-dried using an Edwards Modulyo 4K freeze-dryer (Crawley, UK) prior to storage.

RT-PCR, sequencing and sequence analysis.

Total RNA was extracted from symptomatic leaves of tobacco plants inoculated with selected field isolates using the MagExtractor-RNA Kit (Toyobo, Japan) performed on the magnetic particle processor KingFisher mL (Thermo Scientific, Basingstoke, UK). Reverse Transcription (RT)-PCR for sequencing R1 and R2 regions or typing PVY isolates was performed as described by Mortensen *et al*. (2010) using the primers listed in Table 1. The sequencing reactions and relationships between sequences were analysed by creation of neighbour-joining trees in MEGA (Mortensen *et al*., 2010).

Table 1. List of primers for RT-PCR and sequencing R1 and R2 recombinant junctions.

Primer	Sequence (5'-3')
PVY-R1FWD	GARATGTTATAYATTGCCARGCAR
PVY-R1REV	CTRTGGGTTTTATGWACYGAGTGATAGAG
PVY-R2FWD	YGCMATYCCMAGAACYCTAA
PVY-R2REV	TMGTRCTYGTTTCTGTGATGATYGAYG
DV76FWD (EU-NTN)	CTATAGAGTTGGTGGTATTCCTAAT
DV76REV (EU-NTN)	AAAACCCGCCTTGAATAGG
DV69FWD (NA-NTN)	TTATAGAGTTGGTGGTATTCCTGGG
DV69REV (NA-NTN)	GTGAAAAACCTGCCTTAAACAAC

Biotyping of PVY strains.

PVY isolates were mechanically inoculated onto five week-old tobacco plants *Nicotiana tabacum* cv. White Burley (developing veinal necrosis –VN- symptoms to PVY^N biotypes) and *Nicotiana benthamiana* for propagation. Two lower leaves from each plant were inoculated with 10□l of infectious sap (two plants per isolate). Vein necrosis or mosaic appeared within 10-15 days post-inoculation. Symptomatic leaves were collected to prepare fresh sap (homogenized in 2ml 50mM $NaHPO_4$/ KH_2PO_4 pH7.5 buffer). Similarly, mechanical inoculation of PVY isolates was performed onto *in vitro* potato plantlets at the 6-10 leaf stage of cultivars King Edward, Pentland Crown, Desiree, Maris Bard and Pentland Ivory harbouring differential PVY resistance genes *Nc*, *Ny* and *Nz* (Table 2).

Table 2. Symptoms triggered by PVY strains on potato and tobacco cultivars. HR: Hypersensitive Reaction, S: susceptible, VN: vein necrosis.

	PVY^C	PVY^O	PVY^N	PVY^Z	PVY^E
King Edward (*Nc:ny:nz*)	HR	S	S	S	S
Pentland Crown (*nc:Ny:nz*)	S	HR	S	S	S
Desiree (*nc:Ny:nz*)	S	HR	S	S	S
Maris Bard (*Nc:Ny:Nz*)	HR	HR	S	HR	S
Pentland Ivory (*Nc:Ny:Nz*)	HR	HR	S	HR	S
Tobacco (cv White Burley)	S	S	VN	S	S

2010 Field trial layout

The field trial plot (Pickup *et al*., 2009) consists of 450 virus-free Maris Piper tubers. Twenty one tuber-borne infected potatoes were used as the inoculum source of PVY, of these seven plants were infected with $PVY^{EU\text{-}NTN}$, $PVY^{NA\text{-}NTN}$ and PVY^O isolates (Figure 1). Infector plants were cv. Maris Piper with the exception of $PVY^{EU\text{-}NTN}$ which was cv. Nadine. As the infectors alternate throughout the plot, one of each isolate is positioned at the centre of each of the *Nicotiana debneyi* drills. This should minimise any positional effect on transmission. Week-to-week variation in virus transmission is monitored by changing the *N. debneyi* bait plants each week and testing them by DAS-ELISA.

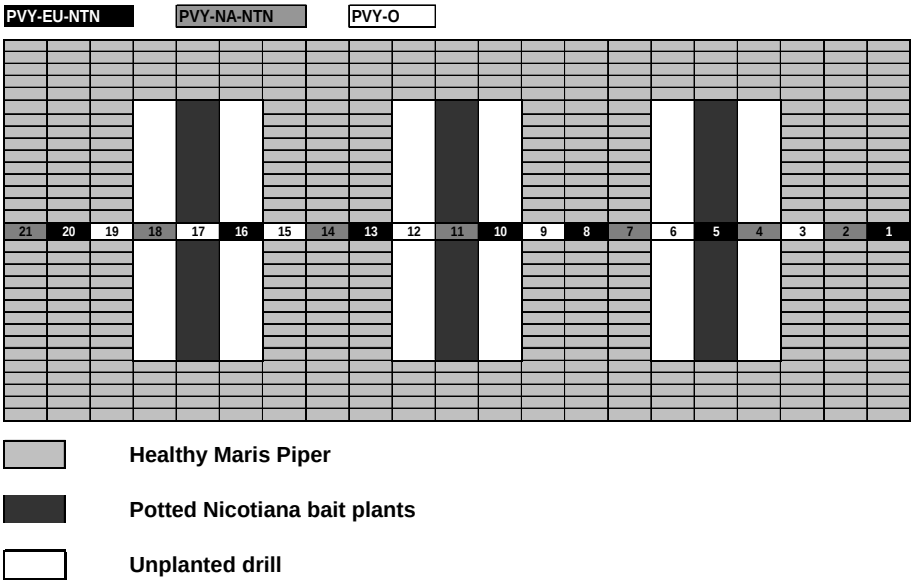

Figure 1. Trial design for investigation of the timing of transmission of PVY.

RESULTS

Molecular Characterisation

A total of 259 leaf samples intercepted in 2009 were tested positive by ELISA for PVY^N and selected for partial sequencing. To maximise the diversity of PVY^N field isolates to be studied, ELISA positive PVY^N leaf samples were selected from a wide range of geographical regions covering all seed potato growing areas and from 66 different potato varieties.

Sequencing of the R1 region and phylogenetic analysis indicates that all isolates are separated into 4 distinct clades (Figure 2). Of the 53 PVY^N isolates sequenced so far, 43 (81%) clustered within the European EU-NTN clade, 6 (11%) in the North American NA-NTN and 3 (6%) in the EU-N clade. The R2 region has also been sequenced and all isolates were found to cluster together in one large clade re-grouping all EU-NTN, NA-NTN, EU-N isolates (data not shown).

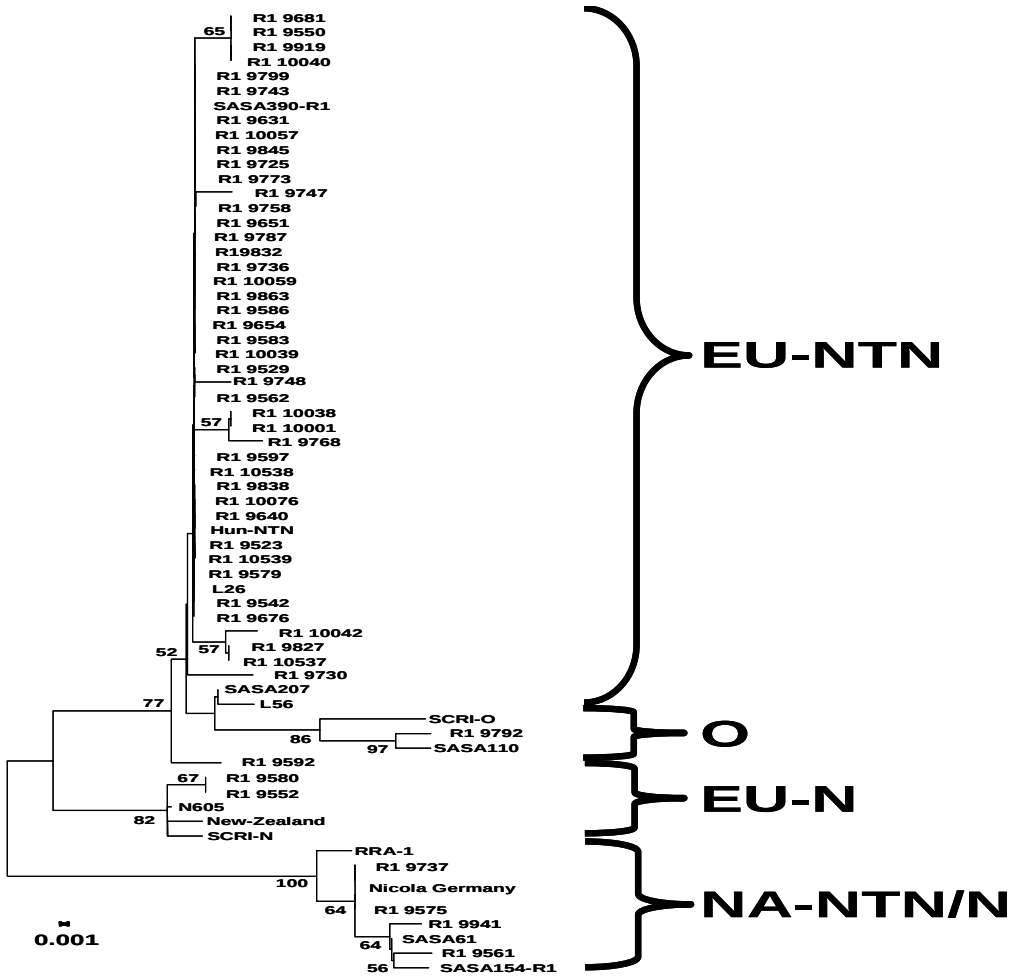

Figure 2. Phylogenetic analysis of Recombinant Junction 1 (R1) regions of PVY field isolates (numbers) and isolates sequence from public database. Phylogenetic trees were generated using neighbour joining method. Bootstrap values >50% are shown. The evolutionary distances are expressed as units of the number of base substitutions per site.

Biological characterisation

The ability of PVY^N isolates to cause the development of either vein necrosis (VN) or mosaic in *N. tabacum* cv. White Burley was assessed. The vast majority (95%, n =106) produced vein necrosis. Three isolates that were serologically PVY^N triggered mosaic symptoms. These three isolates clustered within the EU-NTN clade.

Resistance specificity of differential potato cultivars harbouring PVY-specific resistance genes (*Nc*, *Ny*, *Nz*) allows the classification of PVY isolates into biological strain groups (Singh *et al*., 2008, Table 2). Six representative PVY^N isolates were selected for further characterisation on the basis of their genomic sequence and the symptoms elicited on tobacco plants: (*i*) three isolates from EU-NTN clade that elicit either vein necrosis or mosaic on tobacco (*ii*) two isolates from the NA-NTN clade and (*iii*) one isolate from the EU-N clade. Following infection of the potato cultivars listed in Table 2, none of the isolates triggered a hypersensitive response in the cultivars tested. This suggest that all isolates tested belong to the PVY^N biotype, with the exception of one isolate from the EU-NTN clade failing to produce vein necrosis in tobacco and therefore belonging to the PVY^E strain group.

Transmission and distribution of PVY isolates in field conditions

Weekly transmission of PVY isolates was assessed by ELISA-test of tobacco bait plants across the 2010 season. ELISA can only distinguish between PVY^O and PVY^N serotypes. $PVY^{EU\text{-}NTN}$ and $PVY^{NA\text{-}NTN}$ infected plants are both counted positive for PVY^N. The majority of infected tobacco bait plants (73%) tested PVY^N positive while 27% were PVY^O positive (Figure 3, left panel). Timing of transmission was similar for both PVY^O and PVY^N serotypes (Figure 3), with a pattern matching the overall PVY vector pressure (Figure 3, left panel), suggesting that PVY^N and PVY^O are likely to be transmitted by the same aphid species. Overall, these preliminary results show that isolates belonging to the PVY^N serotype ($PVY^{EU\text{-}NTN}$ and $PVY^{NA\text{-}NTN}$) and PVY^O are comparably transmitted across the season.

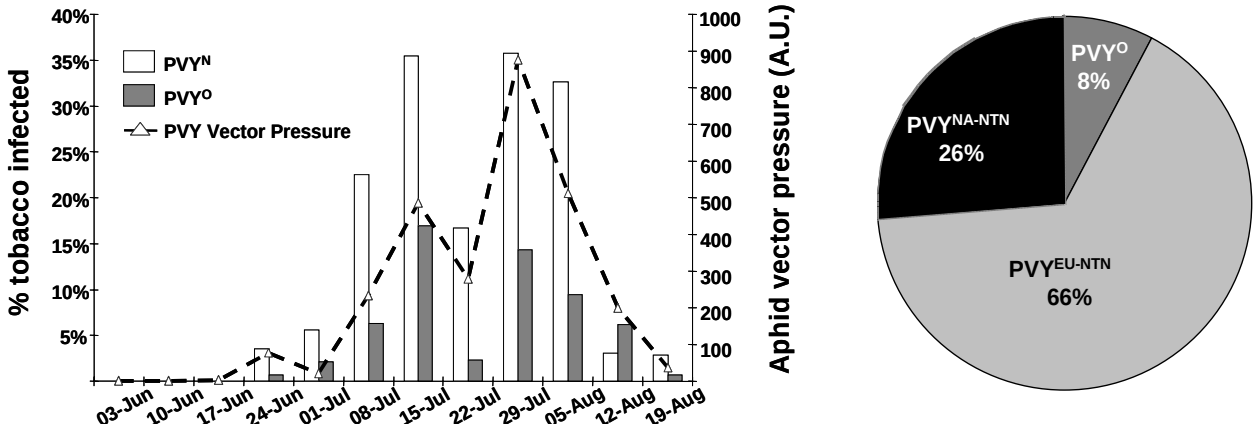

Figure 3. Weekly timing and frequency of transmission of PVY^N and PVY^O serotypes in field conditions. The corresponding aphid PVY vector pressure from the Edinburgh suction trap is presented (left panel). End-of-season distribution in infected bait potato plants (right panel).

Transmission to daughter tubers was monitored in PVY infected plants by RT-PCR using primers specific for $PVY^{EU\text{-}NTN}$ and $PVY^{NA\text{-}NTN}$ isolates (Table 1). A significantly higher proportion of plants (66%) were found to be infected by the $PVY^{EU\text{-}NTN}$ isolate, while only 26% and 8% were infected by $PVY^{NA\text{-}NTN}$ and PVY^{O} respectively (Figure 3, right panel).

DISCUSSION

Over the past decade, numerous studies have reported the occurrence of recombinant PVY^{NTN} strains worldwide affecting potato crops, replacing non-recombinant PVY^{O} and PVY^{N} strains. Partial sequencing of recombinant junctions on the PVY genome has revealed a strong homogeneity of isolates with over 80% clustering within the European NTN clade, demonstrating that the $PVY^{EU\text{-}NTN}$ group is prevalent in Scottish seed crops. Further biological characterisation using six representative isolates, has defined them as belonging to the PVY^{N} biotype with one isolate belonging to the PVY^{E} biotype. The isolate defined as PVY^{E}, falls within the EU-NTN clade suggesting that despite their related genetic background, EU-NTN isolates can elicit very diverse symptoms on their host. The ability of Scottish isolates to trigger PTNRD on a range of potato plants is currently on-going to assess the potential risk they might pose for the potato industry. Preliminary data suggests that transmission to daughter tubers differs significantly between PVY strains. $PVY^{EU\text{-}NTN}$ strain may be fitter and is out competing PVY^{O} and $PVY^{NA\text{-}NTN}$ isolates in the field. Further studies are on-going to understand the mechanisms that drive the prevalence of EU-NTN variant in the PVY population.

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THE DEVELOPMENT OF PENFLUFEN AS A SEED TREATMENT FOR POTATOES IN NORTHERN EUROPE

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Summary: Penflufen is a novel alkylamide fungicide which has activity at low dose rates against fungal diseases of economical importance in many field crops. Penflufen is being developed in the UK and other European countries as a potato seed treatment, providing a high level of protection to plants against the seed-borne *Rhizoctonia solani*, the cause of black scurf. Trial results from across northern Europe using infected seed stocks, demonstrated that both liquid and dry powder formulations of penflufen gave very high levels of control of the symptoms of *R. solani* and a useful side effect against *Helminthosporium solan*i, the cause of silver scurf, without causing any detrimental effects to crop safety.

INTRODUCTION

Penflufen is a novel alkylamide fungicide which has activity at low dose rates against basidiomycete and ascomycete fungi causing diseases of economical importance in many field crops. *In vitro*, penflufen has shown activity on reducing spore germination, germ tube elongation and mycelium growth. At the biochemical level, penflufen inhibits the electron transport in the respiration chain by blocking succinate dehydrogenase (complex II – SDH-inhibitor), thus terminating mitochondrial respiration. This new fungicide belongs to the FRAC Fungicide Group 7 (Carboxamides). In Europe and in other countries, penflufen is being developed as a seed treatment on potatoes for the control of seed-borne *Rhizoctonia solani* and other seed-borne diseases. This paper describes the development of penflufen on potato seed tubers as both a liquid seed treatment for application over a roller table and as a dry powder seed treatment for application through a mechanical metering device at planting.

MATERIALS AND METHODS

Small plot replicated trials were conducted in the UK, Denmark, Sweden and Finland with additional crop safety trials in Germany, France and Poland. All trials were conducted by or on contract to Bayer CropScience unless otherwise stated. Experimental plot sizes ranged from 12.0 to 30.0 m² and treatments were replicated four times in randomised block designs. Both liquid (FS) and powder treatments (DS) were applied in plastic bags using the appropriate weight or volume of chemical to a known weight of seed or using commercial machinery. Liquid treatments were diluted to make up a diluent equivalent to 2 litres per tonne of seed. Trials were planted in commercial potato fields or research stations and received normal commercial applications of crop protection materials. Seed was assessed prior to planting to determine the baseline levels of seed-borne diseases. Non destructive assessments were made

of plant and stem numbers. Where trials were taken to yield, whole or part plots were harvested by machinery or hand digging and the samples weighed after riddling. After a period of storage, progeny tubers were assessed for incidence and severity index of skin diseases. Diseased tubers were graded according to disease infection levels and the disease severity index was calculated as a function of the disease grade and the numbers of tubers within that grade. All assessments took account of the EPPO Guidelines. Individual trials were statistically tested using the Duncan's Multiple Range test.

Efficacy Trials

Rhizoctonia solani
Trials were conducted in the 2008, 2009 and 2010 growing seasons using *R. solani* infected seed tubers. In 2008, three rate determination trials were conducted in the UK using the 2DS powder formulation of penflufen and where the commercial standard treatment used was pencycuron 250g a.i./t (Monceren DS). In 2008 – 2010, 20 trials were conducted in the UK (7), Denmark (6), Finland (5) and Sweden (2) comparing the efficacy of the penflufen FS and DS formulations at 20g a.i./t in comparison to the most effective commercial standard treatment in each trial from either pencycuron 150-250g a.i./t or flutolanil 60-120g a.i./t (Tables 1-4). Two trials contracted to Cambridge University Farms (CUF) and SAC Aberdeen (SAC) also investigated the efficacy of treatments against stem and stolon canker symptoms (Table 5).

Helminthosporium solani
Six trials were conducted in the UK using *H. solani* infected seed stocks in 2009 and 2010. Penflufen FS and DS formulations was tested at 20g a.i./t and the comparison treatment was fludioxonil 100SC applied at 25g a.i./t seed (Table 6).

Crop safety
Eight crop safety trials using seed tubers with no or very low levels of seed borne skin disease present, were conducted in the UK (3), Germany (3), France (1) and Poland (1). The mean results of the major yield parameters are given in Table 7. Penflufen FS and DS at 20g a.i./t was compared to either pencycuron + imazalil 250g+12g or fludioxonil 25g a.i./t seed.

RESULTS

The tables below give the mean efficacy and crop safety results.

Table 1. *R. solani* incidence on progeny tubers in penflufen rate evaluation trials (3 trials, UK, 2008)

Treatment	Rate g a.i./t	08GBR1	08GBR2	08GBR3	mean
		% disease incidence			
Untreated	-	96.3 a	98.3 a	93.5 a	*96.0*
penflufen DS	10	4.0 b	23.5 b	22.5 b	*16.7*
penflufen DS	20	2.5 b	13.5 b	13.5 b	*9.8*
penflufen DS	40	3.3 b	5.5 b	12.0 b	*6.9*
pencycuron DS	250	10.8 b	8.0 b	8.0 b	*8.9*

Different letters indicate a significant difference at p=0.05

The 2008 trial results (Table 1) confirmed that the optimal rate of penflufen was 20g a.i./t seed giving efficacy results that were very similar to those of the commercial standard treatment pencycuron DS (250g a.i./t seed). This rate of penflufen was tested further in 2008, 2009 and 2010 in both the DS and FS formulations.

Table 2. Seed treatment effects on crop establishment in crops grown using seed-borne *R. solani* (black scurf) infected seed stock

Treatment	Rate g a.i./t	Early plant count plants/m row	*relative*	Final plant count plants/m row	*relative*
Untreated	-	2.15	*100.0*	3.32	*100.0*
penflufen FS	20	2.20	*102.1*	3.37	*101.4*
penflufen DS	20	2.22	*102.9*	3.41	*102.6*
standard		2.01	*93.2*	3.39	*102.1*
(number of trials)		(13)		(20)	

The results in Table 2 show that both the DS and FS formulations of penflufen increase the rate of crop emergence and final plant counts in crops with black scurf infected seed.

Table 3. Seed treatment effects on stem numbers and yield in crops grown using seed-borne *R. solani* (black scurf) infected seed stock

Treatment	Rate g a.i./t	Stem numbers stems/m row	*relative*	Yield t / ha	*relative*
Untreated	-	13.9	*100.0*	47.1	*100.0*
penflufen FS	20	14.4	*103.6*	48.8	*103.6*
penflufen DS	20	14.1	*101.9*	50.1	*106.4*
standard		14.4	*103.9*	49.7	*105.6*
(number of trials)		(20)		(20)	

Both formulations of penflufen increased stem numbers and gross yield whilst reducing black scurf symptoms on the progeny tubers.

The following tables present results on the efficacy of the seed treatments against the different symptoms of *R. solani*, *Helminthosporium solani* and crop safety using healthy potato seed tubers.

Table 4. Seed treatment effects on black scurf incidence and severity on progeny tubers in crops grown using seed-borne *R. solani* (black scurf) infected seed stock

Treatment	Rate g a.i./t	Black scurf incidence % infected	*% reduction*	Black scurf severity severity index	*% reduction*
Untreated	-	61.6	*0.0*	1.52	*0.0*
penflufen FS	20	8.8	*85.7*	0.20	*86.9*
penflufen DS	20	6.6	*89.3*	0.18	*88.2*
standard		10.8	*82.5*	0.19	*87.6*
(number of trials)		(15)		(12)	

Table 5. Seed treatment effects on stem canker and stolon canker in crops grown using seed-borne *R. solani* (black scurf) infected seed stock

Treatment	Rate	Stem canker % incidence		Stolon canker % incidence
	g a.i./t	CUF	SAC	SAC
Untreated	-	53.9	18.6	10.1
penflufen FS	20	18.6	18.3	6.0
penflufen DS	20	15.0	11.9	2.3
fludioxonil	25	19.7	20.8	2.8
	S.E.	6.2	ns	ns

Table 6. Seed treatment effects on silver scurf incidence and severity on progeny tubers in crops grown using seed-borne *H. solani* (silver scurf) infected seed stock

Treatment	Rate g a.i./t	Silver scurf incidence % infected	*% reduction*	Silver scurf severity % cover	*% reduction*
Untreated	-	80.8	*0.0*	6.7	*0.0*
penflufen FS	20	38.0	*53.0*	2.2	*67.4*
penflufen DS	20	43.7	*45.9*	2.8	*57.6*
fludioxonil	25	63.2*	*21.8*	4.3*	*35.6*
(number of trials)		(6) (*5)		(6) (*5)	

Table 8. Seed treatment effects on crop safety in crops grown using healthy seed stocks

Treatment	Rate	Final plant count	Stem numbers	Yield
	g a.i./t	plants/m row	stems/m row	t / ha
Untreated	-	3.33	13.4	47.2
penflufen FS	20	3.38	13.9	50.2
penflufen DS	20	3.34	13.8	49.9
standard		3.26	14.1	48.1
(number of trials)		(8)	(5)	(8)

DISCUSSION

Rhizoctonia solani is the most economically important seed-borne disease of potatoes. Not only can the disease detrimentally affect crop yield but also the marketability of progeny tubers for the processing and pre-pack markets by causing variations in tubers size and shape as well as skin blemishes due to the black scurf fungal sclerotia. Since the introduction of effective seed treatments such as pencycuron in the 1980s, the effects of the disease were mitigated (Adam & Malcom, 1988). Advances in fungicide chemistry and formulation technology have progressively reduced the levels of active ingredients required to obtain good control of the disease and in the case of penflufen the amount of a.i. utilised per tonne of seed is approximately ten times less that of pencycuron applied to the same weight of seed.

As with all crop protection products coming under the new EU regulatory processes, the development of penflufen as a seed treatment for potato crops was conducted across a range of European countries encompassing all the different growing conditions, agronomy, pest and diseases in the single European Zone for seed treatments. The results presented in this paper came from field trials undertaken in the more northerly countries in Europe, particularly the UK and Scandinavia. This spread of trials gives great confidence that the product can work under all the conditions likely to be encountered in any particular location.

The trials in the UK in 2008 confirmed that the 20g a.i./t seed rate of penflufen was the most appropriate rate. Doubling the rate produced only a marginal improvement in efficacy whilst halving it caused a substantial drop-off in efficacy. At the 20g a.i./t rate, both the FS and DS formulations of penflufen were similar in reducing the impact of seed-borne disease on the visual parameters of yield (plant and stem numbers) as well as gross yield and tuber skin quality. Not reported here are the effects on progeny tubers size, where results indicate that penflufen has a positive effect in reducing the proportion of under-sized and over-sized tubers thus increasing the marketable yield.

Penflufen is a broad spectrum fungicide with efficacy against other pathogens. Results reported demonstrate that it has a very useful side effect against the skin tarnishing disease *Helminthosporium solani* (silver scurf) and further work is being progressed to determine if other economically significant diseases can be reduced by this treatment.

The commercial introduction of penflufen as a seed treatment for seed potatoes would rank as an important milestone in providing potato growers with a highly effective fungicide using the lowest levels of active ingredient in this sector and presented in liquid or dry powder formulations to suit the requirements of individual growers. Potato growers in the United Kingdom will be the first in Europe to benefit from this advance in crop protection with the introduction of the product in 2012 under the trade name of Emesto™.

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IN VITRO INHIBITION OF SOIL BORNE POTATO PATHOGENS BY ISOTHIOCYANATES

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Summary: Isothiocyanates are formed through the breakdown of Brassica plant tissue and possess a range of anti-microbial properties that can suppress pathogenic organisms; a process known as biofumigation. This study examined the effects of seven different isothiocyanates against the growth and development of three important soil borne potato pathogens, *Colletotrichum coccodes, Rhizoctonia solani* and *Helminthosporium solani* in culture. Results have identified which ones possess the greatest potential for use within a biofumigation system.

INTRODUCTION

With increasing amounts of government pressure put on potato growers to reduce their use of pesticides and soil fumigants, attention has turned to the use of alternative methods for the control of soil borne pathogens. One such method is Biofumigation; a term that was first coined by Kirkegaard e*t al.* (1993), cited in Sarwar *et al*. (1998), to describe the principle of exploiting the natural hydrolysis products of glucosinolates, (plant secondary metabolites) to suppress soil microorganisms (Angus *et al.* 1994). Within the tissues of *Brassica* plants there are both glucosinolates, (Fenwick and Heaney, 1983) and the enzymes, myrosinases, which under normal conditions when the plant's tissues are intact are compartmentalised, however upon tissue disruption both come into contact and glucosinolate hydrolysis occurs. This process results in several compound groups being formed, the most common of which are isothiocyanates (ITC). With regards to the biofumigation of soil borne pathogens, isothiocyanates have been shown previously to be the most toxic towards a range of microorganisms. Ultimately this study will determine which specific isothiocyanates provide the greatest level of *in vitro* control for each of the three fungal pathogens studied here, and the lowest concentration at which control can be achieved.

MATERIALS AND METHODS

Fungal cultures

All fungal cultures (recent isolates from potato tubers, provided by the Potato Section at SASA) were incubated at 22°C with alternating periods of 12 hours darkness and 12 hours light. Cultures were periodically sub-cultured for experimental use. Fresh cultures were made by coring 7mm fungal plugs from the outer margin of mature colonies and transferring onto Petri dishes containing fresh potato dextrose agar medium (PDA).

ITC solutions

All pure ITCs were purchased from Sigma Aldrich, Dorset, UK. Solutions were made using 50% EtOH (aq.), ensuring even distribution of the solutions when incorporated into the PDA. Using a method adapted from Dhingra *et al.* (2004), seven isothiocyanates (Allyl (AITC), Benzyl (BITC), Isopropyl (IITC), Methyl (MITC), Naphthyl (NITC), Phenylethyl (PEITC) and Propyl (PITC), (Sigma Aldrich)), that can form naturally by glucosinolate hydrolysis in *Brassica* spp., were studied under *in vitro* conditions to determine the effects they had on the growth of *C. coccodes, R. solani* and *H. solani.* The inhibition effects were determined by culturing the individual fungus on PDA amended with each of the isothiocyanates at different concentrations, ranging from 125ppm to 10,000ppm. Each ITC amended culture plate was inoculated with a 7mm fungal plug taken from the advancing margin of a mature fungal colony on PDA. Petri dishes were sealed with Parafilm 'M' and incubated at 22°C with alternating periods of 12 hours darkness and 12 hours light. Radial colony growth along four axes from the edge of the fungal plug to the outer margin of new growth, was measured at regular intervals. The trial was completed when the control colonies had reached the edge of the Petri dish, approximately 16 days for *C. coccodes* and *R. solani*, and 44 days for the much slower growing *H. solani*. Mean radius values for each time point were calculated and comparisons between treatments over the time course where made through line graphs, which plotted the mean radial growth of the fungal cultures and the duration of each trial.

RESULTS

Results varied depending upon the specific isothiocyanate incorporated into the agar. In all cases where growth was significantly suppressed by the presence of the isothiocyanate there was an effect of concentration on efficacy as growth decreased as the concentration of the incorporated isothiocyanate increased.

Comparison between the three pathogens highlighted the potential of specific isothiocyanates, mainly BITC and PEITC, to interact with all three screened potato pathogens also importantly experimentation established that some isothiocyanates (IITC and NITC) did not result in any level of control of the three fungal pathogens studied. PEITC in all three trials was shown to establish degree of control upon each of the pathogens tested. However the level of suppression observed varied greatly, dependent upon the pathogen, as well as the concentration of specific ITC incorporated into the PDA.

In vitro* experiments investigating the effect of isothiocyanates on *Collectrotrichum coccodes

Out of the seven isothiocyanates screened against *C. coccodes* PEITC was shown to have the most significant suppressive effect upon the growing cultures. As the concentration of the incorporated ITC increased the resultant colony size decreased, Figure 1.

In vitro* experiments investigating the effect of isothiocyanates on *Rhizoctonia solani

The screening of the seven isothiocyanates against *R. solani* cultures showed varying degrees of efficacy (Figures 2a-d). The most significant suppression was observed on the cultures growing upon the media incorporated with BITC and MITC. In each instance no growth was observed upon any of the treatment plates after 14 days incubation. Results from the assay investigating *R. solani* in response to Propyl isothiocyanate, showed that an increase in PITC concentration reduced the resultant colony size.

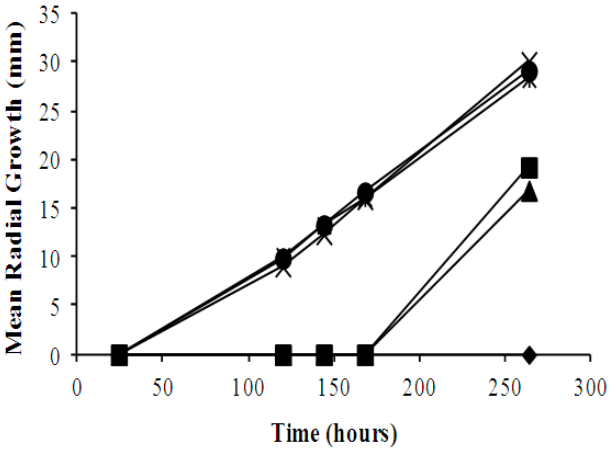

Figure 1a. The *in vitro* effect of varying concentrations of PEITC, incorporated into PDA media on *C. coccodes* growth. Vertical bars show the standard error of the mean. (●) Control, (☐) 125 ppm, (x) 250ppm, (▲) 500ppm, (■) 1000ppm, (◆) 10,000ppm.

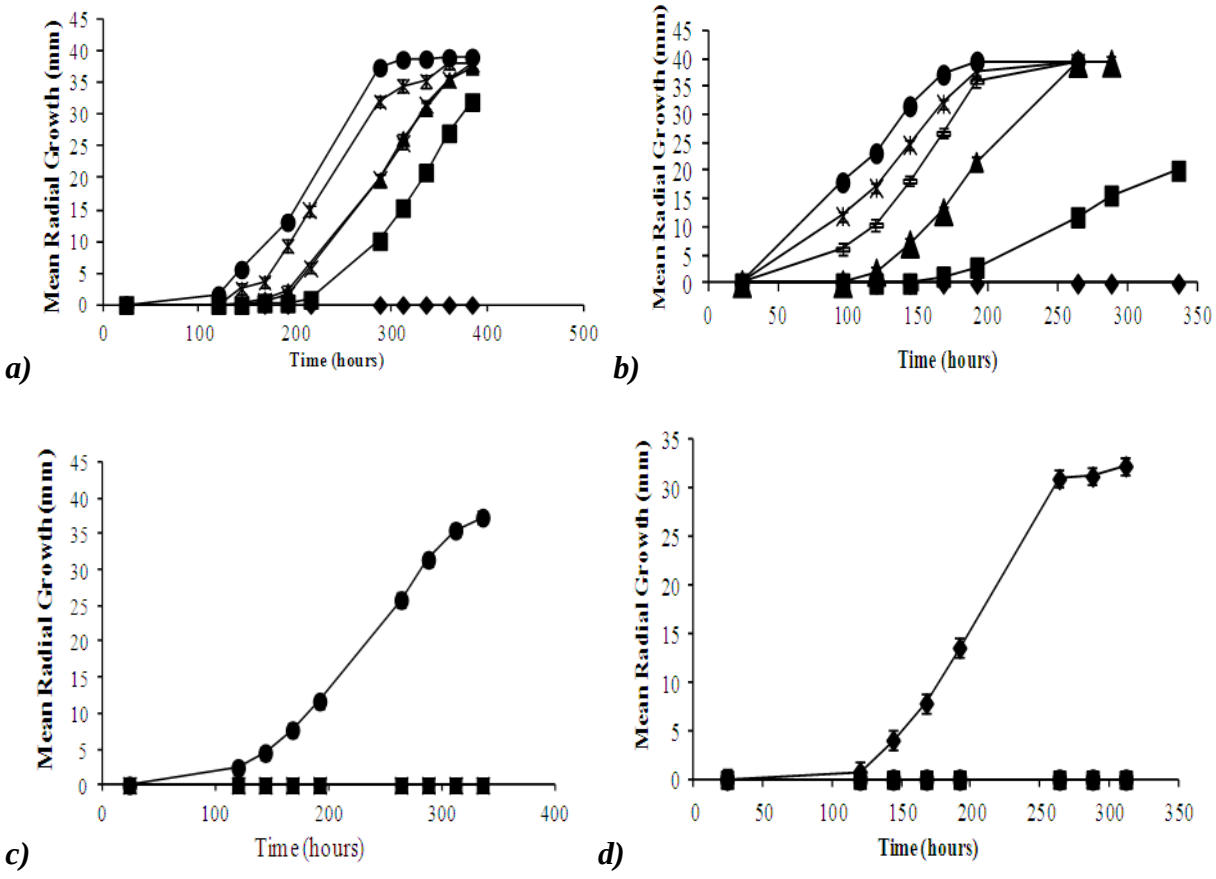

Figure 2 a-d. The *in vitro* effect of varying concentration of a) PITC, b) PEITC, c) BIT, d) MITC, incorporated into PDA media on *R. solani* growth. Vertical bars show the standard error of the mean. (●) Control, (☐) 125 ppm, (x) 250ppm, (▲) 500ppm, (■) 1000ppm, (◆) 10,000ppm.

In vitro* experiments investigating the effect of isothiocyanates on *Helminthosporium solani

Growth of *H. solani* cultures on isothiocyanate media was varied, dependent both upon the specific isothiocyanate within the interaction and the concentration of solution incorporated into the growth media (Figure3a-c). Again the most significant results, in relation to suppression overall growth observed after completion of the experimentation time period, were observed on 2-PEITC media, when growth was only observed on the 125ppm treatment after 35 days. After 44 days of incubation there was no presence of growth on treatments >125ppm. An overall suppression in resultant colony size was also observed in the presence of AITC. In colonies, as the resultant size decreases as treatment concentrations are increased. In addition to this the initial point of growth increases on 1000ppm treatment plates, suggesting a fungistatic response.

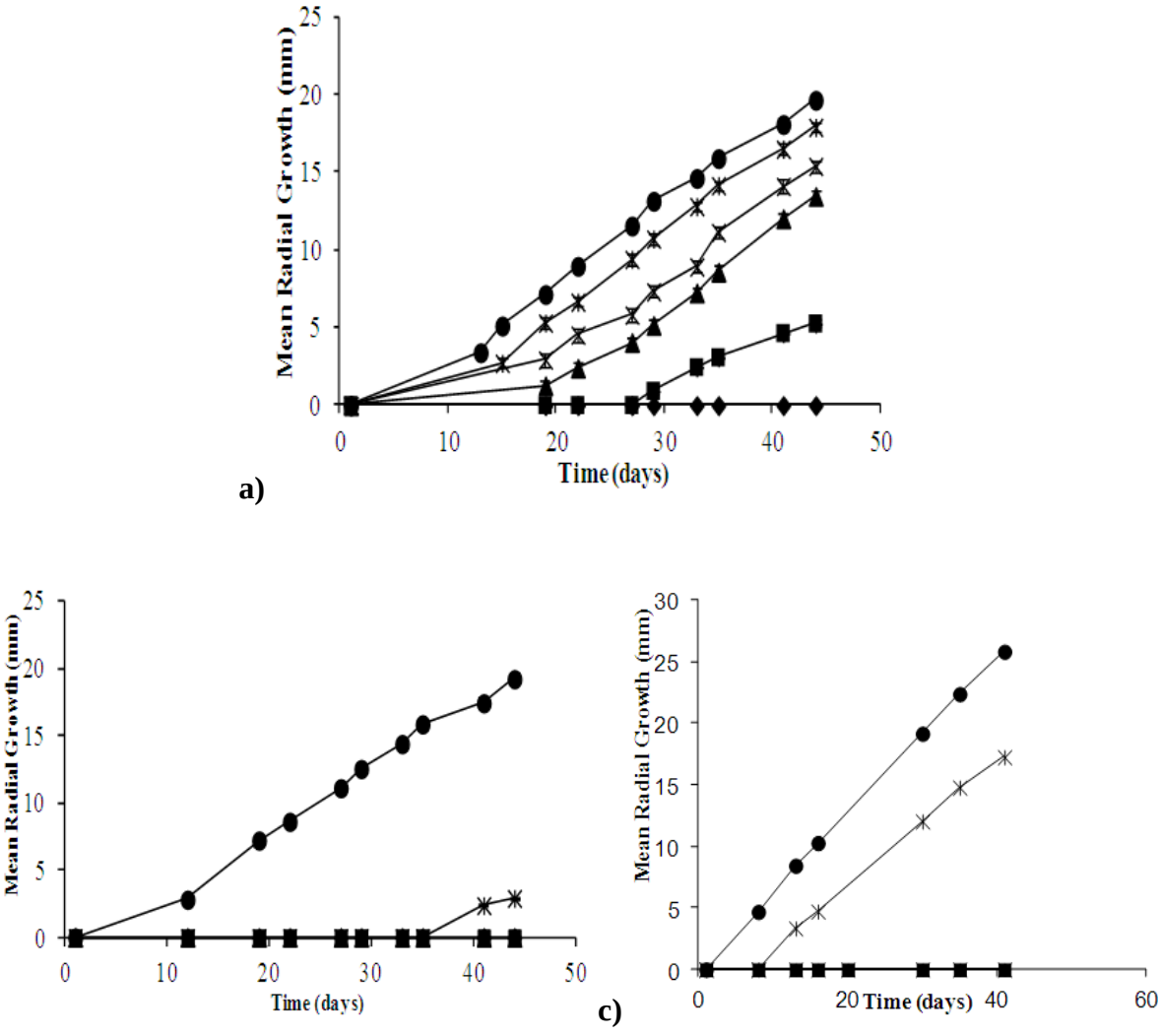

Figure 3 a-c. The *in vitro* effect of varying concentration of a) AITC, b) PEITC, c) BITC incorporated into PDA media on *H. solani* growth. Vertical bars show the standard error of the mean. (●) Control, (☐) 125 ppm, (x) 250ppm, (▲) 500ppm, (■) 1000ppm, (◆) 10,000ppm.

DISCUSSION

The above data highlights some of the key results from this experimentation. The full data set provides evidence that the isothiocyanate – pathogen interaction is one of great specificity. As a large range of effects in response to isothiocyanate incorporation are measured. The overall effect that isothiocyanates have upon fungal pathogens is not only dependent upon the specific structure of isothiocyanate but also the specific fungal pathogen that it is aiming to target. The specificity of the interaction has also been observed within previous studies, Yulianti *et al.* 2006 showed that the level of suppression achieved was dependent upon the strain of the fungus and the type of ITC and the growth.

The results shown here confirm the toxic nature and antifungal effects of ITCs towards soil borne potato pathogens, as has been previously been observed (Sarwar *et al.* 1998). Specifically this study demonstrates that isothiocyanates can have an inhibitory effect upon the growth of *C. coccodes, R. solani* and *H. solani* from which economically important diseases in potato crops results, albeit under *in vitro* conditions. This supports previous data which has shown that glucosinolate hydrolysis products particularly isothiocyanates can suppress the growth of fungal pathogens, (Sarwar *et al.* 1998).

This study has highlighted that 2-Phenylethyl isothiocyanate has toxic properties towards each of the three fungal pathogens examined within this study. Its high level of toxicity has been demonstrated in other studies using a range of different pathogens (Drobnica 1967), specifically *Aspergillus niger, Penicillium cyclopium* and *Rhizopus oryzae*. Significant suppression of the growth of *H. solani* and to a lesser extent *R. solani* was also observed, grown on agar media containing AITC. Growth was not observed on agar containing 10,000ppm until after 10 days of incubation, and on agar containing 1000ppm after 7 days of incubation. Therefore releasing AITC into agricultural soil, through incorporation of Brassica *spp.* green manures, may be a desired method to control levels of soil borne pathogens, particularly *H. solani*. However data on concentrations of specific isothiocyanates is limited, concentrations vary greatly between cultivars. prelimary data by the authors has shown that AITC, BITC and PEITC are common is a number of different Brassica *spp*. ranging in 0.2 to 200ppm.

Previously only a limited amount of research has been carried out the effects of isothiocyanates on the growth and development of *C. coccodes,* and therefore its potential to be controlled through biofumigation. PEITC, which has been identified as being commonly produced by a range of different brassica species, was shown to inhibit the growth of *C. coccodes.* Results in both instances show a relationship between the level of control, and the concentration of the treatment, as the resultant colony size after 13 days decreases as the treatment ITC concentration increases. However in each instance as the concentration of ITC is increased the time for initial growth to be observed also increases, therefore suggesting an initial fungistatic response.

There is little evidence of previous work on *H. solani* and isothiocyanates. Therefore only general comparisons between the trend of results between isothiocyanates and other fungal pathogens can be made here. Again the most promising results for control are observed on PEITC and AITC incorporation studies.

Work by Sarwar *et al.* (1998) found that aromatic ITCs including 2-phenylethyl and benzyl ITC were more toxic towards *R. solani,* than aliphatic ITCs dissolved in agar methyl-ITC, propenyl-ITC, butenyl-ITC, pentenyl-ITC. Yulianti *et al.* (2006) also concluded that 2-phenylethyl

ITC had a higher toxicity level on *R. solani* cultures growing on agar, than Allyl ITC. The above study has produced parallel findings, displaying a range of different levels of control of *R. solani* dependent both upon the concentration of isothiocyanate, incorporated into the agar plate, but probably most significantly is the specific isothiocyanate used within the study. The greatest level of control was observed by Benzyl and Methyl Isothiocyanate, which inhibited growth at all concentration levels used.

2-Phenylethyl Isothiocyanate also exhibited a level of control over *R. solani.* Results showed that as the concentration was increased between 125 – 500ppm the time for the cultures to reach their maximum size was not significantly altered. Yet at 500ppm the time taken for the cultures to begin to grow increased, indicating a level of fungistatic control. At 1000ppm after the 15 day incubation period *R. solani* cultures showed 49.2% inhibition in comparison to control cultures. A similar result was also observed on plates containing agar amended with Allyl Isothiocyanate, where the time taken for growth to be first visible on agar amended with 1000ppm AITC was much greater than that observed on lower concentrations and control plates. These results agree with those presented by Yulianti *et al.* (2006) which show that 2-Phenylethyl ITC is more toxic towards *R. solani* growing cultures than Allyl ITC.

Overall the results show the need to understand the specific interactions occurring between differently structured isothiocyanates and different pathogens. Simple *in vitro* screening of isothiocyanates and their ability to affect the growth of a range of important soil borne potato pathogens allows study and biofumigant crop breeding to be angled towards producing crops that will produce the most effective isothiocyanates that will lead to the highest possible level of control to be achieved. The above study shows that PEITC is clearly the best ITC for providing a broad spectrum of control against the fungal pathogens studied.

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A PROTOTYPE LOW-COST MACHINE VISION SYSTEM FOR AUTOMATIC IDENTIFICATION AND QUANTIFICATION OF POTATO DEFECTS

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Summary: This paper reports on a current project to develop a prototype system for the automatic identification and quantification of potato defects based on machine vision. The system developed uses off-the-shelf hardware, including a low-cost vision sensor and a standard desktop computer with a graphics processing unit (GPU), together with software algorithms to enable detection, identification and quantification of common defects affecting potatoes at near-real-time frame rates. The system uses state-of-the-art image processing and machine learning techniques to automatically learn the appearance of different defect types. It also incorporates an intuitive graphical user interface (GUI) to enable easy set-up of the system by quality control (QC) staff working in the industry.

INTRODUCTION

The British potato industry delivers sales-value of £743m at the farm gate and £3.5bn at consumer level, and potatoes account for 40% of the carbohydrate consumed in the UK. For the fresh market, the main factor affecting consumer preference is physical appearance with clear unblemished skin a significant selling point. Potatoes with defects, diseases and blemishes caused by otherwise benign (to human) skin infections, are strongly avoided. Most potatoes are sorted into different grades by hand, with inevitable mistakes and losses.

This paper reports on the current progress of a project to develop a prototype low-cost machine vision system for the automatic detection, identification and quantification of common defects at near real-time frame rates. The prototype is designed to enable accurate monitoring of individual potatoes as well as aggregation of summary statistics for a sample of potatoes. For example, the summary report lists the proportion of potatoes in a sample affected by different defects, such as common scab, black dot, silver scurf, greening, etc. While the primary intended application of the prototype system is in quality control, the technology also has potential for online application in potato processing and packaging facilities to enable accurate monitoring of an entire harvest of potatoes.

MATERIALS AND METHODS

Hardware Setup

The prototype system uses off the-shelf hardware, including a low-cost machine vision sensor and a standard desktop computer. To enable real-time performance the computer is also equipped with a graphics processing unit (GPU), namely an NVidia GTX 750, which uses parallel processing to accelerate some of the required image processing functions.

Figure 1. Image acquisition - A tray containing potatoes is placed inside the light box, then the box is closed by placing the door on the front (not shown in the picture), attached with magnetic fixings, and an image is captured from the overhead camera for analysis by the system.

Consistent lighting is important for the accuracy and consistency of the system. Therefore, a custom made light-box was built to hold a standard sized tray for quality control purposes (Fig. 1). The floor of this tray measures around 300mm x 400mm, and the tray is capable of holding approximately 12 potatoes, depending on their size. Four battery-powered LED lights were mounted on the ceiling of the light box. Each light was covered with a diffusing plastic material, to ensure reasonably even lighting levels across the tray and to reduce the influence of shadows from the potatoes in the captured images.

The hardware used for image capture is a Logitech C910 HD web-camera capable of taking images with a resolution of 10 megapixels, mounted in a central position in the ceiling of the light box.

Graphical User Interface

A graphical user interface (GUI) was developed to allow the software to be used by quality control experts from the industry (Fig. 2). Within a few button presses the system can be trained to recognise different defect types and then used to analyse potatoes in real-time. An important aspect in the design of the GUI concerns how to achieve an efficient and accurate way for the user to mark-up defective and non-defective areas of a few selected potatoes. This

is important because the system relies on the human expert to provide examples of areas of unblemished potatoes as well as the different defect types, from which the machine learning algorithms used by the system are able to learn the appearance models required to discriminate between the different defect types.

Figure 2. The current prototype interface.

An overview of the GUI is provided in Figure 2, where the two smaller images on the left hand side represent the webcam feed (top) and a visual representation of the user-selected data on which the machine learning classifier has been trained (bottom). The final larger window is used initially for the user mark-up of selected potatoes for training the system, and then for displaying the classifier output when the trained system is used for quality analysis. Finally, the GUI displays a summary report giving the percentage of the classified area for unblemished potato as well as for each defect type (see Fig. 5 for an example).

An image is captured by pressing the "capture image" button and then pressing the "remove background" button to extract the potatoes, setting the background pixels to a neutral colour. There is also a corresponding "learn background" button to learn the appearance of the image background after first taking an image of the empty tray. The tray does not need to be in exactly the same position between images. The user mark-up is carried out by first selecting a user-defined class or category (e.g. "non-defect", "defect type 1", "defect type 2", etc., where the defect types could correspond to common scab, silver scurf, black dot, etc.) and selecting pixel areas within the image that correspond to that particular class to provide training data. The user presses the "train classifier" button when the mark-up is finished. The user can then press the "classify" button to analyse entire images of potatoes in the tray, producing an output image where every part of the potatoes has been colour-coded according to the categories assigned by the trained classifier. The high computational speed of the system, producing

almost instant results, means that the user can interactively refine the trained classifier by adding or removing additional areas of the potato to include in the training data for each class (e.g. parts of the potatoes which were initially misclassified due to insufficient training data).

Once the user is satisfied with the set-up and training of the system, the trained classifier can be used to classify as many trays of potatoes as desired, provided that the potatoes are sufficiently similar to those used to train the system. The system can be quickly retrained to work with different potato varieties, diseases and lighting conditions, etc.

Automatic Defect Identification

The software for image processing and machine learning used for defect detection and identification is based on the earlier work by Barnes *et al*. (2010a, 2010b). In this approach, classifiers are trained to detect and identify defects using colour and texture features extracted from the image. A very large set of candidate features, based on statistical information relating to the colour and texture of the region surrounding a given pixel, is first extracted. A machine learning algorithm (known as AdaBoost) is then used to automatically select the best features for discriminating between defects and non-defects, and also to discriminate between the different types of defects. With this approach, different image features can be selected for different potato varieties, while also handling the natural variation in fresh produce due to different seasons, different lighting conditions, etc.

While the above-mentioned research established the "proof of concept" for this project, the previous work did not include development of any interface for training the system, nor did it include processing of the images in real-time (the previous software developed would take several hours to analyse a single image). Therefore, some of the important developments in this project to realise the prototype system included developing the graphical user interface (as described above) and also the re-implementation of the system using heterogeneous computing. Heterogeneous computing systems use a variety of different types of computational units, including here a general-purpose processor (GPP) with four processor cores and a graphics processing unit (GPU) for accelerating many of the more computationally expensive image processing functions. Consequently the prototype system is currently capable of running at 30 frames per second using the live feed from the web-camera. This means that the system still has spare computational power for further improvements and development of the technology.

RESULTS

The screen capture shown in Figure 3 demonstrates how a human expert would mark-up images by simply selecting areas of particular defects. This data is then passed onto the machine learning algorithm to determine the features that best separate the different classes. The screen capture shown in Figure 4 shows the corresponding output of the trained classifier when used to analyse the whole image of potatoes. Figure 5 shows the corresponding summary report, taken from the graphical user interface, showing the percentage of the classified area for unblemished potato as well as for each defect type.

Once trained, the system can be used to classify potatoes with very similar general features and can be very simply retrained to improve particular class recognition.

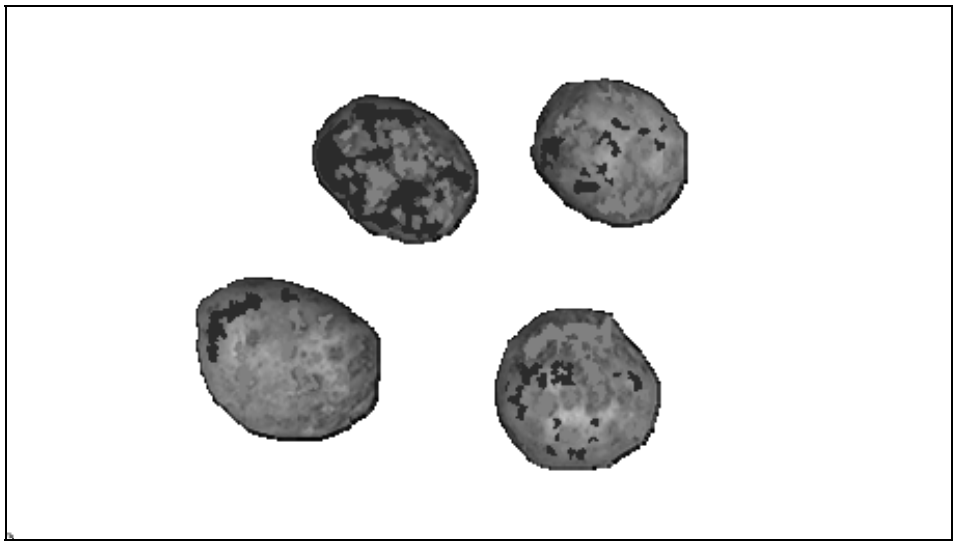


Figure 3. Mark-up of a set of potatoes with common scab (marked in light grey) and unblemished skin (marked in dark grey)

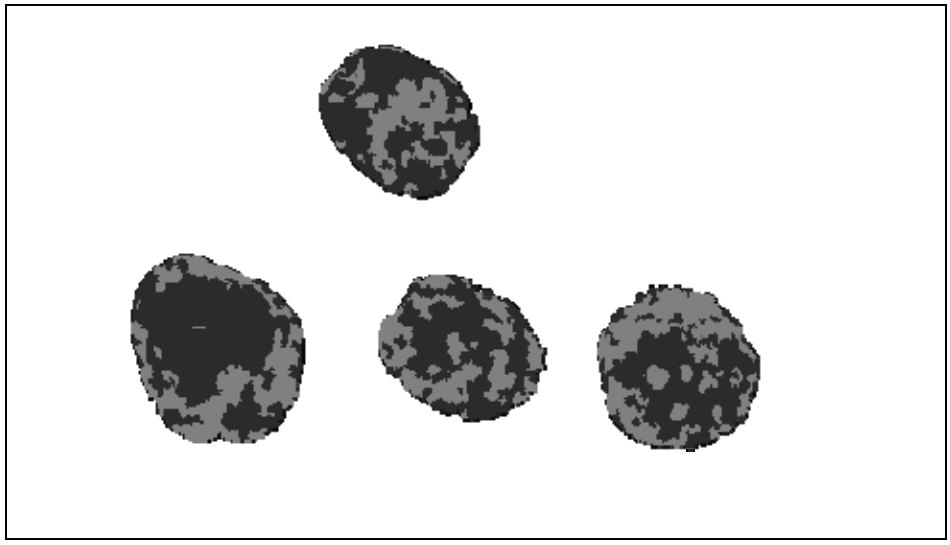
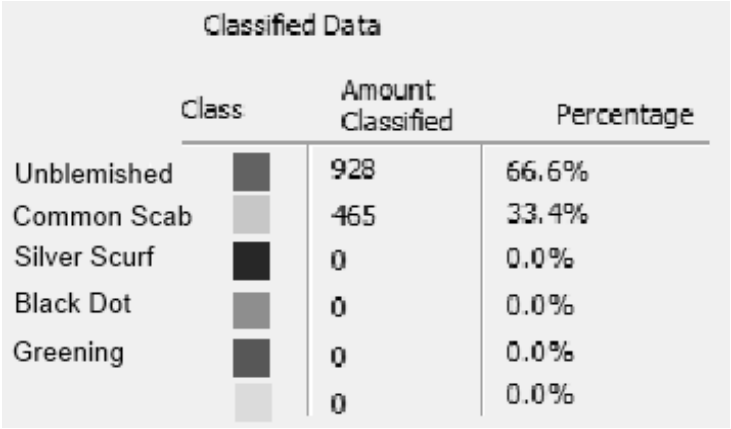


Figure 4. A classified image of potatoes using the classifier trained from the data in Figure 3.

CONCLUSION

In conclusion, a prototype system for automatic defect detection has been developed. A number of planned improvements will enable the system to be more robust with respect to different potato samples and image capture environment. Another extension would be to add a database of previously trained classifiers for different types of potatoes and potato defects, which could then be recalled and re-used as needed, without having to retrain the system from scratch each time.

Overall the system has many benefits to QC staff, improving consistency, speed and accuracy of defect identification and quantification. However, the system is not a replacement for the human element of quality control, as this research aims to produce an aid to experts but not a replacement. Therefore, if the training completed by the human expert is inaccurate and inconsistent, then the system itself will classify and calculate the defective areas incorrectly due to the poor quality training.



Classified Data

Class	Amount Classified	Percentage
Unblemished	928	66.6%
Common Scab	465	33.4%
Silver Scurf	0	0.0%
Black Dot	0	0.0%
Greening	0	0.0%
	0	0.0%

Figure 5. Summary report showing the percentage of the classified area for unblemished potato as well as for each defect type, using the same example shown in Figures 3 and 4.

ACKNOWLEDGEMENTS

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DEVELOPMENT OF A MULTIPLEX REAL-TIME QUANTITATIVE PCR ASSAY TO DETECT *PHOMA* PATHOGENS OF POTATO

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Summary: Gangrene is a storage disease of potato caused predominantly by the soil-borne fungus *Phoma exigua* var. *foveata.* The less virulent pathogen *Phoma exigua* var. *exigua* also causes gangrene-type lesions. Gangrene has been increasing in prevalence in Scottish seed crops in the last three years and, unexpectedly, high levels have been recorded in some early generation Pre-Basic crops. A rapid and sensitive multiplex real-time PCR assay, which can differentiate between the closely related *Phoma* pathogens, would greatly facilitate research into this disease whilst also providing a time-efficient and cost-effective diagnostic. Here, we report on the development of a multiplex real-time assay, based on Plexor™ technology, capable of distinguishing between the two most pathogenic *Phoma* species in a single qPCR reaction.

INTRODUCTION

Gangrene is a storage dry rot of potato (*Solanum tuberosum*) caused predominantly by the soil-borne fungus *Phoma exigua* var. *foveata* (Foister) Boerema (1967), which infects largely through wounds arising at harvest or grading. The less aggressive pathogen, *Phoma exigua* var. *exigua* (Boerema, 1967), is also associated with gangrene. Symptoms start with small sunken thumbprint-like lesions at wounds which then enlarge, becoming irregular in shape. Internally, a dark rot develops and large cavities can be present.

Cases of gangrene have been increasing at a number of seed growers during the last few years (SASA, unpublished data), with even high-grade Pre-Basic 2 (PB2) crops having undesirable amounts of gangrene recorded. Such high gangrene levels, at this early stage of seed multiplication, are unexpected and suggests that contamination of PB crops may be occurring much earlier than previously thought. Carnegie *et al.* (1981) found that the incidence of these fungi on first and second year VTSC clones was less than on older clones. However, it is possible that the loss of the fungicide 2-AB as a control measure and sulphuric acid as a haulm desiccant, possibly combined with large tonnages being produced at the earlier stages of production, may be impacting on the spread and development of the pathogens responsible for these diseases.

For research and diagnostic purposes, it would be useful to have an assay which can rapidly distinguish between the *Phoma* pathogens. In culture, *P. exigua* var. *foveata* and *P. exigua* var. *exigua* are morphologically similar, except that *P. exigua* var. *foveata* produces a yellow-brown anthraquinone pigment. However, pigment production can be lost making it difficult to differentiate between *P. exigua* var. *foveata* and *P. exigua* var. *exigua* (Boerema, 1967). At the

molecular level there is considerable similarity between these two closely related *Phoma* varieties, with no genetic differences being found in the internal transcribed spacer (ITS) regions 1 and 2 (MacDonald *et al*., 2000). MacDonald *et al.* (2000) developed a RAPD-generated PCR-RFLP marker to distinguish between the two varieties, but the use of restriction enzymes makes this assay time-consuming and relatively expensive. Cullen *et al.* (2007) developed conventional and quantitative PCR assays for the detection of *P. exigua* var. *foveata*, but the primers also detected the closely related *P. exigua* var. *exigua.* Aveskamp *et al.*, (2009) developed specific primers which can differentiate the two varieties with conventional PCR, but this is more time-consuming and less sensitive than real-time PCR. Real-time quantitative PCR (qPCR) also allows for a simpler quantification than conventional PCR (Cullen *et a*l., 2002). Therefore, the aim of this study was to develop a rapid, multiplex quantitative real-time assay which could distinguish between the main *Phoma* potato pathogens in one reaction, thus saving on time, labour and reagents.

The assay was designed on Plexor™ technology (Promega) which is a real-time PCR methodology offering straightforward multiplexing of quantitative PCR, with the benefit of increased throughput and reduced cost compared with standard real-time PCR. For Plexor-based assays, one of the species-specific primers is both labelled with a fluorescent dye and modified with methylisocytosine (iso-dC) residue at the 5' end. The other primer is not modified. Each set of species-specific primers is labelled with a different fluorescent label to create a multiplex reaction. The qPCR reaction buffer (Promega) includes dabcyl-iso-dGTP (iso-dG); during thermocycling this becomes incorporated at the position complimentary to the iso-dC label, effectively quenching the fluorescence over time (Frackman *et al*., 2005).

MATERIALS AND METHODS

Primer design

DNA Sequences were generated from the isolates listed in Table 1 for the purpose of designing primers specific to the *Phoma* pathogens by comparative sequence analyses. Sequences were amplified using primers derived from RAPD-PCR fragments (Phoma 2 and Phoma 7) (MacDonald *et al,* 2000) and elongation factor 1a (EF1a) gene-specific primers (Schoch, unpublished data) (Table 2). The PCR amplifications were conducted in 20µl reactions in an Applied Biosystems Veriti thermocycler, with 5µM primers and 1ng purified template DNA in a Jumpstart ready mix (Sigma). PCR products were visualised on a 1% agarose gel, then PCR-amplified products were sequenced using ABI BigDye Terminator kit version 3.1 (Applied Biosystems). Sequencing (in both directions) was carried out using the ABI 3130*xl* DNA Analyser and the resulting sequences were analysed on SeqMan and aligned with Mega 5.03, prior to primer design. In addition to the sequences generated above, the *Phoma* sequences published by Aveskamp *et al*., (2009) were also used to design primers. These sequences are published on Genbank as accession numbers EU880838 (*P. exigua* var. *exigua)* and EU880839 (*P. exigua var. foveata)* respectively. Both of these sequences were generated from DNA amplification fingerprinting fragments derived from arbitrary mini-hairpin primers (Aveskamp *et al*., 2009). The primers generated in this study were designed using the Plexor™ primer design software (Promega).

Table 1. Scottish seed crop *Phoma* species used in the primer design and to test the Plexor™ assay

Phoma species	Culture Reference	Origin
P. exigua var. *foveata*	P61	Galashiels, 2005
P. exigua var. *foveata*	P52	Grampian, 2006
P. exigua var. *foveata*	P26	Grampian, 2007
P. exigua var. *foveata*	P54	Aberdeenshire, 2004
P. exigua var. *foveata*	P21	Grampian, 2009
P. exigua var. *foveata*	PF HG	Morayshire, 2011
P. exigua var. *foveata*	FONTANE 3	Perthshire, 2011
P. exigua var. *foveata*	CAB 1	Aberdeenshire, 2011
P. exigua var. *foveata*	CAB 2	Aberdeenshire, 2011
P. exigua var. *foveata*	CAB 3	Aberdeenshire, 2011
P. exigua var. *foveata*	CAB 4	Aberdeenshire, 2011
P. exigua var. *foveata*	CAB 5	Aberdeenshire, 2011
P. eupyrena	P70	Morayshire, 2010
P. eupyrena	POT 1	Aberdeenshire, 2009
P. eupyrena	POT 2	Aberdeenshire, 2009
P. exigua var. *exigua*	P55	Aberdeenshire, 2008
P. exigua var. *exigua*	P53	Fife, 2008
P. exigua var. *exigua*	15	Fife, 2010

Table 2. Primers used to amplify *Phoma* DNA for sequencing purposes.

Primer name	Specific target	Sequence 5' to 3'	Size
Phoma 2 Phoma 7	RAPD-PCR derived marker specific to *Phoma sp.*	GGACCCCTGTACTGACGTC AGCGGCTAGGATAGACAGGCG	474bp
EF1-1Fa EF1-1Ra	EF1a gene	GCTGGTATCTCCAAGGATG TCRGTGAARGCCTCAAC	~870 bp

The Plexor™ primers generated, PFov-R and PEx-R (Table 3), were labelled at the 5' end with TEXAS RED (peak emission at 620nm and peak excitation at 584nm) and fluorescein phosphoramidite (FAM; peak emission at 516nm and peak excitation at 492nm) respectively.

Additionally, both primers were modified at the 5' end with an iso-dC residue. Primers PFov-F and PEx-F were not labelled or modified.

Quantitative PCR amplifications were conducted in 25µl reactions in a Stratagene MP3005P thermocycler, with the *P. exigua* var. *exigua* primers at a final concentration of 100nM, the *P. exigua* var. *foveata* primers at a final concentration of 200nM and <100ng purified template DNA. Reactions were carried out using 2x Plexor™ qPCR system master mix (Promega). The following amplification protocol was used: initial denaturation of 2 minutes at 95°C, followed by 40 cycles of 95°C for 5s and 60°C for 35s, then 1 cycle of 60°C for 15s and 95°C for 5s. Following amplification, results were analysed using Plexor™ Analysis Software (Promega).

RESULTS

The *Phoma* specific primers (Phoma 2 and Phoma 7) (MacDonald *et al,* 2000) and elongation factor 1a (EF1a) gene specific primers (Schoch, unpublished data) (Table 2) generated fragments with too little heterogeneity between the two *Phoma* species to allow the design of specific qPCR primers based on these sequences. Therefore the Genbank sequences published by Aveskamp *et al*. (2009) were used in the primer design and the resulting primers are listed in Table 3.

Table 3. Plexor™ primers generated in this study specific for *Phoma exigua* var. *foveata* and *Phoma exigua* var. *exigua* based on DAF-generated PCR-specific marker sequences.

Primer name	Target organism	Sequence 5' to 3'	Size
PFov-F	*P. exigua*	**GGTGAACTCTGTGCTCGATATGC	80bp
PFov-R	var. *foveata*	ATGACAGGAGTGAGACGATGATAGT	
PEx-F	*P. exigua*	*AATCTAGAGCAACATTAGCAATCCTGT	80bp
PEx-R	var. *exigua*	TGGTCTCCACTTGTAAACGTTAGAATCA	

*Modified at the 5' end with iso-dC and FAM
**Modified at the 5' end with iso-dC and Texas Red

The multiplex assay amplified each of the *Phoma* isolates listed in Table 1 accurately, achieving the same level of sensitivity as the individual monoplex reactions. The multiplex detection limit was 80fg for the *P. exigua* var. *foveata* assay and 160fg for the *P. exigua* var. *exigua* assay. The standard curves produced for each species showed high correlation coefficient (R^2) values, of 0.98 and 0.97 for *P. exigua* var. *foveata* and *P. exigua* var. *exigua* respectively, indicating linear responses in detection related to the increasing DNA concentration, which can be used to estimate DNA concentration of unknown samples.

DISCUSSION

Here we present a quantitative real-time PCR assay based on Plexor™ technology (Promega) which can accurately detect the two main gangrene pathogen species found in Scotland, *P. exigua* var. *foveata* and *P. exigua* var. *exigua*. The significant degree of sequence homology of these two pathogens can cause issues when designing primers for molecular diagnostics, yet at the pathogenicity level these two pathogens are very distinct, with *P. exigua* var. *foveata* being significantly more aggressive than *P. exigua* var. *exigua*. MacDonald *et al.* (2000) required the extra step of restriction enzyme digestion following RAPD PCR to distinguish the two pathogens, Cullen *et al*. (2007) produced a real-time assay that could detect *Phoma* pathogens at low levels, but unfortunately it could not distinguish between these two gangrene pathogens. To date, Aveskamp *et al*.(2009) have produced the most discriminatory assay, but this is based on conventional PCR which lacks quantification, is less sensitive and is more time consuming than real-time qPCR as it requires an additional gel electrophoresis step to visualise the results.

The sequences generated in this study failed to identify sufficient polymorphisms between the *Phoma* species for successful Plexor™ primer design and therefore we based our primers on the sequences already published by Aveskamp *et al.* (2009). Several combinations of primer sets were generated using the Plexor™ primer design software and we evaluated each of them prior to selecting those shown in Table 3 (data not shown). Primer concentrations were modified during the optimisation of the assay, but no other parameters required modification. The sensitivity of the multiplex and monoplex reactions was established and compares well against conventional PCR assays.

Standard curves were generated for multiplex and monoplex reactions indicated a strong relationship between the DNA concentration and detection limits of this test and there was little loss of sensitivity when comparing results of the multiplex versus monoplex reactions.

Additional genes from *Phoma* species have now been sequenced (data not shown) in a search for potential sites for primer design, e.g. actin, calmodulin, ITS, 18S rRNA and 28S rRNA. Data from some of these genes appear to have sufficient heterogeneity between *Phoma* species to permit future development of a triplex assay which includes detection of *P. eupyrena*.

Therefore we conclude that this multiplex real-time qPCR assay, based on Plexor™ Technology offers significant improvements over the currently available diagnostics for the gangrene pathogens, in that it is time and cost- efficient when compared with conventional PCR or monoplex real-time qPCR assays. With the increases in gangrene levels being found in seed crops in the last three years, this assay will provide an excellent resource for both diagnostics and research purposes.

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GENE EXPRESSION OF *GLOBODERA PALLIDA* IN DIFFERENT POTATO GENETIC BACKGROUNDS

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Summary: The aim of this study was to determine key genes that are differentially expressed in *Globodera pallida* in different stages of the life cycle and during the initiation of the feeding site in susceptible or partially resistant potato genotypes. For this purpose, two microarray experiments were designed, in the first of which a a comparison of eggs, infective juveniles of second stage (J2) and sedentary parasitic stage J2s was made, in the second a comparisons of sedentary J2s at seven days after inoculation in the susceptible cultivar Desirée and two partially resistant lines. The results show differential expression of *G. pallida* genes during the different stages, including previously characterized effectors. Also higher differences in gene expression were detected between the sedentary J2 nematodes in the susceptible cultivar compared to those infecting partially resistant lines; these differences were smaller when two partially resistant lines were compared.

INTRODUCTION

Globodera pallida, the white potato cyst nematode, is the most economically important plant-parasitic nematode in the UK, and also causes problems for growers in many other parts of the world (Jones *et al*., 2009). *Globodera pallida* and *Globodera rostochiensis*, the golden potato cyst nematode, are collectively known as the Potato Cyst Nematodes (PCN). Both species have complex interactions with their hosts and these interactions involve changes in gene expression in both the nematode and the host plant. Generally, the life cycle of cyst nematodes begins when the vermiform J2 hatches and invades the roots of host plants. Each individual nematode feeds on a group of cells in the pericycle, cortex or endodermis, transforming them into a syncytium or transfer cell. The nematode remains sedentary, as it passes through two more juvenile stages to become either male or female. Males are active and leave the root to find and fertilize females. Females remain in the root, and retain the eggs inside their bodies and the first moult from J1 to J2 occurs in the egg. When the females are fully mature they die and their cuticle hardens and turns brown to become a protective cover (the cyst) around the eggs.

Some *G. rostochiensis* pathotypes can be controlled by the use of cultivars containing major resistance genes, however, repeated use of these cultivars has led to selection of *G. pallida* in the UK, and this species is now present in 65% of the fields used for potato ware cultivation in England & Wales (Minnis *et al*., 2002). The reduction in availability of effective nematicides due to changes in EU legislation and consumer pressures may exacerbate these problems in future. Little is known about the molecular interactions between the plant and nematode in this complex interaction and even less in plants that have some level of resistance. How nematode

metabolism and development is altered by host resistance is not understood and this study was designed to examine the effect of resistance on nematode gene expression.

Microarrays offer the possibility of studying a broad number of genes while using less starting material than high throughput RNA sequencing platforms, an important consideration when the scarcity of sample material is a major issue. Examination of plant-parasitic nematode gene expression using microarrays has not been extensively used to date. A few studies have been conducted on *Heterodera glycines* in different stages and different populations (Klink *et al*., 2009a; Klink *et al*., 2009b; Elling *et al*., 2007, Ithal *et al*., 2007) and searching for novel putative parasitism associated genes (Elling *et al*., 2009) identifying putative parasitism-associated genes and differential genes between virulent and avirulent populations to a specific plant resistance gene.

The aims of this study were to understand which genes were modified in expression during the development of different *G. pallida* stages and to examine which were modified in sedentary nematodes in susceptible or partially resistant potato plant genetic backgrounds.

MATERIALS AND METHODS

Nematode inoculum, plant material, and experimental design

G. pallida (population Lindley) was cultured at The James Hutton Institute (JHI). Three potato genotypes have been used which react differently to this population; susceptible reaction (cv Desirée) and resistant reactions (breeding lines 11305 and 11415) with different genetic background. The partially resistant line 11305 has resistance derived from *Solanum vernei* and line 11415 has two resistant sources, one of which is the *H1* gene (complete resistance to *G. rostochiensis* pathotypes Ro1 and Ro4) from *S. tuberosum* ssp. *andigena* CPC 1674 with partial resistance to *G. pallida* conferred from *S. tuberosum* ssp. *andigena* CPC2802.

Two microarray experiments were conducted, using either different stages in *G. pallida* development (hydrated eggs for 24 h, infective second stage juveniles (J2) and sedentary nematodes from roots after 8 days post-inoculation (dpi) with the susceptible cv. Desirée) or with sedentary nematodes 8 dpi with the susceptible (cv. Desirée) and partially resistant lines (11305 and 11415). Plants were inoculated close to the roots with 3000 J2s suspended in 3 ml of sterile distilled water.

RNA extraction, amplification and hybridization

Total RNA was extracted using RNeasy® Plus Micro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA digestion was conducted on glass fibre during RNA extraction using RNase-Free DNase set (Qiagen) following the manufacturer's instructions. Total RNA was quantified using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) using Agilent Small RNA kit and RNA amplification and hybridization was performed using Quick Amp Labeling, two-color kit (Agilent) following the manufacturer's instructions. Data were extracted from scanned array images using Agilent FE software, and data quality control (QC), normalisation and analysis performed using program GeneSpring GX 7.3 (Agilent).

Microarray analysis

Microarray gene expression analyses were conducted using a custom-designed 8 x 15k Agilent array representing 5683 *G. pallida* genes with a total of 14649 probes. Probes were designed using different EST libraries (Jones *et al*., 2009) and sequences from public databases. After normalization of the intensities obtained, a one way ANOVA test was performed using Bonferroni multiple testing correction. For comparison between partially resistant genotypes a less restrictive statistical analysis was also performed using a volcano plot with fold change of ≥ 2 between both genotypes and a p-value cut off of 0.05. K-means clustering was used to group gene expression profiles in both microarray experiments for those genes significantly differential expressed.

Real-time RT-PCR

Six genes differentially expressed respective to their different stages or genotypes and one gene without differential expression were studied along with three housekeeping genes for validation of the microarray.

RESULTS

Sequence BLAST hit identities with a threshold of 1e-04 identified 43% of genes (2458 genes from 5747 genes from 14649 probes spotted onto the microarray). Validation of the microarray by real-time RT-PCR gave gene expression matching the microarray results.

Microarray analysis of developmental stages

The majority of the genes passed the QC filtering. Comparison of the different stages (egg, parasitic J2 and sedentary nematodes at 8 dpi) identified 7.5% of the total genes which were significantly modified after ANOVA statistical analysis. K-means clustering of genes modified in the different stages produces four main significant groups. The first cluster K1-stages represented the genes highly up-regulated in egg and down-regulated in sedentary J2; cluster K2-stages represented genes modified in parasitic J2 in comparison to egg and sedentary J2 stages; cluster K3-stages represented genes up-regulated in sedentary stages in comparison to egg and parasitic J2; and finally, cluster K4-stages represented genes up-regulated in parasitic J2 and down-regulated in egg stage.

Microarray analysis of sedentary nematodes in different potato genetic background

Comparison of interaction of resistant lines (11305 and 11415) and the susceptible cultivar Desirée to *G. pallida* gives 7.3% significantly modified genes after ANOVA statistical analysis using Bonferroni multiple testing correction. Nematode genes differentially expressed between potato genotypes produced two main significant groups. One set (K1-genotypes) showed higher expression in the resistant lines and the rest of genes (K2-genotypes) were clustered by lowered expression in resistant lines in comparison to the susceptible cultivar Desirée. Of these genes, only a small subset was differentially expressed between the two resistant lines.

DISCUSSION

K-means clustering allowed the differentiation in expression of gene groups with similar profiles between different nematode stages. Annotation of these gene groups allowed us to infer biological meaning. The K1-stages group was more related to the nematode survival, as they were preferentially expressed in nematode eggs and the sedentary stage detected in nematodes at 8 dpi. K2-stages genes were more related to mobility and the first stages of parasitism in plants. K3-stages were more related to sedentary stages of nematodes with metabolism and body changes associated to this period (loss of mobility and digestion process). Finally, K4-stages were more associated to activation of some genes in the mobile parasitism stages of the nematode and not associated with the survival egg stage. Genes up-regulated in mobile J2 are clustered in K2-stages and K4-stages, indicating important biological process of these genes in parasitism.

Similarities in the sedentary nematode expression in the different genetic potato backgrounds could be related to similar response of the plant to nematode infection in both lines in comparison to the susceptible cultivar. The K1-genotypes group of genes showed an increase in the metabolic enzymes, stress and some effectors in the nematodes inoculated to the partial resistant lines in comparison to the susceptible lines. Some of these genes may be responsible for overcoming the plant defences and resisting this harsh environment. While K2-genotypes genes showed effects in the down-regulation of putative effector genes and a broad number of functions which may affect resistance in the nematode. The comparison of parasitic stage gene expression between both partially resistant lines showed a lower gene expression of the statistically significant genes in line 11415 than line 11305.

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GENETIC ANALYSIS OF POTYVIRUS RESISTANCE IN *SOLANUM TUBEROSUM* GROUP *PHUREJA*

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Summary: To make the potato breeding process more efficient, it is desirable to find genetic markers for use in a marker-assisted selection (MAS) strategy. In this study, the chromosomal location of the major gene conferring resistance to PVY in *Solanum tuberosum* group *Phureja* was identified by microsatellite analysis. From this work we have identified one microsatellite marker located in the middle of chromosome IX that shows linkage with extreme resistance (ER) to the potyvirus, PVY. The location of this marker on the genetic map suggests that resistance to PVY is conferred by a gene different from other known extreme resistance genes, specifically Ry_{sto}, Ry_{adg} and Ry_{chc}, found in other member of the genus *Solanum*.

INTRODUCTION

Potato virus Y (PVY) belongs to the *Potyvirus* genus (family *Potyviridae*) and is one of the most important plant viruses affecting potato. PVY is responsible for heavy crop losses, and can reduce potato yield production by up to 80%, as well as decrease plant productivity and tuber quality (De Bokx & Huttinga, 1981; Brunt, 2001). Breeding to produce resistant cultivars has become more important and is an effective strategy to achieve protection against many pathogens, including plant viruses.

Besides several hypersensitivity-type resistance genes (Cockerham, 1970), extreme resistance (ER) genes to PVY have been identified from three different sources: Ry_{sto} from *Solanum stoloniferum* Schlechtdl. & Bouché. (Cockerham, 1943), Ry_{adg} from *S. tuberosum* L. subsp. *andigena* Hawkes (Munoz *et al.,* 1975) and Ry_{chc} from *S. chacoense* Bitt. (Asama *et al.,* 1982). Potato ER genes can confer resistance to single viruses, e.g. Rx and potato virus X (PVX), or two or three viruses, e.g. Ry_{sto} that confers resistance to PVY, PVA and PVV, although the latter operates *via* two or three genes organised in a very tight linkage group (Barker, 1996). The Ry_{sto} locus has been mapped to chromosome XII, co–segregating with the SSR marker STM0003 (Song *et al.,* 2005). A Japanese study into cv. Konafubuki mapped an extreme resistance gene to PVY (Ry_{chc}) to the distal end of the chromosome IX (Hosaka *et al.,* 2001).

The study presented here exploits the use of microsatellite markers to map the location of a newly identified PVY resistance gene that exists in a population of *Solanum tuberosum* group *Phureja*.

MATERIALS AND METHODS

Plant Material

A family of 190 diploid F1 hybrids (O8H1 potato population) were derived from a cross done at JHI between the clone DB 375 (1), which is extremely resistant to PVY, and the susceptible clone 842 P75.

Resistance Assay to PVY

Resistance to PVY of the F1 hybrids and their parents was evaluated by mechanical inoculation of the plants with a PVY^O strain, obtained from virus collection at JHI and maintained in *Nicotiana tabaccum* cv. White Burley by repeated sap inoculation. The inoculated potato plants were tested, 3 weeks after inoculation, by triple-antibody sandwich-enzyme liked immunosorbent assays (TAS-ELISA). The absorbance at 405 nm (A_{405}) was measured after 1 h using an ELISA microplate reader (Multiscan® Ascent). Samples were deemed positive if their absorbance value was more than twice that of the non-infected control plants.

Plant DNA Isolation, PCR and Electrophoresis

Total DNA was extracted from fresh potato leaves using the Qiagen DNeasy Plant Mini Kit® as recommended by the manufacturer. PCR amplification was performed in a total volume of 10 µl containing 1 X PCR buffer, 10 pmol of each primer, 30 ng of potato genomic DNA, 2 µM of each of the four dNTPs, 1 unit of *Taq* polymerase (Roche) and sterile distilled water. The PCR reaction parameters were: 94°C for 1 min followed by 35 cycles of denaturation at 94 °C for 30 S, extension for 1 min annealing at XºC as appropriate for each primer pair for 30 S. and extension at 72°C for 5 min. The above temperature programme was used throughout with only the annealing temperature varying. Amplified products were then resolved on a 1% agarose gel and visualised with ethidium bromide staining. In total, 45 primer pair were used for detection of genetic diversity in the parental clones and their progeny in order to map the location of PVY resistance gene.

Mapping of the Phureja PVY Resistance Gene

Any of the SSR markers that revealed a polymorphism between the resistant and susceptible parents was used to test the whole progeny population. Linkage analysis was performed using the GeneMapper v3.7 software programme.

RESULTS

Identification of the Marker Linked to the PVY Resistance Gene

To map the group *Phureja* PVY resistance gene, the O8H1 population derived from a cross between clone DB 375 (1), which is resistant to PVY, and clone 842 P75, which is susceptible to PVY, was utilized. Of 45 amplified marker loci, only one SSR marker (651229), which maps to chromosome IX, was polymorphic between the resistance and susceptible parental

plants (Fig. 1), producing a 272 bp fragment in the resistant genotypes which was absent in the susceptible genotypes.

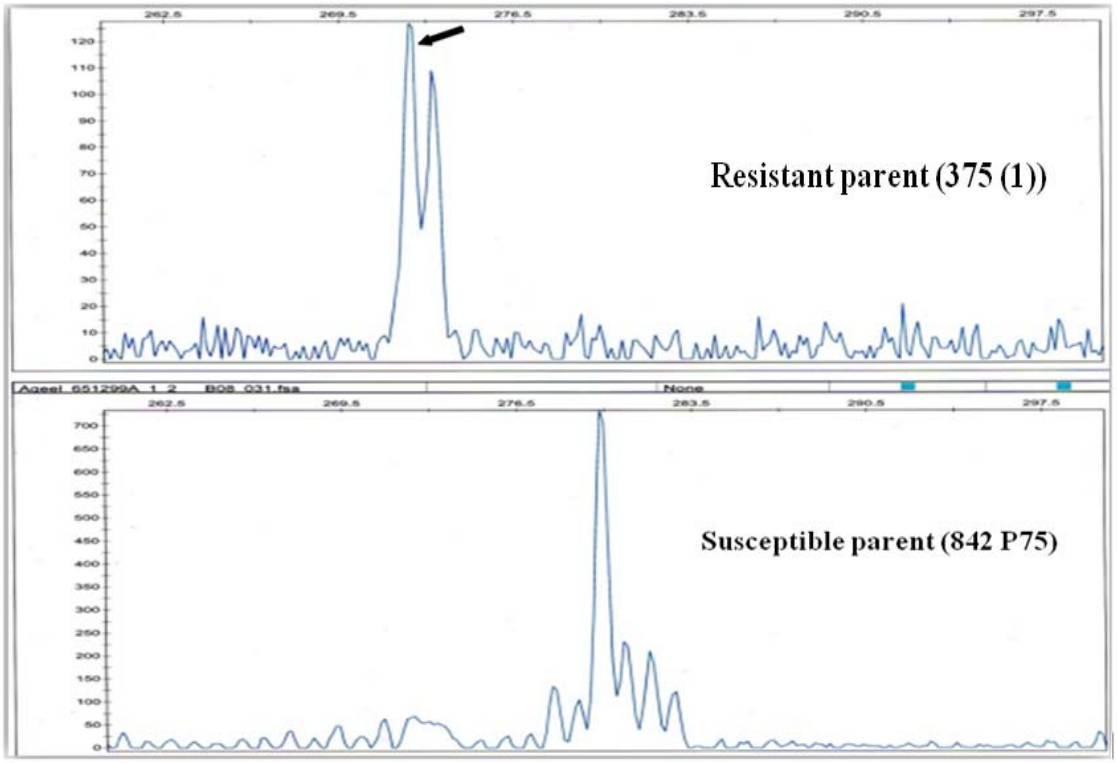

Figure 1. SSR peak generated from resistant and susceptible parents (clone DB 375 (1), and clone 842 P75, respectively) with the SSR marker 651299. DNA fragments are represented by blue peaks, the 272 bp fragment is amplified from the resistant clone DB 375 (1), indicated by an arrow, but not amplified for susceptible clone 842 P75.

A total of 190 individual potato plants were screened by ELISA for their resistance or susceptibility to PVY, and from these 85 were found to be resistant and 93 to be susceptible. DNA from these virus-tested plants was PCR screened using primers for the SSR marker 651299, showing that the 272-bp SSR PCR product was amplified from 81 of the 85 virus-resistant plants but not amplified from 4 of these plants. Of the 93 plants that were virus-susceptible, 81 failed to amplify the 272-bp SSR fragment, whereas the fragment was amplified from 12 of these plants. A further 12 of the initial 190 plants gave inconsistent PCR results and were disregarded from the analysis.

To date, our work supports the localization of a PVY resistance gene in group *Phureja* potato on chromosome IX (Fig. 2), which serves as a starting point for the positional cloning of the *Ry* gene. For this purpose and for fine mapping, saturation of the genetic map by increasing the amount of markers at the chromosomal region of interest is in progress.

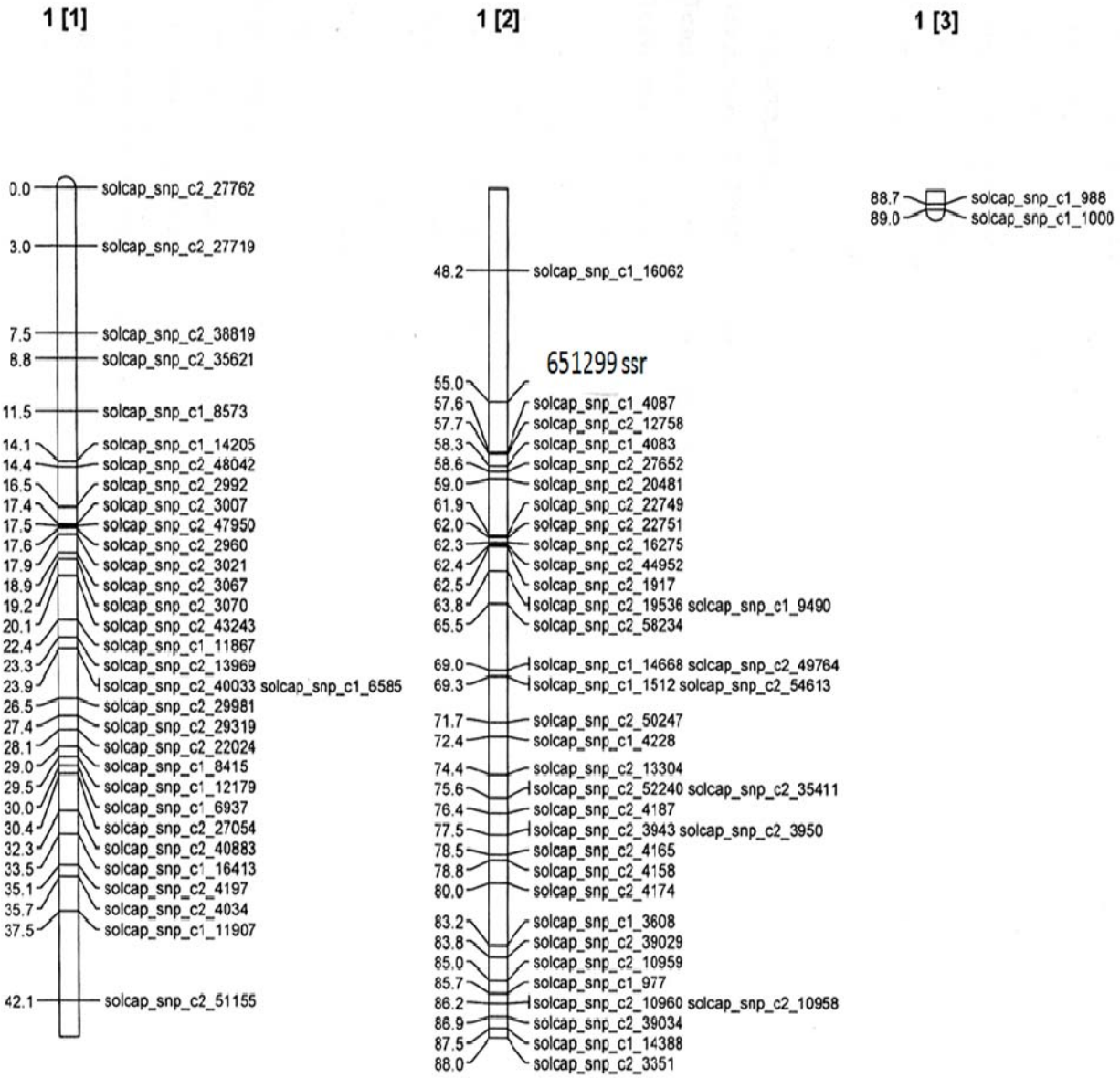

Figure 2. Chromosome IX linkage map of the O6H1 population used to map the PVY resistance locus in the O8H1 potato population. The SSR marker 651299 linked to PVY resistance gene was located in the middle of chromosome IX. Genetic map distances are shown in centimorgans (cM) to the left of each location mapped.

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INFLUENCE OF TEMPERATURE ON THE LIFE CYCLE OF THE POTATO CYST NEMATODE

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Summary: The potato cyst nematodes (PCN) *Globodera rostochiensis* (Stone) and *Globodera pallida* (Woll) are major parasites of potatoes and other members of the *Solanaceae* family. Damage caused by PCN is affected by a range of abiotic and biotic factors. The aim of this work is to examine the relationship between temperature and population dynamics of PCN and particularly to assess the effect of temperature increases associated with climate change on nematode reproductive rates.

INTRODUCTION

The potato cyst nematodes (PCN) *Globodera rostochiensis* (Stone) and *Globodera pallida* (Woll) are major parasites of potatoes and other members of the *Solanaceae* family. According to EPPO, PCN have been detected in 71 (*G. rostochiensis*) and 44 (*G. pallida*) countries (EPPO, 2011). In the UK the direct and indirect crop losses caused by PCN have been valued at ~9% of yield annually (Evans, 1993). The economic cost of PCN to the UK potato growing industry was estimated at more than £43M in 1998 based on lost yield alone (Kerry *et al*., 2002). This value accounts only for direct costs; indirect costs include additional expenditure for fertilizers and irrigation which are used to compensate for poor crop performance (Kerry *et al*., 2002). The direct costs of managing a field infested by PCN also include expenses for pre-planting testing and the use of nematicides. Sampling costs have increased in Scotland due to the latest PCN Directive (2007/33/EC), which requires increased pre-planting soil testing for all seed crops. In fields where PCN are detected, the directive prohibits the growing of seed potatoes and ware potatoes may only be grown under an officially approved control program which includes using resistant varieties, nematicides or other control measures and rotation. (Hockland *et al*., 2000).

Damage caused by PCN is affected by a range of abiotic and biotic factors including soil texture, crop nutrition, water status, potato cultivar and potential interactions with other pathogens and pests. The relationship between soil temperature and PCN development and the subsequent effects on nematode multiplication and plant damage has not been thoroughly investigated. Many nematodes are adapted to particular temperature ranges and temperature is often a key environmental factor affecting their biology.

Nematodes require different optimum temperatures for feeding, hatching, reproduction and survival (Neilson and Boag, 1996). *Globodera pallida* populations typically hatch and develop more quickly at cooler temperatures than *G. rostochiensis* populations, although *G. rostochiensis* is more successful than *G. pallida* at temperatures above 20 ^{o}C (Franco, 1979). In northern Europe, there is usually one main generation of potato cyst nematodes per year (Jones, 1950), however there are reports of the occurrence of a partial second generation. Greco (1988) recorded a completed second generation of *G. rostochiensis* at Avezzano in Italy and Jimenez-Perez (Jimenez-Perez *et al*., 2009) observed a second generation of *G. rostochiensis* at soil temperatures of 18 ^{o}C in Venezuela and a lack of entry into diapause.

The effect of temperature on the life cycle of potato cyst nematodes has been investigated. The data obtained so far has shown that the optimal temperature range for hatching of *G. rostochiensis* is 15-21 ^{o}C which is higher than for *G. pallida*. Additionally, at soil temperatures of 17 ^{o}C and 20 ^{o}C a second generation of juveniles was observed on the susceptible cv. Desiree within 14 weeks of planting. Field experiments were established in 2011 in the contrasting temperature environments of Luffness (East Lothian) and Newport (Shropshire) (unpublished data). This data will be used to develop a dynamic temperature based model for the life cycle of PCN.

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IDENTIFYING FREE LIVING NEMATODE POPULATIONS

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Summary: In addition to direct damage caused by feeding, some species of trichodorids and *Longidorus* can transmit plant viruses, causing significant economic losses to the agricultural industry. Accurate identification of these and other nematodes such as *Pratylenchus* is essential but difficult due to the minute morphological differences between species, the requirement for specific taxonomic expertise and the time required for morphological identification. An integrated battery of molecular diagnostics capable of distinguishing between nematodes of interest to the UK potato industry will be developed, validated and deployed. We present preliminary results of field-population analysis, primer development and validation of a specific PCR primer set for the genus *Longidorus*.

INTRODUCTION

The phylum Nematoda represents a highly diverse group with over 26,000 described species (Hugot *et al*., 2001). It has been estimated that plant parasitic nematodes cause approximately 12% of global crop losses (Sasser & Freckman, 1987). In addition to direct damage, feeding by some plant parasitic nematodes can lead to secondary plant diseases including virus infection, and hence these are often referred to as virus-vector nematodes (Taylor & Brown, 1997). Free living plant parasitic nematodes (FLPPN) of the order Dorylaimida (specifically species of *Longidorus, Paralongidorus* and *Xiphinema)* transmit Nepoviruses whereas those of the order Triplonchida (specifically *Trichodorus* and *Paratrichodorus*) transmit Tobraviruses (Taylor & Brown, 1997). *Tobacco rattle virus* (TRV) can cause spraing (also known as corky ringspot) symptoms, appearing as brown, necrotic arcs or lines found within tuber flesh. This does not necessarily reduce the overall yield but greatly reduces the value of the crop. Economic costs can result from lack of uniformity in tuber shape and size, changes in sugars and subsequent fry quality of tubers, which results in the devaluation of the crop or rejection by packers and processors. Relatively low levels (>5%) of TRV infections can render entire crops unsaleable, both for the fresh and the processing industries, causing an estimated loss to the UK potato industry of >£13m p.a. All sectors of the UK potato industry are affected by the impact of FLPPN.

Trichodorids, also called stubby root nematodes, are widespread and abundant in freely draining, sandy soils and have been recorded from many parts of the world (Alphey & Boag, 1976). The first *Longidorus* species, *L. elongatus*, was described in 1876 and the genus now includes 139 nominal species that are ectoparasites of many crops throughout the world (Ye & Robbins 2004). Currently, species discrimination is based primarily on morphometrics but the

high degree of overlap among species can increase the potential for mis-identification. This uncertainty over identification, due to population variation in their morphometrics, has led many taxonomists to regard *L. elongatus* as a species complex and it has been the subject of nine re-descriptions (Taylor & Brown, 1997). Root-lesion nematodes, *Pratylenchus* spp., are amongst the most economically damaging plant-parasitic nematodes (Al-Banna *et al*., 2004). They have a wide range of hosts and occur throughout temperate regions (MacGuidwin & Stanger, 1991); their geographic distribution being mainly dependant on the prevalence of host plants (Castillo & Vovlas, 2007). They exhibit little morphological diversity and yet present sufficient intraspecific variability in certain morphological characters so that the taxonomic separation of the various species is difficult (Roman & Hirschman, 1969).

FLPPN are able to travel vertically through the soil column (Taylor & Brown 1997). Their movement is dependent on the presence of water films on the surface of soil particles but they are highly susceptible to desiccation or mechanical disturbance, leading to a rapid decrease in their numbers in the upper layers of soil during drought conditions. Stable populations can, however, persist below the level of moisture or mechanical stress (below 20-30 cm). Whilst their lateral movement is typically less than 0.5m per year (Cooper & Harrison, 1973) long-distance dispersal is possible by other means such as farm equipment and tillage (Boag 1985; Taylor & Brown, 1997). They have extensive host plant ranges and can survive for extended periods in plant-free soil and this ability to persist makes management difficult using crop rotation methods as populations do not diminish rapidly (Taylor & Brown 1997).An important consideration in managing FLPPN is that their aggregated distribution varies in space and time, as seen for *Xiphinema* (Taylor *et al*., 1994). Similar observations have been made for cyst nematodes (Webster & Boag, 1992). Thus, the design of nematode sampling strategies is important (Marshal *et al*., 1998) as nematode foci could be missed with an ill-designed sampling strategy. Knowledge of the location of these clusters would allow localised treatment with nematicides thus contributing to sustainable agronomic practices (Evans *et al*., 2003). Accurate identification of FLPPN nematodes can be problematic and accurate observation of morphological characteristics and measurements of taxonomically-indicative parameters is essential though this is compounded by an ever decreasing skill base (Coomans 2002). However, with the advancement of molecular biology many of the taxonomic issues can be overcome by applying an integrated battery of molecular-based diagnostics. Several routes towards non-microscopic identification of nematodes have been investigated including isozyme analysis, monoclonal antibody and DNA-based approaches (Jones *et al.,* 1997). Amongst the DNA-based approaches, attempts have been made to develop PCR primers that specifically amplify targeted nematode species. These primers are often based within the ribosomal DNA (rDNA) repeat unit as this is one of the most informative genomic regions for evolutionary and diagnostic purposes for a wide range of organisms (Boutsika *et al*., 2004) and extensive information for this region exists on public databases. A highly useful development in molecular biology was the ability to perform quantitative PCR which allows not only the qualitative detection of target organisms but also enumeration of the targets in a sample. For example, Holeva *et al.* (2006) developed a quantitative PCR diagnostic for 2 species of trichodorid nematodes and for TRV producing accurate and sensitive molecular information on both virus and vector populations.

Here we present preliminary data concerning the spatial distribution of *Trichodorids* in field-plots along with initial results for the development of primers specific for the genus *Longidorus*. These results were developed during the first year of a five year project (Project 292-249), a component of which is developing molecular diagnostics for FLPPN.

MATERIALS AND METHODS

Field scale spatial distribution

Soil samples were collected from 240 plots, 3.66m (2 beds/4 rows) wide by 6m long at 4 field sites located in some of the key potato growing areas of the UK; Tayside, Yorkshire, Shropshire and East Anglia. The actual area sampled for FLN was 1.83m (1bed) by 5m long which represents the harvest bed (2 rows) from the centre of each plot. FLN sampling was done post ploughing, bedforming, destoning (if required) but before nematicide application/incorporation and planting. From each plot, approximately 800g soil was collected and thoroughly mixed. Nematodes were extracted from 200g soil using a modified Baermann funnel for 48 hr (Brown & Boag, 1988) and the final volume reduced to 10ml. Nematodes were identified (to genus-level) and enumerated using low-powered light microscopy. Field scale spatial distribution maps were produced for each site using GENSTAT. Once enumerated, samples were preserved by freeze-drying and stored at-20° C for later molecular analyses.

Species- or Genus-specific primer design and testing.

Total DNA was extracted from freeze dried nematode-extracts using the method of Donn *et al.* (2008). Amplification of 18S rDNA was achieved using universal primers (Floyd *et al*., 2005; Donn *et al*., 2011; www.nematodes.org/research/barcoding/sourhope/nemoprimers.html) from individual identified nematodes obtained from a sub-set of the above soil samples. Full-length 18S rDNA sequences (obtained in both directions to minimise sequencing errors) were aligned with published sequence data and analysed to identify regions unique to the target species. Once these regions were identified, primers were designed to amplify target species only. These species-specific (or genus-specific, in the case of *Longidorus*) assays were tested on samples of known nematode-populations.

To validate the specificity of putative *Longidorus* genus-specific primers on environmental samples, soil samples from 4 locations at JHI, Dundee and 3 locations at Luffness (East Lothian) were collected and 200g processed as above to extract nematodes. Samples were analysed using light microscopy to detect and enumerate any *Longidorus* present. Sample volume was reduced to 2ml and dehydrated by freeze-drying before extracting total DNA (Donn *et al.*, 2008). Extracted DNA was amplified using potential *Longidorus* genus-specific primers and the products separated on a 1.5% agarose gel.

RESULTS AND DISCUSSION

Field scale spatial distribution

Consistent with cyst (Webster & Boag, 1992) and other FLPPN (Boag & Topham, 1984; Boag *et al*., 1989), trichodorids have an aggregated distribution with discrete foci (Fig. 1). Environmental factors, for example, abiotic factors such as elevation (Háněl, 1996), clay content (McSorley & Frederick 2002), nitrogen content (Kimpinski & Welch 1971), calcium (Kandji *et* al., 2001) but not soil pH, and biotic factors including the plant community (Háněl, 1993) have a significant role on nematode distributions. The data from this site and three others will provide an opportunity for the first time to determine damage threshold levels for a range of potato cultivars and target FLPPN.

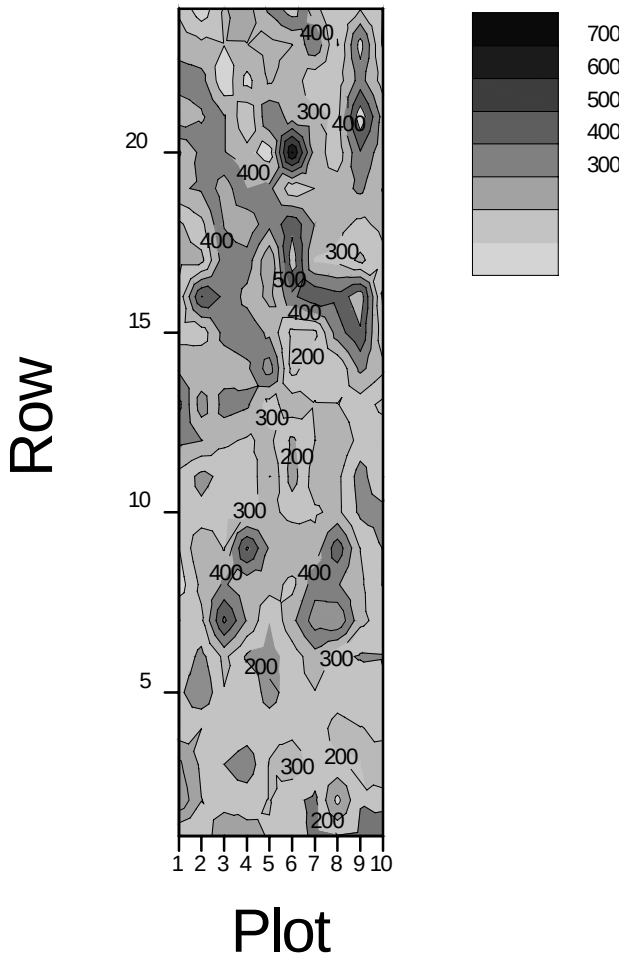

Figure 1. Spatial distribution of trichodorids within a 240 x 50 m potato field experiment. Numbers on scale refer to nematode abundance from a 200 g soil sample.

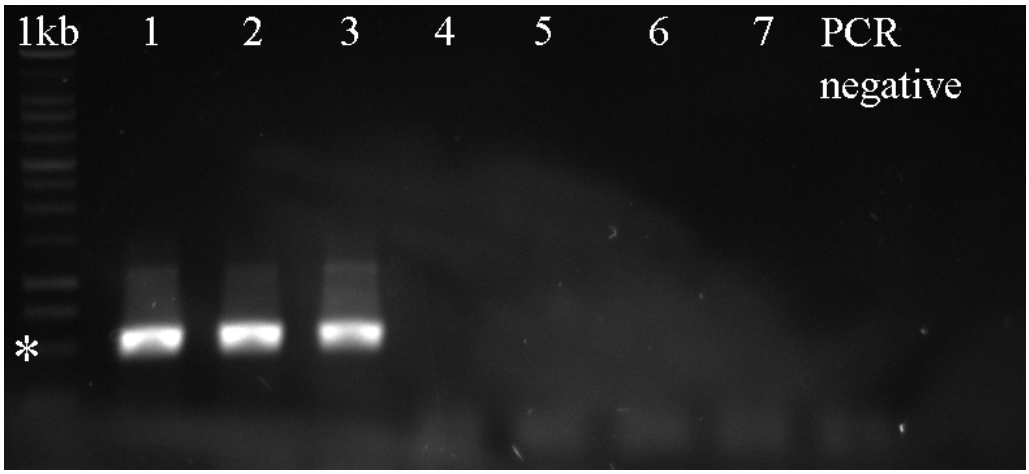

Figure 2. Preliminary validation of a potential *Longidorus* genus-specific primer from field samples. Lanes 1-3 contain *Longidorus* whereas lanes 4-7 are *Longidorus* deficient. * indicates position of the 500bp size standard.

Species- or Genus-specific primer validation.

Taxonomic analysis of Baermann funnel-extracted nematodes indicated that Dundee sites 1, 2 and 3 contained *Longidorus* at low numbers (<10 individuals in 200g soil), whilst the remaining 4 sites (1 Dundee and 3 Luffness) contained no *Longidorus*. The expected PCR product (540bp) is only seen for samples 1, 2 and 3 (Fig. 2). Specific PCR (Hübschen *et al.*, 2004) and sequence analysis of the products confirmed the presence of *L. elongatus* in samples 1, 2 and 3. *In silico* analysis of the tested primer pair indicates it will produce amplification products for all known UK *Longidorus* species but not from related genera. These primers (and others) will be further tested against a wider range of soil samples during the validation period of the project.

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ABBREVIATIONS

The following abbreviations may be present, without definition, in the papers in this and previous editions of the Proceedings of the CPNB Conferences.

acid equivalent	a.e.	litres per hectare	litres/ha
active ingredient	a.i.	logarithm, common, base 10	log
approximately	c.	logarithm, natural	ln
body weight	b.w.	low volume	LV
boiling point	b.p.	maximum	max
centimetre(s)	cm	maximum residue level	MRL
coefficient of variation	CV	metre(s)	m
colony-forming unit(s)	cfu	metres per second	m/s
compare	cf	milligram(s)	mg
concentration x time product	ct	milligrams per kg	mg/kg
concentration required to kill 50% of test organisms	LC_{50}	millilitres(s)	ml
		millimetre(s)	mm
correlation coefficient	*r*	Minimum	min
cultivar	cv.	minimum harvest interval	MHI.
cultivars	cvs.	minute (time unit)	min
day(s)	d	moisture content	M.C.
days after treatment	DAT	molar concentration	M
degrees Celsius (centigrade)	DC	more than	>
degrees of freedom	df	no significant difference	NSD
dose required to kill 50% of test organisms	LD_{50}	not less than	|<
		not more than	|>
emulsifiable concentrate	EC	page	p.
enzyme-linked immuno-sorbant assay	ELISA	pages	pp.
		parts per billion	ppb
fast-protein liquid chromatography	FPLC	parts per million	ppm
for example	e.g.	parts per trillion	ppt
freezing point	f.p.	pascal	Pa
gas chromatography-mass spectrometry	gc-ms	percentage	%
		polyacrylamide gel electrophoresis	PAGE
gas-liquid chromatography	glc		
genetically modified	GM	polymerase chain reaction	PCR
genetically modified organism	GMO	post-emergence	post-em.
gram(s)	g	pre-emergence	pre-em.
growth stage	GS	pre-plant incorporated	ppi
hectare(s)	ha	probability (statistical)	*p*
high performance (or pressure) liquid chromatography	hplc	relative humidity	r.h.
		revolutions per minute	rev/min
high volume	HV	second (time unit)	S
hour	h	standard error	SE
integrated crop management	ICM	standard error of the difference	SED
integrated pest management	IPM	standard error of the mean	SEM
kilogram(s)	kg	soluble powder	SP
kilogram(s) per hectare	kg/ha	species (singular)	sp.
kilometres per hour	km/h	species (plural)	spp.
least significant difference	LSD	square metre	m^2
less than	<	subspecies	ssp.
litre(s)	litre(s)	suspension concentrate	SC
systemic acquired resistance	SAR	mega (x 10^6)	M

tandem mass spectrometry	MS-MS	kilo	($x10^3$)	k
technical grade	tech.	milli	($x10^{-3}$)	m
temperature	temp.	micro	($x10^{-6}$)	μ
thin-layer chromatography	tlc	nano	($x10^{-9}$)	n
time for 50% loss; half life	DT_{50}	pico	($x10^{-12}$)	p
tonne(s)	t			
tonne(s) per hectare	t/ha			
ultralow volume	ULV			
vapour pressure	v.p.			
variety (wild plant use)	var.			
volume	**V**			
water dispersible granule	WG			
weight	*wt*			
weight by volume	*wt/v*			
weight by weight	*wt/wt*			
wettable powder	WP			