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LOSS OF PRODUCTS, LOSS OF BIODIVERSITY, CLIMATE CHANGE: THREE PROBLEMS - ONE SOLUTION

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Summary: Agriculture has three big problems which are superficially antagonistic; reduced availability of plant protection products, loss of biodiversity and the impact of climate change. This presentation will explore a mutually beneficial solution to the three problems through a more targeted approach to production. Novel mechanisms for incentivising better outcomes; namely payment for ecosystem services and the IUCN Net Positive Impact approach will be described. Specific practical examples of control strategies with mutually beneficial outcomes will also be discussed.

INTRODUCTION

Conventional agriculture is under considerable pressure as a result of the combined factors of market forces and production constraints. On the market forces side: price volatility, competition for market share, quality demands from the supply chain and oversupply issues in an increasingly global market are significant challenges. On the production side increased input costs, reduced availability of plant protection products, and more challenging growing seasons are increasing pressure on farmers and agronomists. In combination these factors make for uncertainty. Taking the dairy sector as an example, some have responded by entrenchment in their established practices, my own family farm would be a case in point, and others have withdrawn from production entirely, as several of the neighbouring farms have done. A few have used innovation to reinvent their businesses and leverage new technology or alternative production systems to create an economically sustainable business. Conspicuous examples are Mackie's of Scotland and Cream o' Galloway. These businesses also exemplify production systems that set environmental sustainability at the heart of their business models.

PLANT PROTECTION PRODUCT AVAILABILITY

Market research by the crop protection industry consistently highlights the decline in global spend on research and development targeted towards the EU. For example a report for the ECPA indicated that EU focused research has gone from 33% of the global spend in the 1980's to just 7.7% in the period 2005 – 2014. However they also reported a global trend in reduced development of AIs with 28 products in the pipeline in 2012 compared to 70 in 2000, a fall to less than half (Phillips McDougall, 2013).

Europe has lost out in R&D spend for two reasons: firstly it is seen as a mature market with a harsh regulatory framework and secondly, current R&D is focused on seeds and traits much of

which is delivered using genetic modification which the EU electorate has, so far, rejected.

In addition to the reduction of products in the development pipeline there is also increased pressure from the regulators particularly as focus has moved from risk to hazard but also due to water framework directive requirements and MRL exceedances. The UK industry has reacted strongly with the Healthy Harvest campaign launched in June 2014 (NFU, 2014).

BIODIVERSITY LOSS AND CO_2 EMISSIONS

Human activity causing environmental degradation in general and biodiversity loss in particular are not new phenomena – indeed man has been altering the planets environment for tens of thousands of years. Nobel laureate Paul J. Crutzen has proposed that the current geological epoch be named the "Anthropocene", such is the extent to which man is currently driving conditions on our planet. He proposed the late 18th Century as the starting period for this epoch as at this point human-generated greenhouse gasses became detectable in ice cores. From a biodiversity perspective man has had a clear and measurable impact on biodiversity from 50,000 years ago, but the reason the Anthropocene timeline is important in the context of CPNB is that from 11,000 years ago onwards humans have been farmers. By 8,000 years ago extensive agriculture was widespread in Europe and Asia and whilst having substantial benefits for humans this activity also had a measurable impact on ecosystems and greenhouse gasses.

Everything changed in1909, when Fritz Haber and Carl Bosch invented the industrial process which converts atmospheric nitrogen to ammonia. It is this process in conjunction with agriculture that has allowed humans, in only 81 years, to go from a population of 2 billion in 1930 to 7 billion in 2011 (United Nations Department of Economic and Social Affairs). This can be seen as an astonishing success for modern agriculture, however, one of the key drivers of arable agriculture as a contributor to climate change is this use of synthetic nitrogen which according to FAOStat sat globally at 92 million tonnes in 2009. Use of synthetic fertilisers accounts for 15% of agricultural (CO_2 equivalent) emissions in the UK and is the largest source after enteric fermentation and manure. In terms of emissions N_2O (Nitrous Oxide) is 300 times more potent than CO_2 as a greenhouse gas and agricultural soils produce 69% of UK emissions, amounting to 65 kilotonnes in 2013 according to the UK National Atmospheric Emissions Inventory. For this reason the Scottish Government is actively working up policies to reduce N_2O emissions from farmland.

Another problem faced by agriculture is the continuing biodiversity loss associated with farming. Scotland's State of the Environment Report, hosted on Scotland's Environment Web, rates farmland as of Moderate Condition and declining. The report says "Scotland's farmland is highly varied and contains a wide range of habitats for wildlife. However, populations of some birds and insects are in decline. Intensive land management is the main challenge to farmland wildlife."

The report goes on to describe the main sources of the pressure on biodiversity from arable and grassland production systems. These are predominantly associated with intensive production methods using pesticides, fertilisers, high stocking rates and a limited range of species either as crops or as components of grass swards.

The report also cites the general improvement for agricultural production as going hand in hand

with a decline in landscape features such as, scrub land, hedges, trees and ponds. Though there has been some compensation from agri-environment schemes, the lack of coherent management at the landscape scale for these features has negated the benefit.

The principle data set showing the decline is the British Breeding Bird Survey, 2012. This shows that of the 61 species for which Scottish trends can be calculated nine declined significantly between 1995 and 2011: kestrel (-57%); oystercatcher (-30%); lapwing (-56%); swift (-57%); rook (-34%); skylark (-19%) starling (-40%); and meadow pipit (-29%). It is noteworthy in this context that some threatened farmland species, such as grey partridge, tree sparrow and corn bunting, are now so scarce that the breeding bird survey cannot measure them.

SCHOOLS OF THOUGHT

There are two main schools of thought when it comes to using our resources sustainably these are the "Critical Limits" view and the "Competing Objectives" view (Farrell and Hart, 1998). Tait and Morris, 2000 described how these concepts can be developed to produce measurements for sustainable agriculture: put simply the Critical Limits view focuses on the fact we have only one planet and we need to live within the ecological limits that comes from being a small habitable ball of rock in a large empty universe. The Global Footprinting Network perhaps sums this argument up most succinctly with Earth Overshoot Day which in 2015 was 13th of August. The Competing Objectives view is more pragmatic and seeks to balance the needs of humans, including the farmers need for profit, with ecological outcomes.

These two views can be characterised by movements such as hard line degrowth advocates on the one side, for example NGOs like Greenpeace and prominent thinkers like George Monbiot, with the Eco-modernism movement espoused activists such as Mark Lynas and the natural capitalists led by Dieter Helm on the other side. There is a good deal of science advocacy in the debate on both sides of the argument.

But farmers do not produce food in schools of thought, so the important thing here is not the difference of opinion between these groups, but the common thread that is shared by the two ways of viewing the problem. For us, this is the need to produce our food in a way that sustains not only the production system but also the ecosystem. So the question becomes how this thinking will emerge into a change in practice in our food production systems.

POLITICAL CONTEXT

Farming is a heavily subsidised industry and the pesticide industry is closely regulated, as a result decision making around land use and production is substantially influenced by policy.

A good example of the importance of the political context is in the emerging policy area of natural capital accounting and payment for ecosystem services. In November 2015 Edinburgh hosted the second World Forum on Natural Capital during which Scotland First minister (Sturgeon, 2015) set out Scotland's position saying: "In Scotland we are determined to play a leading role in developing the thinking about the concept [of natural capital] and its application." She outlined that Scotland is at the forefront of developing natural capital

accounting and that this concept explicitly sits within Scotland's 2020 Biodiversity Strategy. In practical terms we became the first country to establish a natural capital asset index in 2011. She went on to outline the value of natural capital as being worth more than £20 billion each year and directly supporting more than 60,000 jobs and indirectly being responsible for many more, for example in the tourism sector. Her speech explicitly mentioned peatlands and forestry but did not mention agriculture at all.

In political terms this is not just a Scottish left of centre issue. As long ago as 1988 former conservative prime minister, Margaret Thatcher said, "The last thing we want is to leave environmental debts for our children to clear up" "No generation has a freehold on this earth. All we have is a life tenancy – with a full repairing lease."

Philip Hammond the UK foreign secretary invoked this Thatcher reference on the 14th November 2015 in his speech to the American Enterprise Institute in Washington where he himself said, in relation to climate change: "The worst case is even more severe: a drastic change in our environment that could see heat stress in some areas surpass the limits of human tolerance, leaving as the legacy of our generation an unimaginably different and more dangerous world for our children and grandchildren. So the costs of doing nothing are, potentially, catastrophic – beyond anything that can easily be quantified in economic terms."

So across the political spectrum the rhetoric is strong and unequivocal. What is interesting to us, is that both Rt Hon Hammond and Scotland's First Minister went on to be completely explicit about monetising the *externalities* in order to correct for the fact that pollution is a cost and nature has a value. Hammond put it this way: "we have allowed CO2 emissions to be a "free good" to the polluter even though they impose costs on society. With any other waste, we pay for it to be taken away. We don't let people just dump it in the street." and Sturgeon said "We intend to ensure that our approach to economic development, takes account of the custodianship of our natural resources."

Clearly there is political momentum for those generating profits at the expense of our ecosystems to pay the environmental costs. So there is a big stick here for industries with a poor environmental track record. There is also a significant carrot for farmers, since farming delivers a vital ecosystem service of feeding the planet's human population. Moreover our industry is powered by the (essentially) infinite resource of sunlight. Farming in Scotland can and does deliver many other ecosystem services whilst also generating profit to the farmer, but needs to substantially improve our approach to both delivering these ecosystem services and getting paid to do so.

NATURAL CAPITAL AND ECOSYSTEM SERVICES

In order to properly account for our impact on natural systems we need to have a framework for valuing it. Natural Capital has been defined as the world's *stocks* of natural assets which include geology, soil, air, water and all living things. It is from these *stocks* that we derive the *flow* of ecosystem services that sustain people. Farming is very familiar with this concept since natural capital (*stock*) is the land and the ecosystem service farmers are paid for is food production (*flow*). The industry is less familiar with other ecosystem services that also have a value, such as buffering and filtering water, soil carbon storage, pollination services and maintaining the scenery that underpins the tourist industry. Since these flows of services have

value the emerging system of natural capital accounting could, if harnessed properly, deliver that value as income to those who can demonstrably protect and improve the flow of these services. Likewise those businesses able to maintain and enhance their stock of natural capital will be best placed in the future to reap the rewards of their investment. One model through which this could be achieved is the No Net Loss, and Net Positive Impact model proposed by the International Union for Conservation of Nature (IUCN).

THE IUCN MODEL

This new approach to the problem of commercial exploitation of natural systems was recently proposed by the IUCN in their report (Aiama *et al.,* 2015) they propose that activities can be managed in such a way as to have no net loss of biodiversity or indeed a net positive impact defined as: "diversity within and among species and vegetation types; long–term viability of species and vegetation types; and, functioning of species assemblages and ecosystems, including ecological and evolutionary processes". The proposal is simple in that it proposes that any negative impact on biodiversity caused by an activity be balanced (no net loss) or outweighed (net positive impact) by compensating actions in the region of the activity. The proposal has at its heart the mitigation hierarchy (Figure 1.).

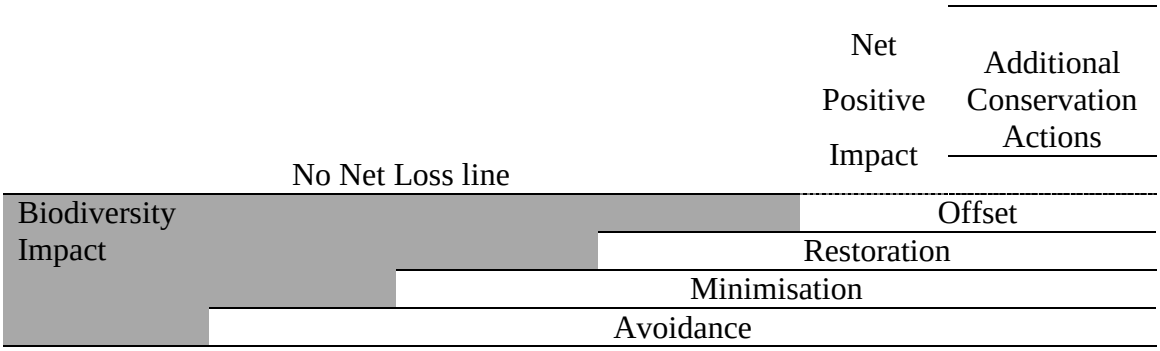

Figure 1. The mitigation hierarchy.

The mitigation hierarchy actions, which should be taken in order, are: avoidance of the impact, its minimisation, restoration of damage, then finally offsetting. Additional conservation actions can also be taken to create a net positive impact resulting from the activity.

What does it mean in practical terms for protecting crops? Taking insecticide use as an example of an action with a biodiversity impact: Avoidance would be to manage insect pests without insecticides, minimisation would be to only use insecticides when absolutely necessary for example as part of an integrated pest management plan, restoration could be to compensate for insecticide use in the cropped season with incorporation of habitats beneficial to insects in the rotation following the crop, offset would be provision of an insect beneficial habitat or food source elsewhere on farm during the cropping season. Additional conservation action could be provision of permanent habitats beneficial to insects on unproductive areas of the farm.

The great benefit of the net positive impact approach is that it allows for the intensification of production in land best suited to generating yield and profit for the grower, provided the negative impacts of that production are compensated for. As the model is scalable it also allows for actions on one farm to be compensated for by actions on another farm. This presents the

opportunity for producers with alternate approaches to work together for mutual benefit, i.e. an intensive arable farmer could work with an organic neighbour to have a net positive impact in their local area.

This hierarchy fits into a wider framework which in turn provides a mechanism for decision making. Firstly both the biodiversity measures and impacts that are to be considered within scope should be defined at the outset. These are then considered within a specific geographical region and specific measurable goals set. So for example this might be the impact of sward management on ground nesting birds in a particular area with outcomes measured by bird survey.

The following three additional elements should also be incorporated. What are the limits of this approach i.e. some features can only be protected by wholly avoiding an activity in a particular area. What is the appropriate time scale, in agricultural systems crop rotations can be over many years and single seasons can be more affected by weather than any management action so sufficient time is needed to determine a true picture. Transparency is the final element, being publicly open about the goals set and progress made towards them builds trust between the stakeholders particularly where outcomes are measured by an independent third party.

APPLYING THE IUCN MODEL IN PRACTICE

The question then for the industry is how this approach fits into the emerging thinking around management of agricultural land and not withstanding any policy level interventions how does an individual producer use this approach to help inform their own management decisions.

National Level

Nationally the model can be used to set goals at the country, region, or catchment scale. Specific projects can then be taken forward within this framework. The Scottish Biodiversity Strategy (SBS) is in the process of doing this in Scotland and has set out its route map to 2020 including twelve priority projects. Of these, two relate explicitly to agriculture: Priority Project 10: Improving ecological connection and Project Priority 11: Sustainable land management. Other specific activities are included such as preparation of Scottish Pollinator and Plant Health Strategies. This means that many of the requirements for the IUCN framework are already in place, particularly the biodiversity scope, goals and specific quantifiable measures. Therefore the crop production sector can take the opportunity to build on this work using the IUCN approach at a landscape scale to set out how it proposes to maintain and improve production whilst having a net positive impact, with the explicit goals of maintaining access to pesticides and securing payments for ecosystem services. The industry can build on existing stewardship and agri-environment schemes to formulate a strategic outcomes based approach.

Local level

At the farm scale the model can be used to set a wider context for decision-making as it would bring the natural systems into focus when making production decisions. However, farmers are unlikely to change their behaviours in a radical way whilst their businesses are producing an acceptable income. The following examples will show that changes in practice, which deliver biodiversity benefit, can be brought about because producers reach a tipping point even from a narrow production economics perspective.

Tuber moth control in Victoria Australia

In Victoria Australia potato growers were suffering major problems due to potato tuber moth (PTM). The growers' representative bodies contacted a locally based entomologist, Paul Horne, who had been very successful in implementing IPM strategies for horticultural crops. He was able to show the growers that there was an abundance of naturally occurring biological control agents for PTM notably parasitic moths. The problem being that these beneficial insects are very susceptible to the broad spectrum insecticides being used to control virus vectors. By eliminating or minimising use of insecticides, with a switch to use of virus tested seed, Victorian seed potato growers are now able to control not only PTM but maintain control of virus issues in their crops. The results for the growers were dramatic, including total avoidance of insecticides over a period of 10 years, compared to 7 insecticides per crop prior to the IPM approach (O'Sullivan and Horne, 2000).

Potato Virus Control in the Community Grade Seed region of Finland

A similar picture has emerged closer to home in the community grade region of Finland. In the early 2000's the growers and authorities saw a large number of downgrades and rejections of seed crops due to PVY. They commissioned research work to establish how best to tackle the problem. The findings were that PVY infection was driven by early season transmission vectored predominantly by the aphid *Aphis fabae*. Insecticides were found to be not effective in controlling PVY transmission. However, control could be achieved by discouraging aphids from feeding on young plants using a straw much. In conjunction with healthy seed, growers in Finland are gaining control of PVY using this method (Kirchner *et al*., 2014).

Rush control in the UK

Control of the soft rush (*Juncus effuses*) has become a major issue for grassland management particularly in the west over the recent wet seasons. Here the conservation objectives are more explicit than in the potato examples and specifically provide income to the farmer though agri-environment schemes. There are many rush species and these are beneficial to biodiversity but an infestation of soft rush is bad for both farmers and biodiversity outcomes. The Ayrshire Farmland Wader project, Caithness Wetlands and Wildlife Initiative and the Strathspey Wetlands and Waders Initiative are all examples of successful partnership working between farmers and conservation bodies, facilitated by SAC consulting. There has also been a great deal of interest in field labs organised on this subject by the soil association. The result of this activity is that farmers have access to high quality and tailored advice which benefits both biodiversity and their bottom line (SAC 2015, AHDB 2014, RSPB, 2015).

CONCLUSION

Revising our approach to crop protection decisions to explicitly consider the impact on natural capital (stocks) and ecosystem services (flows) can be a successful strategy to improve farm income. All of us from working scientist, agronomists to industry bodies need to discuss how we use tools like the IUCN model and natural capital accounting to maximise the potential gains for both production and conservation. In particular we need as an industry to develop the models discussed here into fully functioning framework. By demonstrating that we are able to use our plant protection products and fertilisers whilst having a net positive impact on our environment we will maximise the returns to our industry into the long term.

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HOW WILL EU PESTICIDE LEGISLATION IMPACT THE CROP PROTECTION INNOVATION PIPELINE?

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Summary: By 2009, the implementation of EU Directive 91/414/EEC resulted in the loss of 75% of Active Substances (ASs) previously on the European market. In 2011, this directive was replaced by Regulation EC 1107/2009, the key change being from a risk-based to a hazard-based assessment system. Because of this change in approach, it is estimated that in the UK there are 45 high risk and 38 medium risk ASs, many of which are also registered across Europe. The use of these ASs may be restricted in the future or in some cases removed from the market. The resulting impact on the loss of effective crop management tools is likely to have a significant impact on the future innovation cycle. This paper discusses the evidence for this, what the impact may be on the agricultural industry in Europe and what the options may be for the future.

INTRODUCTION TO THE EUROPEAN REGULATORY ENVIORNMENT

In 1993, the EU Directive 91/414/EEC was implemented to harmonize pesticide approvals across Europe with Active Substances (ASs) having to meet assessment criteria on the basis of safety and efficacy via a process referred to as Annex 1 inclusion. This programme ran until March 2009 with some review work extending until 2012. The net result of this process was the loss of nearly 75% of ASs from the European market. Many of those ASs lost were simply not supported as they had been superseded by technically superior actives or were uneconomic rather than judged unsafe under the EU Directive (King, 2014).

ASs that were positively included in Annex 1 (under Dir. 91/414/EEC) were given approval for 10 years with a requirement for a second review to be completed before the end of the 10-year period, ensuring that risk assessments were kept up to date. The EU Regulation EC 1107/2009, which came into force in 2011, replaced Directive 91/414/EEC and it is under this regulation that the next renewal process takes place (Anon, 2009). However, in the new regulation there was a key change from a risk-based to a hazard-based assessment whereby the intrinsic properties of the active substance itself are key criteria for whether or not it remains approved. There are defined properties, referred to as “cut off criteria” which mean that if applicable to the AS under evaluation, no further assessment will take place and approval will not be granted. These criteria include potential impacts on human health if a substance has Carcinogenic, Mutagenic and/or Reproductive toxicity properties (CMRs 1a/1b). Potentially, those ASs with Endocrine Disrupting (ED) properties will also fulfil the “cut off criteria” however; the definition of ED has still to be clearly defined in the EU (Anon, 2014). In addition, the cut off criteria include factors such as being a Persistent Organic Pollutant (POP),

Persistent Bioaccumlative Toxin (PBT) and very Persistent and very Bioaccumulative Toxin (vPvB).

The renewal process of ASs under EU Regulation 1107/2009 is being carried out in stages (AIR 1 – AIR 4 – Anon, 2007, 2010 & 2012) depending on the original timescale of the Annex 1 inclusion and hence the expiry date of the active substance. Fig. 1 shows the approximate timelines of the AIR renewal programmes and the number of active substances involved.

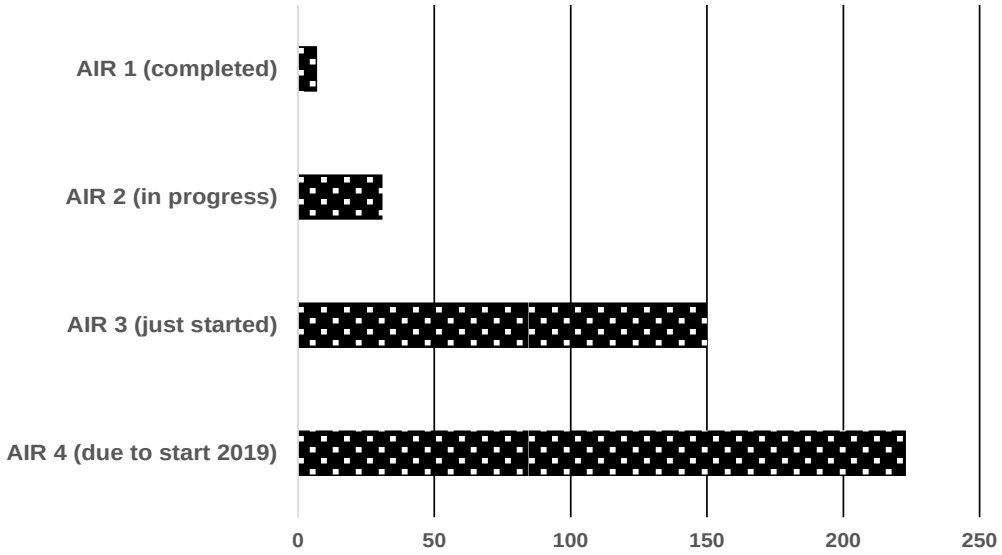

Figure 1. Approximate timelines for AS renewals under EU regulation 1107/2009 (AIR 1- 4, Anon 2007, 2010 and 2012 – AIR 4 still to be described)

In addition to the renewal process for ASs further assessments to reduce risk are also included, the main one being Comparative Assessment (CA) (Article 50 and Annex IV of EU Reg. EC 1107/2009) (Williams, 2011). This assessment differs from the renewal process in that it focuses on products containing ASs which are on a Candidate for Substitution (CfS) list and is also carried out at a member state level (AIR submissions are made at an EU level). The CfS list published in 2015, presently contains 78 active substances. The CA process only started in August 2015, a useful guidance document, SANCO/11507/2013 rev. 12 published in 2014 provides further details.

REGULATORY IMPACT ON THE AVAILABILITY OF CROP PROTECTION PRODUCTS (CPPs)

It is hard to predict how many ASs will be lost over the next 5-10 years as the AIR 3 programme, covering the first large wave of ASs (approx. 150 – Fig. 1) has only just started and AIR 4 substances (approx. 220 – Fig. 1) have yet to enter the process. A proportion of these ASs are those that have more recently been introduced to the market and are vital tools in the crop protection armory for the farmer. Also, important “cut-off criteria” such as ED have yet to be clearly defined making future estimates difficult. However, it is clear that the move from a risk-based to a hazard-based approach has significantly increased the risk of losing key ASs. In the UK alone, it is estimated that at least 45 ASs are at high risk and a further 38 medium risk, many of these are also registered across EU-28 countries so the impact will be far

reaching (King, 2014). The inevitable knock-on effect on the number of registered products across Europe in the future will be even greater.

The aim of Reg. EC 1107/2009 (Anon, 2009) was to have a harmonized approach to pesticide regulation throughout the EU but some countries have imposed additional restrictions at a national level. In Denmark, where ground water is of particular concern several ASs that are commonly found in other European countries are not registered and those on the market are subject to a pesticide tax. This tax is based on a Pesticide Load Index (PLI) - the higher the PLI the higher the tax. In France, the government has taken a different approach, starting with the "Grenelle" programme, which led to nearly 40 ASs being banned. Ecophyto I and now, Ecophyto II, which is aiming to reduce pesticide use by 25% by 2020 and 50% by 2025, supersedes this programme.

IMPACT ON THE INNOVATION CYCLE

Between 2003 and 2011, Europe was the leading regional agrochemical market Worldwide; in 2012, it was overtaken by Asia. In terms of growth and sophistication, the European market is divided into EU-15, new EU-13 and East European markets, with the former presently having the highest value but the slowest growth. Coupled with the increasingly complex and unpredictable regulatory environment of the EU-28 countries there are now very serious challenges to the Innovation Cycle (Fig. 2) for Crop Protection Products (CPPs) and the tools that will be available to the farmer to achieve an economic return on his cost of production. The Innovation Cycle can be split into 5 phases; the evidence of the impact of EU 1107/2009 on these phases is described below

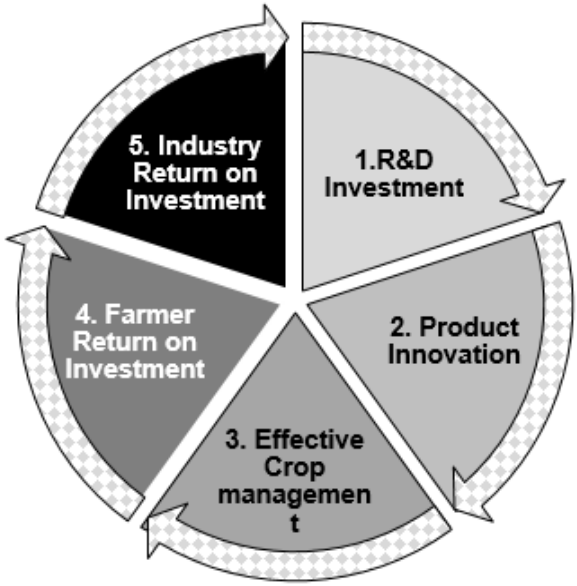

Figure. 2. The phases of the Innovation Cycle for the development of CPPs

R&D Investment

The R&D investment required to bring a new AS to market has more than doubled in the past 20 years from approx. €100 million to €200 million. This is not solely due to inflation or higher labour costs but due to the vast number and complexity of regulatory studies required plus time

and resource requirements for the submission process itself. For R&D companies there is a significant additional cost burden due to the renewal process resulting from Reg. EC 1107/2009. It is hard to put a value on the cost of AS renewal as data requirements can differ widely. However, as never before, R&D companies have to balance the cost of developing new ASs that may have a risk of regulatory failure with a high level of investment required to defend ASs already on the market but also with regulatory risks.

Product Innovation

Most R&D companies are committed to innovation; however, the level of investment at a European level is under threat due to regulation, slow growth in the EU-15 and conflicting demands for development of new AS and the defence of existing ASs. This is shown by the decline in the number of new ASs in Europe compared with the rest of the World (Fig. 3). These investments also need to be considered alongside the need for newer "green" solutions such as biological-based products, developments in new technologies and plant breeding targeting pest and disease resistance.

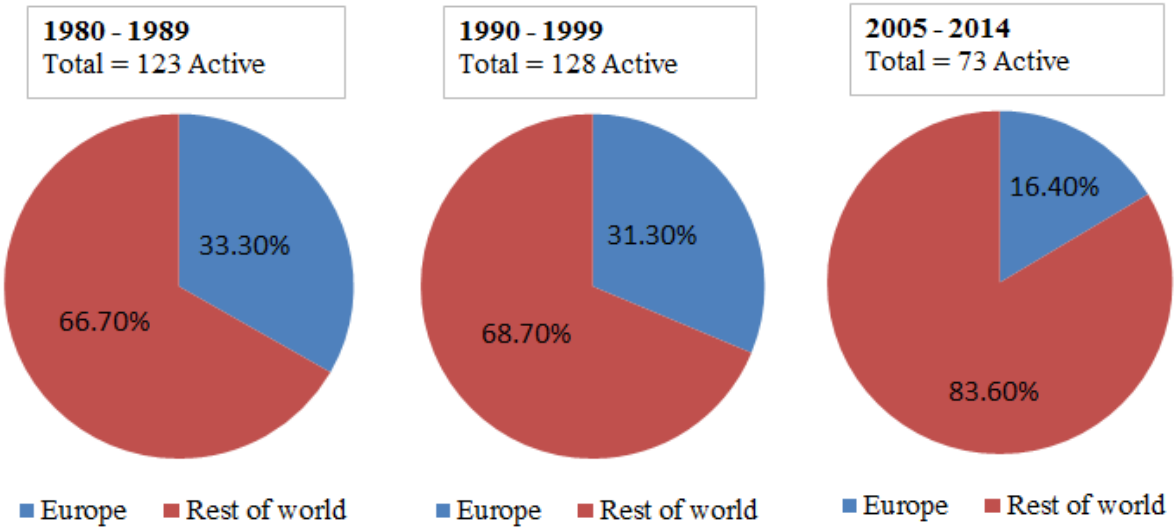

Figure. 3. Split of AS developments between Europe and the Rest of the World, 1980 – 2014 (Philips McDougall, 2013)

Historically, a key driver of CPP Innovation in West Europe, was resistance of the target pest, weed or disease to ASs. In the case of cereal fungicides, the loss of control of the cereal disease, Septoria tritici by the strobilurin fungicides led to the "quick" development of a new class of fungicides, the Succinate DeHydrogenase Inhibitors (SDHIs). These are now potentially under threat due to the possible occurrence of resistance in the future. The azole class of fungicides generally act as resistance management partner to vulnerable ASs such as the SDHIs; however, they are potentially at risk due to the ED cut off criteria, which has still to be clearly defined. It is ironic that within the EU regulation 1107/2009 it is stated in Article 50 that it is important to "maintain chemical diversity ….to minimise the occurrence of resistance to the target organism" when at the same time, the strict "cut off criteria" will severely limit chemical diversity and the farmers ability to manage resistance.

Effective Crop Management

The scale of the impact of the loss of ASs on the ability of European farmers to manage crops to achieve a return on investment is hard to assess. It is clear that over the next 5-10 years, the loss and/or restriction of CPPs will be significant and the number of tools available to crop growers will be severely reduced.

There is now a greater emphasis on the breeding of more pest and disease resistant varieties but these are generally still reliant on conventional chemistry to achieve a return on investment (ROI) for the farmer. Varietal resistance can often be overcome and the rapid break down of cereal varieties to yellow rust is a good example of this. There are high levels of private and government research investment to look at alternative tools for the farmer including mechanical weeding, biological control solutions, remote sensing technologies and hybrid crops. However, these are unlikely to fill the gap to compensate for loss of CPPs.

Farmer Return On Investment

The loss of tools for effective crop management will compromise farmer's ability to secure a return on investment. An example of economic assessment on the loss of just one group of ASs, the azoles fungicides, estimated that yield loss across Europe would be approx. 18.6 million tonnes in 2020 alone being equivalent to a loss of value of 4.6 billion euros. In addition, this would result in Europe being unable to satisfy its internal demand and unable to maintain its 100% self-sufficiency rate. In the next 5 – 10 years, it is likely that farmers across Europe will have to re-think how they grow crops. The whole crop rotation may need to be re-thought, fertiliser inputs managed to reduce pest and disease impacts, cultivation techniques changed to reduce the impact of weeds and cropping timings adapted.

Industry Return On Investment

The market growth for CPPs in Europe has already slowed in EU-15 and the further loss of tools across EU-28 countries will impact on the ability of all agrochemical manufacturers to secure their ROI from CPPs. There are unlikely to be "quick wins" possible in the future. Europe has already suffered from the loss of R&D investment in GM technology due to "political" pressure; it would have a major impact on European agriculture if the extreme regulatory burden further impacts on the industries R&D investments. Perhaps a further illustration of the contradictory nature of the EU Reg. EC 1107/2009 is the key part of its main objective to "safeguard the competitiveness of European Agriculture" when it is increasingly hard to see how this will be achieved in the future.

DISCUSSION AND CONCLUSION

The mantra of the need for food security and the Worlds ability to feed 50 billion people by 2050 is regularly debated. Europe, compared with many other regions of the World has an efficient and effective agricultural industry supported by leading edge scientists and an agricultural industry willing and able to invest in the innovation pipeline. Regulation is of course vital and the first part of the objective of EU 1107/2009 is to "to ensure a high level of protection of both human and animal health and the environment". However, the extreme risk adverse nature of the regulations and the way that they are now being implemented across

Europe runs the very real risk of reducing innovation and having a significant impact on the competitiveness of the agricultural industry across Europe. It would be a brave person who can predict what the European agricultural industry will look like by 2020. The impact of loss of CPPs is likely to be one of slow erosion and restriction rather than very dramatic loss in most cases. As an industry, we must wake up to what the changes may mean and how we deal with them. Innovation in CPPs may be slowing down but that also presents new incentives and opportunities to industry and scientists alike. ROI will come from different approaches and innovations but this change of direction needs to start now if innovation if to help solve the agronomic challenges of the future. It is only by working together with a clear understanding of the challenges the industry face will meaningful solutions be found in the future.

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IPM RESEARCH INTO PRACTICE; A LONG TIME COMING

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Summary: IPM is now central to crop protection strategies in all EU member states; The Sustainable Use Directive (SUD) of pesticides resulted in mandatory National Action Plans (NAPs). These vary in content and ambition across the EU. Some opt for basic IPM measures to apply pesticides more precisely at field scale, in order to reduce human and environmental impacts. Others opt for more comprehensive use of multiple IPM tools (the 'IPM toolbox' approach), sometimes implemented at regional scale over multiple seasons. These are aimed at developing more sustainable cropping systems linked to 'ecosystem service' delivery. We will focus on key opportunities and constraints, using case studies to illustrate why IPM uptake has been generally slow, despite increasing research effort. Complex 'ecological engineering' approaches are not rapidly convertible to practical IPM solutions in terms of efficacy, ease of implementation, cost and on-farm advice, requiring long term 'co-innovation' between researchers and diverse stakeholders.

IPM (Integrated Pest Management) aims to reduce dependency on pesticides for crop protection, thereby reducing potential harm to consumers and the environment. EU policy and various Directives (e.g. 91/414/EEC, 2009/128/EC and the SUD) implemented over several years now ensure that all Member States have a NAP (National Action Plan), based on 8 general principles of IPM (Barzman et al., 2015). These include: (1) Design of robust cropping systems; (2) Availability of local monitoring, warning and forecasting systems; (3) Decision support systems for guiding short and long term IPM interventions; (4) Combination of IPM tools that act in complementarity or synergy to reduce pesticide inputs; (5) Use of IPM technologies, including biocontrol agents; (BCAs) that minimise impacts on human health, the environment and biological regulation of pests; (6) Reduced pesticide usage is effectively combined with other IPM tools and tactics; (7) Root causes of pesticide resistance are addressed as part of IPM; (8) Integration of multi-season effects and trade-offs in evaluation criteria to promote more sustainable crop and environment protection solutions, including important pollinators (now referred to as 'IPPM' to ensure that pollination is included as a protected ecosystem service within IPM strategies).

IPM requires a holistic understanding of the whole agroecosystem, including multiple crops on each farm, their associated foodwebs and the multiple interactions of plants, pests and natural enemies with soil, water, agronomy, climate and surrounding landscape features at regional scale (including other farms and semi-natural habitats). However, there has been slow uptake of IPM schemes in commercial agriculture. Research input via experimentation is typically limited by funding, other resources and timescales, with career pressure to produce scientific papers read by other scientists but often not easily understood by farmers, advisory services,

consumers and policy makers. At EU level, research funders now perceive that scientists sit on ever increasing 'information mountains' of complex genomic, ecogenomic, phenomic, metabolomic and other –omic datasets that often fail to deliver practical solutions to end users in time to make an impact, other than in diagnostics. Other constraints include lack of the wide scale uptake of biotechnology in the EU (eg GM crops with pest/disease resistance) due to consumer resistance and industry-perceived policy barriers. Conventional plant breeding routes have been very successful at JHI and elsewhere, but typically take >10 years to develop a commercially acceptable pest resistant variety. During this time pests, farmers, economics and policies don't stand still. As an example, aphid-resistant raspberry varieties bred at JHI are now overcome by virulent aphid biotypes faster than plant breeders can introduce new resistance gene combinations, even when using selectable genetic markers (a 'tipping point'). Similarly, insects, pathogens and weeds have developed multiple forms of resistance to pesticides and to approved GM crops (Bt and HT), due to over-reliance on available and relatively cheap crop protection products, exemplified by the 'pesticide treadmill' effect in the 1st Green Revolution and GM crop 'silver bullet' approaches; (Birch, Begg and Squire, 2011). All these are drivers for development more sustainable cropping systems, incorporating IPM at their centre.

In reality, IPM design, testing and practical implementation is a continuum which is constantly evolving to meet evolving pest threats, changing climatic conditions, economic incentives, new policies, farmer education needs, advisory capacity and consumer demands. True 'co-innovation' is needed to ensure that IPM research is more readily translated into practical and timely solutions, particularly at a critical time of climate change, food, water and energy insecurity, rising human populations and more demanding pesticide use restrictions.

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CROP SPECIFIC IMPLICATIONS OF YIELD AND ENERGY USE EFFICIENCY IN NON-INVERSION TILLAGE SYSTEMS

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Summary: This paper reports how non-inversion (reduced) tillage impacts energy consumption and crop yield, utilising 8 years of replicated field trials undertaken by The New Farming Systems study in the East of England. Tillage regimes include: (1) plough, (2) shallow non-inversion (typically 10 cm), and (3) deep non-inversion (20-25 cm) within two rotations of either (1) winter sown / spring sown crops or (2) winter sown / spring sown + autumn cover crop.

Energy use per ha (highest to lowest) was: plough > deep non-inversion > shallow non-inversion. Crop specific and temporal yield responses were observed. Winter sown crops responded favourably to deep non-inversion tillage, and yields improved as the trial progressed. When considered in combination with lower energy input per hectare, energy efficiency increased relative to the plough-only control. Yield response to shallow non-inversion tillage was variable. Spring sown crops declined in yield and therefore overall energy efficiency.

INTRODUCTION

Reduced or non-inversion tillage has been cited by a number of authors as a potential means to improve the efficiency and resilience of arable cropping (for example Lal *et al*., 2007). Arable cropping has traditionally used plough-based systems that invert the soil with a mouldboard plough, followed by a secondary cultivation prior to drilling (Bell, 1996; Gajri *et al*., 2002). The potential degradation associated with sustained ploughing, for example, soil erosion and a decline in biological activity, have been cited as contributors to reduced crop productivity and a decrease in the resilience of the system (Lal *et al*., 2007; Morris *et al*., 2010; Natural England, 2012).

Alternatives to ploughing include non-inversion tillage. Carter *et al*. (2003) describe this as being either shallow (5-10 cm) with crop residues remaining mostly on the soil surface, or deep (15-20 cm) where a proportion of residues are incorporated into the topsoil. Soil compaction, a potential risk associated with reduced cultivations, may be removed by deep non-inversion tillage using a subsoiler (Batey, 2009). Non-inversion tillage is reported to be advantageous due to decreased operational time and decreased energy input per ha (Cannell, 1985). A failure to take account of potential reductions in crop yield, however, risks endorsing a strategy that increases energy consumption per t of crop output. Knight *et al*. (2012) report an initial

decrease in crop yield immediately after conversion to a non-inversion tillage system, that then increases and stabilises over time. A key question to address is whether this yield reduction reduces energy efficiency, and if so, in which crops and for how long.

The New Farming Systems (NFS) research programme is comprised of several long-term field trials that aim to develop bio-sustainable cropping systems for conventional arable cropping. The programme is funded by The Morley Agricultural Foundation (TMAF) and The JC Mann Trust and is being carried out at Morley (Norfolk) on a sandy clay loam soil. The research programme started in 2007 and is currently in year 8 of what will be a minimum 10 year trial. This paper reports on the impact of non-inversion tillage on energy consumption per unit of crop yield accounting for crop type, and temporal variability in crop yield, relative to time after implementation. The importance of long-term field trial research is highlighted.

MATERIALS AND METHODS

Tillage treatments

The NFS trials are a complete or incomplete factorial design of four replicates 12 m × 36 m in size (Stobart and Morris, 2011). Samples were taken in central plot areas. The specific tillage depth and secondary cultivation(s) varied according to crop and season (Table 1). The shallow non-inversion trial was typically 10 cm in depth using a tine and disc based approach. All crop trials followed local best agronomic practice. Where a cover crop is present, fodder radish (*Raphinus sativus*) was sown at 10 kg ha^{-1} either in late August or early September, then destroyed and incorporated before drilling the spring sown crop.

Energy consumption

A Life-Cycle Assessment (LCA) approach has been followed drawing on previous assessments of energy consumption for agricultural commodities (Hülsbergen and Kalk, 2001; Tzilivakis *et al*., 2005; Williams *et al*., 2009). The system boundary extends to pre-harvest. Operations associated with the tillage trials include indirect emissions from agro-chemical manufacture (Audsley *et al*., 2009; Williams *et al*., 2009), especially inorganic nitrogen (N) fertiliser manufacture (Brentrup and Pallière, 2008), and from farm machinery (Hülsbergen and Kalk, 2001; Williams *et al*., 2009). Energy consumption attributed to each scenario (Table 1) has been derived for:

1. Direct (on-farm) from machinery operation (Scope 1): pesticide spraying, fertiliser spreading, tillage depending on soil type, and depth and the type of crop sown (Table 1).
2. Indirect from product manufacture (Scope 3): pesticides and fertilisers, their packaging, storage and transport (to farm).
3. Indirect from machinery manufacture (Scope 3): estimation of depreciation per operation or hours of use (Table 1).

Table 1. Energy consumption (MJ) attributed to direct (*D*) and indirect depreciation (I_d) components of field operations. D_{min} and D_{max} refer to the minimum and maximum values within the range.

Operation	$D_{\min}$	D_{max}	I_d	Treatment (primary)	Treatment (secondary)
chain harrow	233	-	143	-	[a]CC
cultivator drill	914	-	227	all	-
Einbok rake	182	185	152	-	[b]CC
plough (20 cm)	717	1026	143	Pl	-
plough (25 cm)	998	-	143	Pl	-
roll	87	197	28	-	[c]CC
shallow disc cultivation	277	387	28	-	all[d,e]
subsoil (35 cm)	828	1612	143	D-NI[f]	-
non-inversion deep (20 cm)	253	513	152	D-NI	-
non-inversion shallow (10 cm)	164	-	76	S-NI	D-NI[f]

[a]with cover crop 2011; [b]with cover crop 2009; [c]with cover crop 2009 & 2011; [d]all treatments in 2012, 2013, 2015; [e]*2 in plough *1 D-NI and S-NI in 2012; [f]spring oilseed rape only. Pl (plough); S-NI (shallow non-inversion); D-NI (deep non-inversion).

RESULTS

Tillage treatments

Energy consumption was equal for pesticides and fertiliser in all treatments. Variables correspond to differences in tillage and the presence or absence of a cover crop. The energy input ratio given in Table 2 is calculated as:

$$\text{Energy input ratio} = \frac{\text{energy per unit of yield (GJ t}^{-1}\text{) for treatment x in year n}}{\text{energy per unit of yield (GJ t}^{-1}\text{) for plough-only (control) in year n}}$$

A ratio greater than or equal to one (normal text) indicates either no change or a decrease in energy efficiency (greater energy consumption per unit of yield). A ratio of below one (bold text) represents energy consumption less than the plough-only control.

The plough-only control treatment had the highest yields during the early phase of the field trials, especially in the spring sown crops (spring beans and spring oilseed rape), coupled with the lowest energy input per t of yield. Yield improvements and an increase in energy as indicated by a ratio of below one (Table 2), were evident later in the non-inversion treatments post 2011 onwards, especially the deep non-inversion in winter wheat. The energy input associated with cultivations is summarised in Figure 1. The greater input to the plough treatments in 2012 reflects the two shallow disc cultivations in addition to the primary tillage operation.

Table 2. Energy input ratio (GJ t^{-1}) for each treatment relative to the conventional plough (control) treatment (bold text denotes reduced compared to control).

Operation	SOSR 2009	WW 2010	SBN 2011	WW 2012	SBRLY 2013	WOSR 2014	WW 2015	WW mean
Plough	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Deep NI	1.28	1.00	1.40	**0.93**	**0.97**	**0.89**	**0.91**	**0.95**
Shallow NI	1.17	1.06	1.72	**0.93**	1.04	**0.82**	**0.98**	1.00
Plough + CC	1.31	1.02	1.77	1.01	1.10	1.14	1.01	1.01
Deep NI + CC	1.49	**0.98**	1.65	**0.93**	1.08	**0.98**	**0.95**	**0.95**
Shallow NI + CC	1.23	1.07	2.30	**0.90**	1.04	**0.89**	**0.97**	**0.99**

SOSR (spring oilseed rape); WW (winter wheat); SBN (spring beans); SBRLY (spring barley); WOSR (winter oilseed rape); S-NI / D-NI (shallow / deep non-inversion); CC (cover crop).

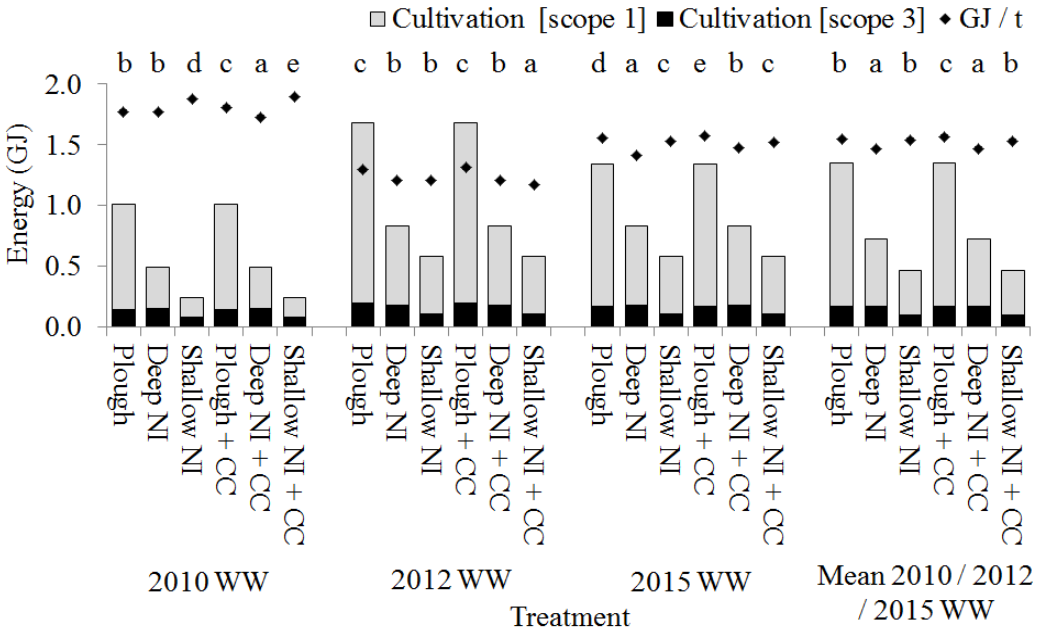

Figure 1. Energy input (GJ ha^{-1}) from cultivations and mean energy input per unit of yield (GJ t^{-1}) ± 1 SE (standard error) of the mean. Different letters denote >1 SE (to 2 decimal places) of the mean GJ t^{-1}.

This improvement is evident for the three years of winter wheat overall. The shallow non-inversion tillage has an equal input per tonne compared to ploughing, although this is partially

skewed by the higher value during 2010, early in the trials. Yields in the shallow non-inversion tillage treatments were more variable, although the addition of a cover crop appeared to be beneficial in 2012 and 2015.

DISCUSSION

Energy inputs to the non-inversion tillage treatments, with the exception of deep non-inversion spring oilseed rape when a sub-soiler was used to break up a pan identified by a penetrometer, were lower per ha compared to conventional ploughing, supporting the conclusions drawn by Cannell (1985). Energy input was lower due to a shallower cultivation depth (typically 20 cm or less as opposed to 23cm) and not inverting the soil. An interesting output was the apparent crop specific response to reduced tillage, with greater benefit realised by the winter sown crops. Secondly, the deeper non-inversion tillage appeared to be a more effective approach than shallow non-inversion tillage. The deep non-inversion treatment in the winter sown crops produced consistently higher yields compared to the conventional plough treatment post 2011 onwards (0.8 to 9.1%) and relative to the shallow non-inversion treatment (2.0 to 30.3%) with the exception of winter oilseed rape (-5.5%). It concurs to a degree with Knight *et al*. (2012) who report that yields tend to improve and stabilise after an initial decline. Energy efficiency also improved per tonne of yield relative to the plough-only control, as the trial progressed.

Of the crops considered, spring beans had the least positive response to non-inversion tillage, with the largest yield reduction relative to ploughing (-51.1 to -52.5%), combined with the energy associated with a cover crop where applicable. Morris *et al*. (2014) also observed that yield loss in non-inversion tillage systems appeared to manifest itself mainly in the spring break crops. Yields might, according to Knight *et al*. (2012), be expected to improve with time, as illustrated by winter wheat in this study. Indeed, Godwin (2014) report that non-inversion tillage has a negligible impact on spring bean yield, therefore, a further assessment of spring beans grown at a later stage in the trial would be beneficial. The yield improvements recorded in winter wheat and the potential crop specific impact of non-inversion tillage emphasise the importance of long-term field trials, which may have been otherwise overlooked if considered over a shorter timescale. The deep non-inversion tillage approach appears to offer benefits both in terms of reduced energy consumption and improvements in yield for winter sown crops.

A review of the literature by Morris *et al*. (2010) concludes that non-inversion tillage is generally more suitable for self-structuring clay soils, where the risk of excessive clod formation post-cultivation of wet soils is decreased. In terms of energy consumption, the decrease in fuel noted on the sandy clay loam soil in these trials might be decreased further if implemented on heavier clay soils (Hulsbergen and Kalk, 2001; Williams *et al*., 2009). This would also be more applicable to the winter sown crops in which yield improvements were typically observed. Winter crops tend to dominate areas where heavier soils are present, due to the limited potential for machinery to gain access to the field during the spring if wet.

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SEPA MONITORING; A TARGETED APPROACH TO PESTICIDES

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Summary: SEPA undertakes pesticide monitoring in response to multiple drivers. Historically, this monitoring has been largely confined to monthly samples, often collected from end-of-catchment locations. SEPA considers that better use can be made of this monitoring effort to help to identify and resolve environmental problems, and are reviewing the national monitoring network with an over-arching aim of introducing a more agile and flexible approach to environmental evidence gathering.

INTRODUCTION

SEPA undertakes pesticide monitoring in response to multiple drivers; WFD compliance, regulation, response to pollution events and to inform policy development. 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 source of water quality and ecology downgrades. Historically, this monitoring has been largely confined to monthly samples, often collected from end-of-catchment locations. Latterly, however, to meet the requirements of Scotland's River Basin Management Plan (RBMP), SEPA has been engaged in a more comprehensive programme of pesticide monitoring to identify areas of impact and provide information on potential sources and pathways of these compounds within priority catchments. Going forward into the second RBMP, this monitoring is currently under review, developing a 'campaign' to pesticide monitoring to provide a comprehensive, risk-based, targeted approach that provides flexibility to respond to new/ withdrawn substances and newly designated priority substances. Some key changes will include;

- need for monitoring being assessed based on likelihood of presence in the environment
- monitoring at time of use
- monitoring at specific locations to provide overview of impacts
- monitoring to provide baseline data or for assessing risk.
- more coverage of non-agricultural sources
- new substances of concern e.g. glyphosate and adding neonicotinoids to the watch list

Pesticides and the WFD

The Water Framework Directive (WFD) requires Member States to achieve good ecological status in all waters by 2015. Good ecological status is quantified in terms of the quality of the biological community present in comparison to what might be expected with minimal anthropogenic impact. Good ecological status involves the assessment of biodiversity, hydrological and the chemical characteristics of a water body in relation to the "Reference Conditions". The impact of pesticides and other chemical pollutants are integrated by

identifying the most important chemicals to consider. These are known as Priority Substances (PS).

PSs are selected at a European Level as they pose a significant risk to the water environment. A subgroup of PSs exists, known as Priority Hazardous Substances (PHS). PHSs are identified on the basis of their chemical properties which make them more damaging to the environment than PSs. The properties considered are their persistence, bioaccumulation and/or toxicity. Together these criteria are referred to as PBT.

Specific Pollutants (SP) pose a lower risk to the water environment and are identified by each member state. These chemicals are formally known as river basin specific pollutants. So far 33 PSs and 28 SPs have been identified. These include a range of industrial chemicals, plant protection products and the compounds of metals. About half of these are (or were) pesticides.

Good chemical status is defined as compliance with all of the quality standards established for substances at a European Level. This ensures a minimum chemical quality everywhere in the European Community. The WFD uses Environmental Quality Standards (EQS) to protect the environment. The EQS is based on ecotoxicology data so that when it is met there should be no environmental degradation from that chemical. The EQS is also intended to be used to regulate the limits contained in discharges so that the EQS is not exceeded in the receiving water. For PSs the EQS values are listed in Directive 2008/105/EC. A water body will fail the good chemical status test when the EQS for any of these substances are exceeded. In the event of a failure, there is a requirement to reduce the quantities of the substance reaching the environment through a reduction in discharges, emissions and losses until good chemical status is achieved. For PHSs, there is also a requirement to stop these substances reaching the environment through the cessation and/or phasing out of inputs.

When a water body is to be used as a source of drinking water it is designated as a Drinking Water Protected Area (DRWPA) and the drinking water standards for pesticides (a.i.) apply (i.e. 0.1 µg/litre per a.i. or 0.5 µg/litre sum of all a.i.). In Scotland, all groundwater bodies are defined as DRWPA but for surface water, only limited areas of rivers and lakes are designated. Seven of the 516 surface water DRWPAs in Scotland have been identified as being at risk of deterioration from pesticides (1.4% of all such areas). At the present time no EQS failures have been noted.

SEPA pesticide monitoring

Pesticides are an important group of chemicals for SEPA. Some are used in very large amounts and across a wide area and as a group they encompass a wide variety of chemical types and properties with a similarly large variation in environmental risk.

The main questions regarding pesticides and the water environment are:

- To what extent are pesticides a risk to the environment?
- Which pesticides require monitoring effort, and to what extent?
- Which pesticides may become an issue in the future?

SEPA takes two approaches to monitoring for pesticides and their impact on the water environment; ecological and chemical. SEPA carry out monitoring of the ecology of surface waters which can be indicative of pesticide impacts. If this monitoring indicates an

environmental impact, chemical monitoring can be carried out to authenticate the issue(s) responsible.

Almost 10,000 water chemistry results were reported for pesticides from 35 locations in 2013, but more than 90 % of these results were below the limit of detection (LOD). Monitoring during 2013 at sampling locations detected 39 active ingredients above the LOD. SEPA consider that better use can be made of this monitoring effort to help to identify and resolve environmental problems, and are reviewing the national monitoring network with an overarching aim of introducing a more agile and flexible approach to environmental evidence gathering.

To identify the pesticides of greatest concern for Scotland's environment, SEPA is taking a tiered "weight of evidence" approach. This approach considers information on the approval status of the pesticide (i.e. can it be used legally, when and in what amounts and by whom), information on land use, environmental concentrations found, and finally what the WFD requires. This process flags pesticides to be prioritised for monitoring.

Additional evidence is used to identify pesticides that may be of emerging concern. For example, glyphosate has usage figures of 200 tonnes per annum (pa) in Scotland but information on levels or impacts of this substance in Scotland's environment is currently lacking. Therefore, SEPA are developing a monitoring plan to confirm whether or not there is an environmental risk from this pesticide.

CASE STUDY: Metaldehyde

Metaldehyde is a cyclo-octane molluscicide used to controls slugs and snails. It is approved in the UK as a Plant Protection Product (PPP) for use on a wide variety of crops in the agricultural, amenity and home and garden sectors. It is normally used as a pre-formulated baited pellet. Metaldehyde is not formally listed under WFD. However, it is still of concern to SEPA as it has the potential to cause significant issues in rivers and lakes designated as drinking water protected areas due to the low limits for all pesticides. Metaldehyde is difficult to remove during the treatment phase so the best approach for compliance with the standard is to reduce the concentration in raw drinking water.

Currently Scottish usage is around 3,000 kg/pa (c. 5 % of the UK total). Amenity usage for the whole of the UK is estimated at 400 kg/pa (2012 Figures) (Goulds, 2015). The use of this pesticide has decreased by about 75 % from 2009 in Scotland. Four crops account for > 80 % of the total use. Ware potatoes (2315 kg/pa, peak use July and August), winter wheat (1973 kg/pa, peak use October), winter oilseed rape (1347 kg/pa, peak use September), and seed potatoes (318kg/pa, peak use September) (Watson et al, 2013). The drastic reduction in usage is not matched by an obvious drop in environmental concentrations measured at this aggregated scale. However, the percentage of LOD results in this data shows a clear increase from 30 % in 2009 to over 60 % in the years 2011 to 2014 (data not shown). Figure 1 shows the monthly pattern of concentrations reflectling the pattern of use. The percentage of samples with concentrations less than the LOD (< 0.005 µg/litre) is also interesting. Shortly after the period of use metaldehyde detections decline sharply to more than 75 % of samples with no traces.

This creates an issue for SEPA in terms of monitoring efforts and what can be learned from the data collected. If adopting an approach of only monitoring metaldehyde concentrations during the highest periods of use (August to November), observed average data would be skewed by

the higher concentrations found at this time – e.g. the monthly average concentration for October was above the DRWPA limit of 0.1 µg/litre. (N.B. this data includes rivers which are not within DRWPA) Therefore, SEPA need to careful to ensure that monitoring data is fit for purpose. When judging compliance against a statutory limit, data is required that is not biased either by peaks or troughs in the environmental concentrations. Therefore, data for this purpose needs samples throughout the year. However, when investigating if a pesticide is a cause for concern, it is desirable to monitor at periods of highest use only to observe the worst case environmental concentrations. These different scenarios can apply to the same pesticide.

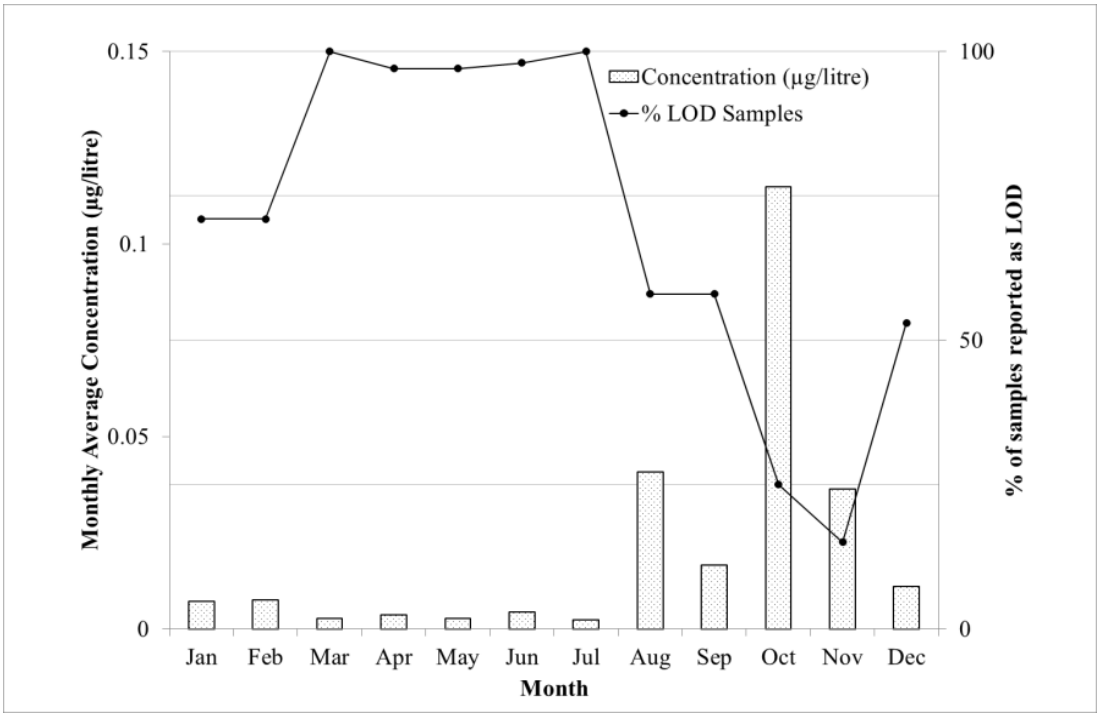

Figure 1. Mean concentration of Metaldehyde in Scottish rivers between 2009 and 2014 and percentage of samples recorded below the limit of detection.

CASE STUDY: Glyphosate

Glyphosate is a phosphonoglycine herbicide with a very broad spectrum, and is one of the most widely used herbicides worldwide. It is approved in the UK as a PPP for use on a wide variety of crops in the agricultural, amenity and home and garden sectors. Glyphosate can also be used to control weeds such as non-native species (e.g. Japanese knotweed) on river banks or aquatic weeds in watercourses (these uses require prior approval from SEPA or the Environment Agency as appropriate). Glyphosate has recently been designated as a SP for the WFD. The EQS is 196 µg/litre for surface waters (Anonymous, 2014).

The current usage in Scotland is 175,000 kg/pa (c. 10 % of the UK total) (Watson et al, 2013). Amenity usage for the whole of the UK is estimated at 348,000 kg/pa (Goulds, 2015). The usage of this pesticide has steadily increased both in terms of area treated and weight applied. There is also a significant usage in the home and garden sector but data is not available.

Four crops account for over 80 % of the total weight applied. These are spring barley (74,000 kg/pa, peak use August), winter oilseed rape (31,000 kg/pa, peak use July and August), winter wheat (23,000 kg/pa, peak use August) and winter barley (21,000 kg/pa, peak use July)

(Watson et al, 2013). Glyphosate has two major uses of crops. The first is to prepare the seed bed and the second is to desiccate the crop for harvest. It has been estimated that over 90 % of the spray area treated with glyphosate is for the second use (J. Hughes Pers Comm).

The combination of high usage in a narrow window for any chemical gives SEPA some cause for concern. To some extent this is mitigated by the environmental fate and behavior of glyphosate. Glyphosate is considered to have a low potential for leaching into water. In water, glyphosate is chemically stable and does not photo-degrade. The half like in water varies from 2 to 91 days. Degradation of glyphosate is largely microbial and the major degradation product is aminomethylphosphonic acid (AMPA).

SEPA currently do not have any data on the concentrations of glyphosate in Scottish surface waters. Data collated from EU Member states, on behalf of Monsanto (Horth and Blackmore, 2009); shows that in the UK, between 1993 and 2007, 92 sites were monitored and 2,809 samples were analysed. Roughly 10%, 297 samples were found to contain glyphosate. The maximum concentration encountered was 8.8 µg/litre. The data compiled in this report suggest that there is a very low probability of glyphosate causing EQS failures.

One area of concern remains with respect to the DRWPA. For this reason we will be conducting a monitoring survey to assess the risk properly based on current concentrations including in DRWPA's. This type of monitoring is targeted to look at worst case scenarios. We will be monitoring within catchments with a high proportion of arable agriculture during the periods of maximum use. The aim of the survey is to assess if SEPA need to be routinely monitoring for glyphosate because it causes environmental degradation as defined by EQS failures or breaches DRWPA limits.

CASE STUDY: Pesticides in the Diffuse Pollution Priority Catchments

Scotland is widely recognised as having one of the leading approaches in Europe for dealing with rural diffuse pollution (now the single largest pressure on Scotland's water) – via an integrated 'diffuse pollution priority catchment approach'. Diffuse pollution priority catchments (PC's) have been identified by SEPA as catchments currently failing (or predicted to fail) to meet environmental standards due to rural diffuse inputs, selected using a risk based approach. Scotland's land managers and public bodies are currently working together (since 2009) to drive improvements in land use management practices in 12 rural diffuse pollution priority catchments, and for the 2nd RBMP cycle (2015-2021) a further 32 catchments will be included.

The main pollutants of concern in these PC's are nutrients, sediment, faecal indicator organisms (FIOs), and pesticides. Going forward, a total of 5 catchments have been selected by SEPA for targeted pesticide monitoring at a water body scale to demonstrate the effectiveness of improvements in land management practices through improved water quality.

Pesticide monitoring in the PCs differs from SEPA's current routine WFD monitoring as it is targeted to high use periods and instead of spot sampling, autosamplers collect a sample every three hours for a week with the composite sample being analysed for an extended range of substances.

This approach helps identify the key pesticides of concern in the catchment under study, as demonstrated in Figure 2. It is hoped that monitoring over time will demonstrate reductions in detects as improvements in management practices take effect.

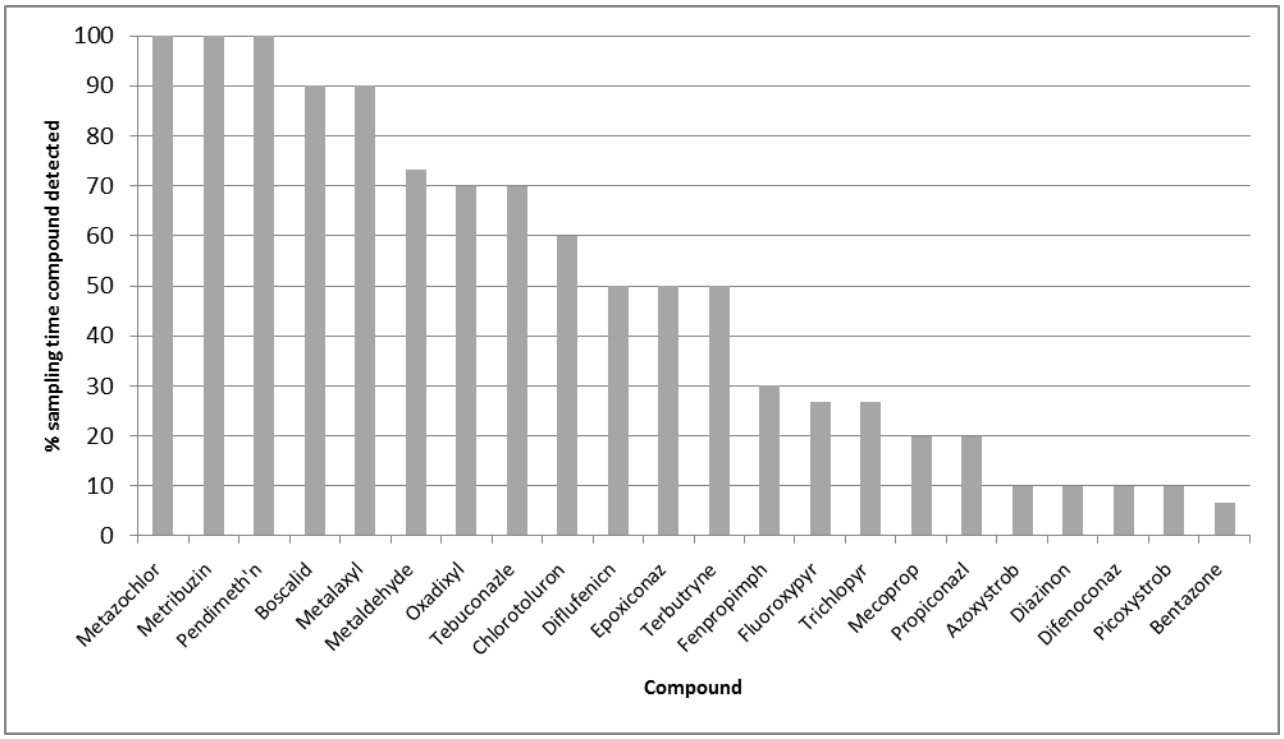

Figure 2. Results from targeted monitoring in a tributary of South Esk (Oct-Dec 2014 and 2015), showing the compounds detected and frequency of detection (expressed as a % of the total sampling frequency)

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The views and opinions expressed in this publication are those of the author and are not necessarily the views or opinions of the Scottish Environment Protection Agency. Whilst every effort has been made to ensure the accuracy of the information provided SEPA cannot be held responsible for any inaccuracies or omissions, whether caused by negligence or otherwise.

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INDUSTRY-LED APPROACHES TO WATER PROTECTION

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Summary: There have been many industry-led stewardship schemes to protect water. Many of these are still active or have evolved into new schemes. All have contributed to improving the awareness of the need to protect water and knowledge of best practice. Stewardship is a valuable alternative to regulation and can give regulators and policy makers more time to evaluate better solutions. "Industry-led" does not always mean the crop protection industry and does not exclude a significant public sector role. Compared with regulation, industry-led schemes offer policy makers flexibility and faster implementation and can represent better value for the national economy. However they can have difficulty achieving universal uptake and thus cannot guarantee 100% compliance. The UK government is considering the introduction of a new industry-led scheme in England to address the oilseed rape herbicides which are being found in drinking water sources.

INTRODUCTION

Since the introduction of the 0.1ppb (0.1ug/l) Drinking Water Standard in 1980, regulators and the farming and crop protection have had to face a challenge not foreseen when the standard was published. Analytical techniques have improved and sensitivity of detection increased, cropping patterns have changed and with a limited range of pesticides, a number of pesticides continue to be detected in drinking water sources, this has in turn resulted in both regulatory pressures on product use and the introduction of industry-led stewardship schemes. In parallel with the concerns about the quality of drinking water sources, regulators have also been concerned about the risk – mainly from drift – to aquatic ecosystems. This paper briefly reviews some of the industry-led initiatives and highlights some of the lessons learnt.

INITIATIVES

Water Company – British Rail/Railtrack/Network Rail Agreement

The residual herbicide atrazine was widely used by British Rail in the 1990s to maintain the safety of the railway infrastructure. However the herbicide was readily leached from railway ballast and, as the sensitivity of detection increased, it become a regular occurrence that the 0.1ppb EU Drinking Water Standard was routinely breached. To tackle this problem, water companies worked with local British Rail regions to identify areas where atrazine and subsequently other residual herbicides would no longer be used. This agreement which is still active has now evolved into a UK wide arrangement between water companies, Network Rail and the Environment Agency.

Spraysafe 1992-97
This Severn Trent initiative was introduced to raise awareness among local authorities of the atrazine ban (1993) and to focus attention on the problems of diuron use on hard surfaces. Highlighted how the quick political/regulatory fix of banning amenity use of atrazine – merely moved the problem to another product diuron. The campaign was able to raise the local water company profile and influence amongst the difficult to reach amenity sector – especially local authorities – encouraging the use of repeat doses of glyphosate.

LERAP scheme
The Local Environmental Risk Assessment for Pesticides (LERAP) scheme was a regulatory initiative developed in consultation with the farming and crop protection industry as a methodology for reducing the risk from spray drift to aquatic ecosystems. Introduced in 1999 it has been supported by industry with product labelling with the LERAP star and advisory booklets and training courses.

Cherwell Study
An ADAS study (1997-99) funded by Bayer CropScience investigating and rectifying the impact of farm yard filling and handling practice on the levels of pesticides reaching drinking water sources. The study showed that through better handling practices farmyard losses could be reduced by 99%. Although focussed on the herbicide isoproturon, the crop protection industry recognised the implications applied to all pesticides. The study has been used as essential supporting evidence for the H2OK? Campaign, the development of Biobeds and the European Crop Protection Association's TOPPSs project.

The Voluntary Initiative (VI)
The VI, led and funded by the farming and crop protection industry, started in 2001. Its aim is to reduce the environmental impact of pesticides across the UK, which meant addressing both biodiversity and water quality issues. The VI originally had over 40 different projects some of which are referenced individually below. Importantly however the VI established three key infrastructure projects the National Register of Sprayer Operators (NRoSO), the National Sprayer Testing Scheme (NSTS) and Integrated Pest Management Plans (IPMP – formerly known as Crop Protection Management Plans - CPMPs). These are now all part of routine farm best practice, adopted as requirements of farm assurance schemes and provide a unique national framework for promoting water protection.

Biobeds
Based on a Swedish concept, the Biobed was introduced to the UK in 1999. Biobeds are a means of controlling and treating small splashes and spills which can occur handling and treating sprayer washings. Regulatory concerns about risks to the groundwater, as well as changes to the status of agricultural waste meant that there were many stops and starts to their adoption. The crop protection industry and regulators funded substantial additional research and a redesign before they were accepted by regulators. Full regulatory acceptance was finally given in 2005 and the support of grants available from Catchment Sensitive Farming and water companies have encouraged farmer adoption. The VI supports the www.biobeds.info website and is responsible for the technical/design manual.

H2OK? Think Water
A Voluntary Initiative (VI) campaign funded by the Crop Protection Association since 2001 promotes best practice measures to protect water. Latest best practice advice is distilled into a

series of advisory leaflets, articles, training activities and presentations. A key element of which are the active substance specific Water Protection Advice Sheets.

VI Pilot Catchment Study
From 2001 to 2005 water companies and the crop protection industry funded a collaborative study in six pilot catchments where pesticides were a problem for water companies. Over a four-year period pesticide manufacturers, water companies, agronomists, local farmers and others worked together both locally and nationally to produce a set of tools aimed at reducing pesticides in water. Improvements ranged from 18-98% in the number of days pesticide levels in raw waters were seen above the 0.1ppb drinking water standard. The work in three of the original catchments continues to be supported by water companies and the crop protection industry, while the tools and approaches have been adopted by Catchment Sensitive Farming.

TOPPS Train the Operators to Prevent Pollution from Point Sources
Starting in 2005 TOPPS was a three year multi-stakeholder project led by the European Crop Protection Association, co-funded by the EU LIFE programme. Using, amongst other sources, UK work notably the Cherwell Study and the H2OK? Campaign, the project identified and promoted commonly agreed Best Management Practices (BMPs), training and information in 15 EU countries targeting farmyards and handling areas, spills, filling practice and the management of spray washings. 24 EU countries have now adopted these measures.

Catchment Sensitive Farming (CSF)
This is a government funded programme which started in 2005/6, encouraging voluntary action on all forms of diffuse water pollution from agriculture. CSF works directly with farmers and also with industry, the latter through Strategic Partnerships. On the pesticide front the VI has worked with CSF since inception, helping prioritise the pesticide issues in the initial 40 catchments and then using tools developed in the VI Pilot Catchment Study to deliver support and best practice targeting in particular at six test catchments. Monitoring by the Environment Agency has shown reductions in the levels of pesticides, delivering a 50% reduction in the overall pesticide levels in these six catchments.

Get Pelletwise
Led by the Metaldehyde Stewardship Group (MSG), this campaign which started in 2008 seeks to reduce the levels of metaldehyde in drinking water sources. Initially the campaign highlighted that many farmers were unaware of the fact that slug pellets were pesticides and that many operators did not have sufficient training. Research funded by MSG has highlighted that although good practice in handling and application is important, the majority of metaldehyde that reaches drinking water sources comes through drain flow. The campaign is working with water companies and other stakeholders to ensure farmers understand and mitigate the risks to drinking water sources associated with the use of metaldehyde.

Campaign for the Farmed Environment
In response to a request from Government to mitigate the loss of the environmental benefits of set-aside and to avoid additional regulation, the farming industry established the Campaign for the Farmed Environment in 2009. The campaign had three main themes resource protection, farmland birds and farm wildlife. Water quality issues are addressed as part of the resource protection theme. Taking a broad approach to water protection from diffuse agricultural pollution, the campaign continues to work closely with The Voluntary Initiative.

Say No To Drift
Expected regulatory changes, potentially requiring aquatic buffer zones of up to 100m for the insecticide chlorpyrifos led manufacturers to embark on the Say No To Drift campaign in 2011. The objective was to raise awareness of the risk of drift, the importance of buffer zones, and the use of drift reducing technology and to demonstrate to regulators that it was possible for farmers to work with wider buffer zones. The campaign is still running although more focussed now on general drift reduction measures and the adoption of low drift nozzles.

TOPPS - Prowadis
Following the completion of the first phase of TOPPS, ECPA supported a second three year programme: Prowadis which looked at diffuse sources of water pollution arising from drift and run-off. Best Management Practices were defined to reduce pesticide losses, diagnosis tools developed and broad dissemination to farmers, advisers and stakeholder was delivered in 7 EU countries; promoting run-off mitigation measures and drift reduction tools. The programme operated in 7 EU countries. To date more than 12000 advisers and farmers have been trained in the theory and practise of reducing the risks of pesticides reaching surface water.

TOPPS - Water Protection
A third phase of the TOPPS programme (Train Operators to Promote best Practices & Sustainability) started in 2015 with the objective of improving and widening the dissemination of the TOPPS best practice advice, Best Management Practises and tools in 12 EU countries. In addition Best Management Practises best practice advice on drainage and leaching losses is being developed. TOPPS is also in discussion with sprayer manufacturers to optimize sprayers on special water protection relevant aspects. A practical 4 day intensive TOPPS academy course on water protection for advisers and stakeholders has also been developed.

Water Company Asset Management Plan (AMP) Periods
Since the privatisation of the water companies in 1989, water companies have had to prepare five yearly Asset Management Plans. In the 1990s these plans were focussed on the building water treatment facilities to help meet the 0.1ppb Drinking Water Standard. Since 2010 a growing number of water companies have had funding approved by the financial regulator, Ofwat, to trial Catchment Management approaches. Working with farmers and agronomists may be a more sustainable long term solution than installing additional treatment processes. The latest AMP 6 period has seen a further increase in activity with many innovative approaches being used to improve plant protection practice and reduce the risks to water.

Oilseed Rape Herbicides
Oilseed rape herbicides (propyzamide, carbetamide, metazachlor, quinmerac and clopyralid) have in recent years been more frequently detected in drinking water sources, while there has been increased awareness of the issue and some reductions in the level detected. Policy makers feel that additional measures are needed to meet the requirements of the Water Framework Directive, in 2014 a set of new and enhanced stewardship proposals targeting these herbicides was submitted to Defra by the Crop Protection Association. A consultation on these proposals is currently expected in early 2016.

DISCUSSION

Most policy makers and regulators recognise – although they are sometimes reluctant to state it - that there are many societal, economic and environmental benefits to the responsible use of pesticides; however they - as does the farming and crop protection industry – recognise that the use of pesticides can result in unintended consequences on the wider environment.

Regulation is a powerful but often blunt, clumsy and inflexible tool and when badly designed can itself result in unintended consequences; in recent years policy makers have turned to collaborative industry-led initiatives to tackle complex problems that are difficult to effectively regulate. These initiatives can act as an effective, practical and timely solution between an unregulated free-for-all and excess regulation. Experience gained from these initiatives can be used to inform and improve on any new initiatives.

A study for the Chemicals Regulation Directorate - Pesticide Stewardship and Regulation PS2816 highlighted 28 different factors that contribute to best practice in designing and delivering pesticide stewardship campaigns. These were grouped into three broad themes: management, research and communication. The study was based on the authors experience of pesticide stewardship and feedback from pesticide manufacturers and organisations who had been involved in stewardship campaigns. The study itself did not specifically highlight individual campaigns, as in this paper, but a number of common themes emerge.

It's not just the Crop Protection Industry

The crop protection industry, especially approval holders may need to take the lead in establishing new initiatives, however initiatives can be led by others especially water companies. Whoever is in the lead, joined up activity from manufacturer, to distributor, to farmer with the support of the agronomist and the involvement of relevant stakeholders is essential for effective communication.

Industry-led initiatives need Government support

Many initiatives started by industry need the specific endorsement of the regulator and encouragement from Ministers in order to continue. Industry has very good connections with large farm business – who account for most product use – but they do not have the same level of contact, influence or sufficient resources to reach every farmer. Where there are wider public and societal benefits, it makes sense that government provides resources to help spread the message to smaller farm businesses.

Initiatives evolve

Most initiatives never actually end, to misquote General Macarthur "Old initiatives never die, they just evolve". Most of the initiatives featured in this paper are still active.

It's not always agriculture

As demonstrated in the first two examples, the amenity sector has had to generate stewardship initiatives and where appropriate other sectors need to be considered as potential contributors to both the problem and providing the solution

Collaboration is best

Working together makes a lot of sense, it ensures a common understanding of the problem, a consistent message and makes best use of resources.

Collaboration is time consuming
Industry initiatives are usually quicker and more flexible to implement than regulation. However the speed of implementation, will depend on the complexity of the issue and the degree of political and stakeholder involvement. Working with partners takes time, and the more diverse the stakeholder groups, number of governments, departments and regulators involved, the longer it takes.

Measures have to be practical, flexible… and economic
Farmers and operators are practical hands-on people who want to adapt advice to fit and work within their own situation. To be adopted on a working farm the solution has to be practical within the existing farming situation. .Farming is also a business and therefore the solution has to be cost-effective, and in that context, the cost of management time and the hassle of making the change is often ignored by policy makers.

"Water" jargon is confusing
Farmers, sprayer operators and others working on the "land" do not always understand water jargon. For example it is not always easy for them to understand the difference between groundwater and surface water or drinking water issues and aquatic life.

Understand the stewardship landscape
With so many initiatives operating it is important to avoid duplication and to work with existing schemes and advice. This can be a problem when a new campaign has plenty of resources but has not taken the trouble to look at the landscape of other initiatives.

Agronomists Matter
The vast majority of crop protection decisions are based on the advice of the agronomist; with some visiting farms more than 10 times a year they have a unique position of influence on farm. Their buy-in is essential and to ensure this they need to be involved from the start.

Three important steps
Almost all initiatives follow a blueprint of: raising awareness, providing solutions, promoting changes in behaviour to ensure an overall improvement in practice on farm.

Be Consistent, Simple and Clear
To avoid confusion within the supply chain and with users it is important that advice and campaign messages are kept consistent, and if needs be gradually evolved over time. Consistent advice also means that materials and advice does not have to be re-printed.

Industry-led Initiatives Work
Industry-led initiatives provide a valuable alternative to regulation, can help keep products available to growers and provide an opportunity to raise awareness of a particular issue, explore new solutions giving policy makers, farmers and the crop protection industry time to review the options and adjust to new circumstances.

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HARPIN CONFERS SOME PROTECTION AGAINST BACTERIAL PATHOGENS OF HORTICULTURAL CROPS

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Summary: Bacterial phytopathogens cause significant damage on horticultural crops. Presently, anti-bacterial agents tend to be limited to products containing copper oxychloride. Elicitors can provide an alternative or supplementary approach, where induction of host defences can be used to control bacterial infection. We assessed the activity of several elicitors, with actual or potential commercial availability in the UK, in controlling bacterial disease of horticultural produce, including *Xanthomonas campestris* pathovar *camprestis* (Xcc) on cabbage and *Burkholderia gladioli* pathovar *allicola* (Bga) on onions. One of the elicitors, Harpin, provided a beneficial effect in controlling symptomatic disease of cabbage from Xcc, and reducing Bga numbers in red onion. Harpin is derived from a bacterial protein HrpN, which acts as a virulence factor in plants. This study shows that it may be beneficial in integrated control strategies for some bacterial phytopathogens of horticultural crops.

INTRODUCTION

Horticultural crops are vulnerable to various bacterial diseases. The plant-microbe interactions vary depending on the plant species and bacterial pathogen. Some interactions are very specific, with a particular plant species susceptible to a particular pathotype within a bacterial species. One of the biggest challenges facing producers for bacterial pathogens is finding treatments for their control. Current treatments tend to be based on compounds containing copper oxychloride, which has environmental toxicity implications, and incorrectly applied can lead to phytotoxicity. Therefore, there is a strong drive to find alternative treatments.

Brassica and allium crops both suffer from bacterial infections, resulting in significant loses in production each year. White and winter cabbage can be infected by *Xanthomomas campestris* pathovar *campestris*, which causes blackened lesions at the leaf margin of outer leaves and in extreme cases causes rot across the entire cabbage head. Red onions are susceptible to *Burkholderia gladioli* pv. *allicolla*, which enters damaged leaves and migrates to the developing bulb. Soft rot is a particular problem for onions that undergo a two-year life cycle, where the immature sets are planted in year 2, after undergoing a 4-week period at relatively high temperature and humidity to prevent bolting. Radish leaves can develop blight-like symptoms following infection with different *Pseudomonas* species, and although the edible bulbs are not diseased, this can reduce sales of radishes sold as bunches with the leaves intact. Finally, soft-rot disease of broccoli is caused by a combination of pathogens, including common soil-borne bacteria, such as certain *Pseudomonas fluorescens* and *Pectobacterium carotovorum* isolates, which can also cause disease on other species.

Elicitors are compounds that induce a defence responsive in plants. They can be roughly grouped in those that occur in nature and tend to trigger pathogen-associated or damage-associated molecular pattern (PAMP / DAMP) responses, and those that mimic plant hormones. Induction of a response that leads to resistance against a broad spectrum of pathogens is termed 'induced resistance', while a 'systemic response' occurs following direct activation of defence responses in systemic tissue as a result of local stimuli and priming, which is more relevant to specific pathogens (Dale R. Walters, 2014). Priming of the defence response results in a faster, more efficient and robust defence response with enhanced resistance to biotic/abiotic stress (Conrath *et al*., 2002).

The aim of the work was to assess compounds that elicit a plant defence response in controlling bacterial disease of horticultural produce. The choice of elicitors included products that are either already commercially available in the UK or available elsewhere with have a good prospect of being sold in the UK. The work is part of an HDC-funded project (FV 417).

MATERIALS AND METHODS

Experimental trials

Experimental field trials for cabbage and red onion were established at the James Hutton Institute, Dundee, Scotland. Treatments were tested in replicate plots of three using a randomised design, and 20 replicate plants were assessed per treatment. Cabbage var. Tundra (a Savoy x White cross) was selected as the most relevant variety for the region and one that is susceptible to *Xanthomonas* and onion var. Red Baron is susceptible to soft rot. Both crops were grown in open-ended poly-tunnels on 100 m x 25 m sites, cabbage in 2013 and 2014, and onion in 2014 only. Poly-tunnels allowed for some degree of control over climatic conditions. The crop was irrigated with a mist irrigation system, 3-times daily for 15 minutes each time.

Applications

Elicitors were applied either applied independently or in conjunction with fungicides. The timing of application was dependent on plant development and all treatments were applied with hand-held sprayer. Four applications of elicitors were applied to cabbage at one-month intervals, while eight applications were applied to onion at 9 days intervals, 11 weeks after sowing, after development of four to five true leaves. The concentration and application rates of the elicitor, additives and fungicides are listed in Table 1 and the treatment schedules and elicitors used are listed in Table 2. Controls included the no-treatment control (NTC), no-bacteria control (NBC) and no-treatment, no-bacteria control (NBNTC), and the standard fungicide programme (SFP).

Disease was assessed visually *in situ*: the incidence of symptomatic disease was scored as 'Healthy' or 'Diseased' and the extent assessed on 5-point scale of symptoms, from no symptoms (0) to symptoms across > 60 % leaf (4). Incidence of disease was scored as presence / absence of symptoms. Analysis of variance was carried out using Excel (Microsoft) or Genstat (VSN International) computer programmes. Disease was not assessed visually for onion bulbs because it is not always an obvious measure of bacterial infection as bulbs can carry a relatively high inoculum without showing visible symptoms. Instead, the bacteria were quantified from onion bulb cores post-storage. Analysis of variance was carried out using Excel (Microsoft) or Genstat (VSN International) computer programmes.

A bacterial inoculum was applied at 10^6 cfu/ml by foliar spray, until run-off. Cabbage plants were infected with *Xanthomonas campestris* pv. *campestris* (Xcc). Bacteria were routinely grown initially in rich media (Luria Bertani) at 28 ^oC to saturation. Prior to plant inoculation, they were sub-cultured into defined media (NYGB, Nutrient Yeast Glucose Broth) designed to optimise expression of virulence factors (at 25 ^oC). The potential for Xcc to cause disease was verified under laboratory conditions, by firstly surface-sterilising Savoy cabbage leaves with 200 ppm hypochlorite and inoculating directly with bacteria. Symptomatic disease was assessed after ~ 7 days, at which point, characteristic disease symptoms became evident. Onion plants were damaged to mimic damage from hail stones by scrapping the leaves lightly with a plastic comb, and infected with *Burkholderia gladioli* pathovar *allicola* (Bga). Bacteria were routinely grown initially in rich media (Luria Bertani) at 28 ^oC to saturation. Prior to plant inoculation, they were sub-cultured into defined media (MOPS supplemented with glycerol and amino acids) designed to optimise expression of virulence factors (at 25 ^oC). The potential for Bga to cause disease on red onion bulbs was verified under laboratory conditions, by firstly surface-sterilising purchased onion bulbs with 200 ppm hypochlorite, and stab-inoculating with bacteria. Symptomatic disease was assessed after 7 days, at which time, characteristic disease symptoms became evident on the onion scales.

Table 1. Concentration of elicitor and fungicide treatments used.

Elicitor	***Working concentration, application rate***	***Applied to***
Bion (ASM = 50%)	1 mM	Cabbage, Onion
SoftGuard + Algal 600	1:600 * ; 1:500	Cabbage, Onion
ProAct (Harpin)	0.15 kg / Ha	Cabbage, Onion
Regalia	4.9 L / Ha	Onion
SiTKO-SA	5 L / Ha	Onion
Tween-20	0.01 %	Cabbage, Onion
Activator-90 wetter	0.05 %	Cabbage, Onion
Fungicides (main a.i.)	**Working concentration**	
Amistar (azoxystrobin)	1 L / Ha	Cabbage
Nativo (trifloxystrobin)	0.4 L / Ha	Cabbage
Rudis (prothioconazole)	0.4 L / Ha	Cabbage
Signum (pyraclostrobin)	1 kg / Ha	Cabbage
Dithane NT (mancozeb)	2.5 kg/ Ha	Onion
Invader (dimethomorph)	2.5 kg/ Ha	Onion
Olympus (azoxystrobin)	2.5 L / Ha	Onion
Unicur (fluoxastrobin)	1.25 L / Ha	Onion
Valbon (benthiavalicarb-isopropyl)	1.6kg / Ha	Onion

* applied to run-off

Table 2 Treatment regime with timings and dates.

Crop	***Application and timing in days (date)***		***Elicitors***
Cabbage (var. Tundra)	Plant transplants	Day 0: 08/07/2013 (Y1), 08/07/2014 (Y2)	o Bion o Harpin o Chitosan & Seaweed extract *applied (i) alone; (ii) + fungicide; (iii) alternating with fungicide*
	Apply bacteria	28	
	Treatment 1 (elicitor +/- Signum)	60	
	Treatment 2 (elicitor +/- Amistar Top)	91	
	Treatment 3 (elicitor +/- Rudis)	122	
	Treatment 4 (elicitor +/- Nativo)	151	
	Disease assessments	122 – 191	
Onion (var. Red Baron)	Sow seeds	19/03/2014 (Y2)	
	Treatment 1 (elicitor +/- Olympus)	77	o Bion
	Treatment 2 (elicitor +/- Unicur, Dithane)	86	o SiTKO-SA
	Treatment 3 (elicitor +/- Valbon)	95	o Harpin
	Treatment 4 (elicitor +/- Unicur, Dithane)	104	o Chitosan & Seaweed extract
	Apply bacteria	111	o Regalia
	Treatment 5 (elicitor +/- Valbon)	114	
	Treatment 6 (elicitor +/- Unicur)	121	
	Treatment 7 (elicitor +/- Invader)	161	
	Treatment 8 (elicitor +/- Invader)	140	
	Harvest and heat treat	161	
	Cold store	182	
	Biomass and disease assessment	210	

RESULTS

Application of Harpin to control black rot of cabbage

Cabbage inoculated with *Xanthomas campestric* pv. *campestris* (Xcc) developed characteristic lesions along the leaf margins and small black lesions on the leaves. Elicitors were applied to cabbage, either alone, mixed with and in combination with the standard fungicide program (SFP) or alternating with the SFP. Harpin used in the absence of SFP had a beneficial effect on the level of disease compared to the other two treatments, Bion and Chitosan + Seaweed Extract (Fig. 1). Both disease severity (i.e. the extent of symptoms) and incidence (the number of plants showing symptoms) was significantly lower with Harpin application in the 2014 trial. The same effect was also evident in the 2013 trial, but to a lesser and not significant extent. The effect was also seen when the Harpin application was alternated with the SFP, but not when Harpin was used together with the standard fungicide treatments. A no-bacteria control

was not included because cabbage transplants are known to carry a degree of inoculum, indeed disease was observed in the un-infected plants in the guard plots (not shown). It is notable that application of any treatment appeared to induce greater disease symptoms and severity, as the lowest level of disease occurred in the no-treatment control. This may have occurred as a consequence of altering the native microflora through the addition of fungicide treatments.

Application of Harpin to control soft rot associated with red onion bulbs

Red onion was grown in a polytunnel from seed to maturity. Elicitors were applied either independently or incorporated into a standard fungicide programme. A bacterial inoculum of Bga was applied mid-way through the treatment schedule, between treatments # 4 and 5 (out of a total of eight), following light damage applied to the leaves, by scraping. Bulbs were harvested, set to prevent bolting (28 °C for three weeks), and cold stored (1-3 °C for 4 weeks) prior to the bacterial load being assessed. Visual disease was not used for the assessment because it can be subjective and is not always a measure of bacterial infection. However, disease was apparent and extensive, such that it was not possible to harvest some individual plants (not shown). Soft rot and the accompanying characteristic smell were apparent during storage and sampling.

Application of elicitors significantly affected the bacterial load. Application of Harpin or Bion alone reduced the levels of bacteria to that seen in the standard fungicide programme (SFP) (Fig. 2). Once again, there was an interaction between the elicitors and fungicides, such that inclusion of Harpin or SiTKO-SA with SFP increased the number of bacteria significantly compared to the SFP control. Application of Chitosan and Seaweed extract or Regalia alone significantly increased the bacterial load, although this effect was reduced with the inclusion of SFP. *Burkholderia* was also present in the NBC control plants. We think that this organism, like the pseudomonads, is able to persist in water droplets, where it can infect other plants via drift. It was also noted that as with cabbage, the process of treatment application appears to encourage infection.

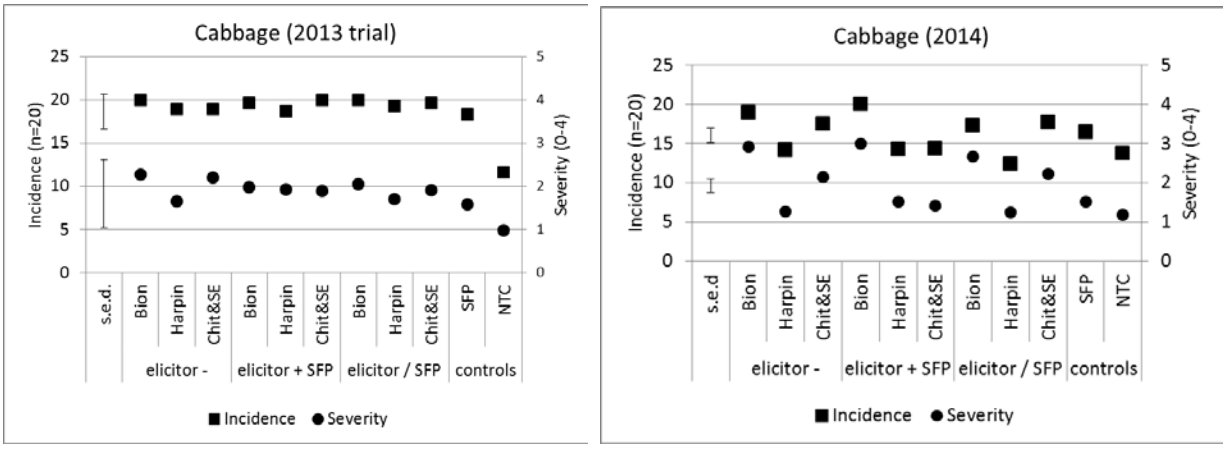

Figure 1. Cabbage treated with elicitors. Disease assessment, showing the level of disease severity per treatment for Year 1 (top, 2013) and Year 2 (bottom, 2014). Disease severity was measured on a 0 (no disease) to 5 (maximum disease) scale and the average shown. Disease incidence relates to the number of plants that showed symptoms in each plot (averaged for n=20). The error bar represents the standard error of the difference. Values are provided for the controls (SFP; NTC).

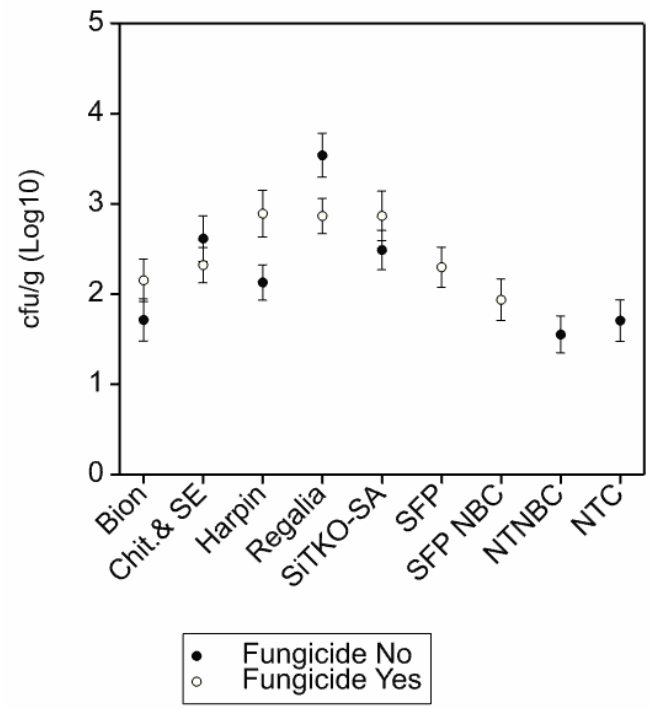

Figure 2. Bacteria recovered from Red onion treated with elicitors. The average number of Bga bacteria recovered from treated plants (n=20 x 3 reps), expressed as cfu per gram of fresh tissue, with the standard error bars shown. The values for the elicitors are presented in the absence (filled circles) or presence (empty circles) of the SFP, here termed 'Fungicide'. Values are also provided for the controls (SFP; SFP NBC; NTNBC; NTC;). The limit of detection in this assay is ~ 1.25 Log_{10} cfu/g.

DISCUSSION

The effect of elicitors was tested on cabbage and onions bulbs infected with phytopathogenic bacteria. Some elicitors had a beneficial effect in reduction of bacterial disease symptoms, in particular Harpin. Interactions occurred between elicitor treatments and standard fungicides. It is possible that the fungicides affect the plant response, the native microbiota or a combination of both, which in turn alters the ability of the bacteria to colonise and cause disease.

Harpin is a protein derived from the secreted protein HrpN (from *Erwinia amylovora*), which acts as a virulence factor once it enters the plant tissue (Wei et al., 1992). It is delivered by the type 3 secretion system, a mechanisms to inject manipulative 'effector' proteins into the plant cell by the bacterium. The protein belongs to a conserved family of haprin proteins in phytopathogenic bacteria. Their main role is as translocators, to facilitate delivery of effector proteins into host cells, although they have other functions and can be perceived as MAMPs (microbe-associated molecular pattern) by the plant (Choi et al., 2013). Importantly, harpins from a number of diverse pythoathogenic bacteria have been shown to elicit a defence response. In our trial, application of Harpin conferred protection in multiple disease systems: Xcc in cabbage and Bga in onion. In addition, proteins of the harpin family have been shown to promote plant growth, which may explain the effect observed in broccoli (data not shown).

SiTKO-SA contains a combination of salicylic acid (SA) and phosphite. There is a reasonable body of work reporting some success using salicylic acid mimics in experimental field trial, for example, the use of ASM in the control of bacterial phytopathogens in orchard trees, lettuce, broccoli and tomato (Balajoo et al., 2012; Graham et al., 2011; Pajot et al., 2005; Yigit, 2011). Furthermore, phosphite has also been shown to induce systemic resistance (Lobato et al., 2011). Chitosan has been well characterised as an elicitor of plant defence as various forms of the polymer are found in fungal cell walls and are recognised by the plant as PAMPs (Trouvelot et al., 2014). Chitosan triggers an alternative defence pathway, through jasmonic acid, which is required for recognition of nectrotrophic pathogens. However, there is feedback and cross-over into other pathways, which may explain the beneficial effect on opportunistic pseudomonads on radish leaves. Regalia is an extract of giant knotweed (*Reynoutria sachalinensis*) and although its mode of action is unclear, it is thought to induce multiple defence pathways in the host plant. It is recognised to have pharmaceutical properties and has been shown to induce phytoalexins which may aid in the control of fungal pathogens (La Torre et al., 2004; Peng et al., 2013).

The finding for Harpin in particular is extremely encouraging for the treatment of bacterial pathogens of horticultural crops, but more work is required to better understand the interaction with fungicides and how best to use Harpin alongside other pathogen control treatments. It was notable that there appeared to be specificity in the response to elicitor application, and interactions with other factors such as fungicides, plant variety and growth conditions. This indicates that due consideration must be given to the whole system: plant, disease agents, treatment strategies (nutrition and pesticides) and environment in order to best promote plant health.

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BENEFICIAL USE OF COMPOSTS AND DIGESTATES IN AGRICULTURE – RESULTS OF A 5-YEAR FIELD EXPERIMENTAL PROGRAMME

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Summary: The aims of the recently completed WRAP/Zero Waste Scotland/Defra-funded field experimental programme on the use of composts and digestates in agriculture (DC-Agri) are outlined and the key findings and practical implications for farmers are discussed. The study showed that compost (green and green/food) applications were a valuable means by which farmers can improve soil quality, ultimately leading to increases in crop yields and improved farm economies. The findings also confirmed that food- and manure-based digestates are an excellent source of readily available nitrogen (RAN), but they must be used with care and applied according to developing best practice guidance in order to maximise nutrient use efficiency and minimise N losses to the wider environment.

INTRODUCTION

The five-year WRAP/Zero Waste Scotland and Defra-funded field experimental programme on the use of digestates and compost in agriculture (DC-Agri) finished in 2015. The overall objective of the research programme was to quantify the effects of food- and manure-based digestate and compost applications on soil and crop quality, crop available nitrogen (N) supply and emissions to the air and water environments. The project had two separate work packages (WP) to achieve this aim, which were:

- WP1: Quantification of the effects of repeated compost and digestate applications on soil and crop quality.
- WP2: Quantification of the N supply characteristics of digestates and composts and their effect on emissions to the air and water environments.

This paper reports some of the key findings from earlier parts of the work. Further practical information, guidance and recommendations are available in project bulletins 1 to 8 which are available from the WRAP website (www.wrap.org.uk/dc-agri), where the full reports will be published in spring 2016, and detailed technical and scientific information will be published in refereed journals over the next 2 to 3 years. A summary of the chemical properties of the composts and digestates used in this study together with an assessment of the legislation relevant to using composts and digestates in agriculture have been previously described in a paper published by Litterick *et al.* (2014).

THE IMPACT OF USING COMPOSTS AND DIGESTATES ON SOIL AND CROP QUALITY

WP1 investigated the effects of repeated applications (i.e. annual applications over 3 years) of compost (green and green/food) and digestate (whole food and manure-based) in comparison with synthetic fertiliser, farmyard manure (FYM) and livestock slurry on soil and crop quality at a range of experimental sites with varying soil types, climatic conditions and cropping. Accordingly, a network of seven experimental sites was established in autumn 2010 across the UK: Aberdeen (Aberdeenshire), Devizes (Wiltshire), Faringdon (Oxfordshire), Harper Adams (Shropshire), and Terrington (Norfolk) which were in arable cultivation, and Ayr (Ayrshire) and Lampeter (Ceredigion) which were grassland sites. The sites at Harper Adams and Terrington were existing experimental platforms which had previously benefitted from applications of FYM, livestock slurry and green compost over a 6-17 year period.

Over the 3 year experimental programme, green compost and FYM application supplied c.16 t/ha organic matter (OM), green/food compost c.11 t/ha OM, livestock slurry c.8 t/ha and whole food-based digestate c.2 t/ha OM. Manure-based digestate was applied at Aberdeen and Ayr in Scotland, supplying 3-6 t/ha OM. At the two sites with a prior history of organic material applications, the range of OM loadings was extended to c.80-105 t/ha of OM from FYM and c.50 t/ha from green compost. There was also an untreated control treatment at each site which received recommended rates of manufactured fertiliser, but no organic materials. Crop yields were determined every year, with a comprehensive programme of soil and crop quality assessments undertaken in 2013.

After the long-term (9 years) application of green compost at Harper Adams and Terrington, the two sites with a prior history of organic material additions, soil organic matter (SOM) had increased by 20-25% relative to the untreated control. Similar increases were measured on the long-term (20 years) FYM treatment at these sites. The repeated compost additions therefore led to a more rapid build-up of SOM compared to FYM due to a higher lignin content, rendering it more resistant to decomposition, confirming its value as a good source of stable organic matter. Where these materials had been applied for just 3 years, there were small but non-significant increases in SOM. The increases in SOM on the long-term treatments were associated with increases in topsoil microbial biomass, nutrient supply (i.e. total N, P, potassium (K), magnesium (Mg) and sulphur (S) status), cation exchange capacity (a measure of the ability of the soil to retain nutrients in a form where they are easily available for plant uptake) and potentially mineralisable N (which measures the N released by microbial breakdown of OM). Decreases in topsoil soil bulk density and shear strength were also measured: these are related to improved rooting and ease of cultivation. The results clearly demonstrated that repeated compost applications are a valuable means by which farmers can improve soil quality, potentially leading to increases in crop yields (due to possible improved rooting and nutrient availability).

Repeated whole digestate applications (both food and manure-based), however, had a limited capacity to improve soil biological and physical functioning, due to the low organic matter loading associated with these materials; nevertheless, the digestates did improve soil nutrient status. There was no effect of compost or digestate additions on soil total metal and organic compound contaminant concentrations or crop metal concentrations. This is an important finding and supports the sustainable use of these materials on crops grown for food production.

The yields of most crops in virtually all years were greater where organic materials had been applied, compared to the untreated control. Indeed, winter cereal yields (averaged over eight sites, monitored over three seasons) increased by 10% on the FYM and digestate treatments relative to the control, by 9% on the livestock slurry treatment, by 8% on the green/food

compost and by 7% on the green compost treatment, amounting to 0.5-0.6 t/ha (Fig. 1). As N inputs were balanced across all the treatments (using MANNER-*NPK* to calculate the additional manufactured fertiliser N required where the organic materials had been applied; Nicholson *et al.*, 2013), these yield increases are thought to be due to the additional P, K and S inputs from the organic materials. This additional nutrient supply was valued at £55-£160/ha, taking into account the value of fertiliser saved and cost of spreading (but not sourcing) the organic materials, and clearly demonstrated the value of an integrated nutrient management plan, using both compost/digestate and manufactured fertiliser.

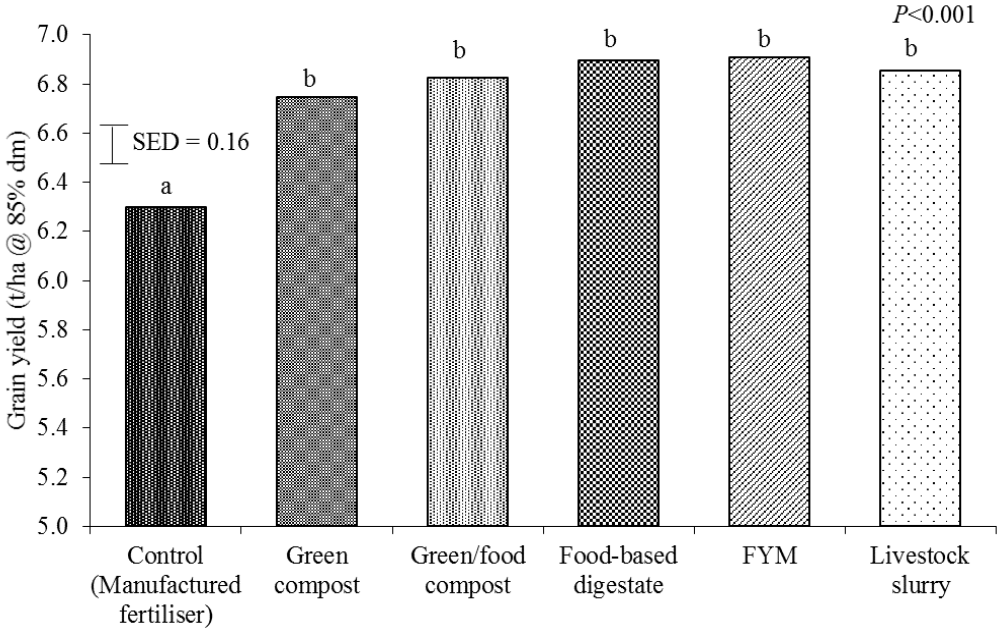

Figure 1. Average winter cereal yields across the five arable sites from 2011-2013 (3 seasons). *Columns with different letters are significantly different (P<0.05) from each other*.

NITROGEN SUPPLY FROM COMPOSTS AND DIGESTATES AND EMISSIONS TO AIR AND WATER

The specific objectives of this work package were:

- (WP2.1) To determine the crop available N supply from quality digestate (food and manure-based) and composts in comparison to FYM and slurry across different application timings to a range of arable and grassland crops throughout Britain;
- (WP2.2) To quantify the environmental emissions, following the application of quality digestate and compost, to the air and water environments; and
- (WP2.3) To quantify the effect of bandspread/shallow injected digestate and slurry application techniques on fertiliser N replacement values, and crop yields and quality.

Accordingly, a network of fifteen experimental sites was established across the UK including three in Scotland and one each at: Wensum (Norfolk), Pwllpeiron (Ceredigion) and North Wyke (Devon). At all the sites, crop N uptake from whole digestate and cattle/pig slurry applications (band-spread to fully replicated experimental plots) was compared with 'bagged' fertiliser N, so that the N use efficiency of the organic materials could be determined. At most sites the digestates and livestock slurry were applied in autumn (before the start of the NVZ closed spreading period for high readily available nitrogen (RAN) materials) and in spring.

At Wensum, North Wyke and Pwllpeiran, whole digestates and slurries were applied using both broadcast and bandspread application technologies; additionally, compost and FYM were

applied. Ammonia (NH_3), nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) emissions were measured from all the organic material treatments using standard methodologies, and at Wensum (a light sandy soil) leaching losses to water of nitrate-N, ammonium-N, P and *E.coli* were measured using Teflon suction cup samplers following organic material applications in autumn 2011. Some of the results from work conducted in 2011 and 2012 are described below.

Nitrogen Use Efficiency

During fertiliser planning and in order to optimise productivity, an estimate must be made of the amount of crop available N supplied by organic materials (sometimes referred to as the N use efficiency or NUE) which should be balanced against the crop N requirement to calculate the amount of mineral fertiliser which needs to be applied.

At Wensum, the NUE of the food-based digestate applied in autumn to winter wheat was only around 20% (Fig. 2). However, in the spring, when the winter wheat was growing actively, the NUE of food-based digestate increased dramatically to around 80%. The NUE of pig slurry and manure-based digestates was also much greater when applied in the spring, compared with the autumn application. Similarly at Pwllpeiran (Fig. 3), the NUE of food- and manure-based digestates and cattle slurry applied to the grass silage crop was greater in the spring than from the autumn application. The higher N use efficiency from the spring applied food-based digestate, compared with the manure-based digestate and cattle slurry reflected the relative RAN contents of the different materials.

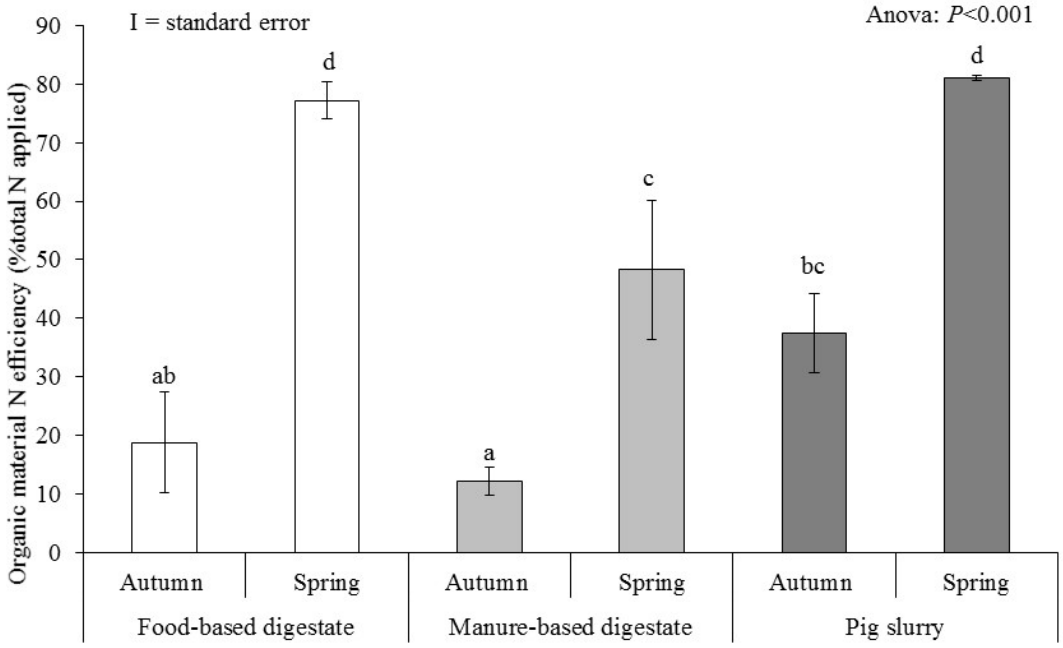

Figure 2. Wensum: N use efficiency - winter wheat 2012 (bandspread). *Columns with different letters are significantly different (P<0.05) from each other*.

Ammonia emissions

Ammonia emissions were higher from food-based digestate than from cattle slurry applications at Pwllpeiran (Fig. 4). This was largely a reflection of the greater RAN content of the digestate (72% of total N applied, compared with 53%). Band-spreading reduced ammonia losses from both the digestate and cattle slurry treatments, compared with surface broadcast application, although emissions were still high.

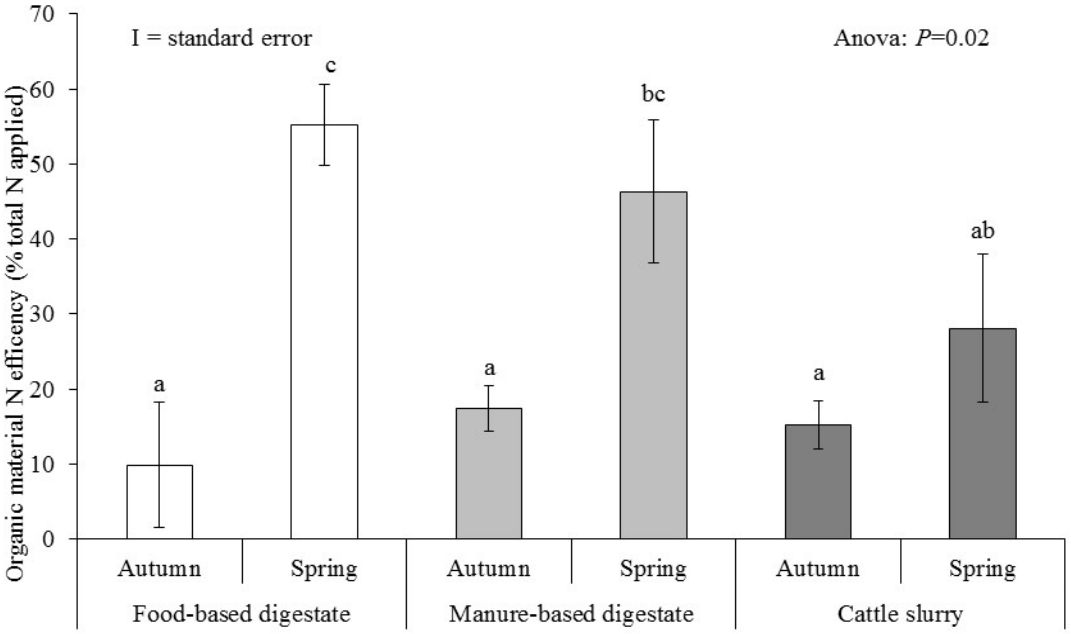

Figure 3. Pwllpeiran: N use efficiency - first cut silage 2012 (bandspread). *Columns with different letters are significantly different (P<0.05) from each other.*

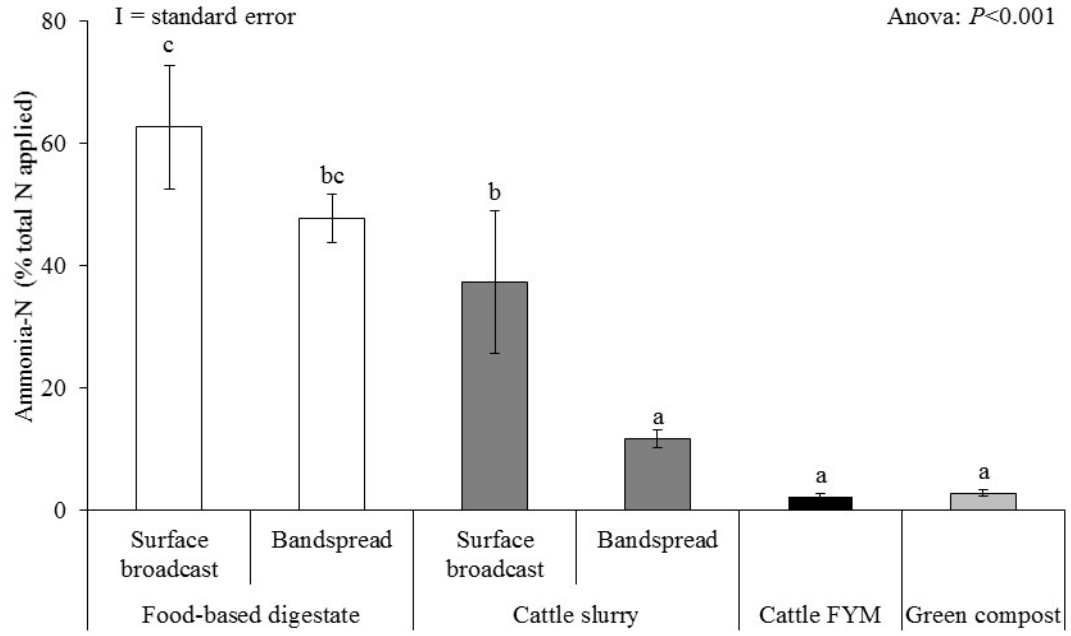

Figure 4. Ammonia emissions following spring 2012 organic material applications to grassland at Pwllpeiran. *Columns with different letters are significantly different (P<0.05) from each other.*

Shallow injection has been demonstrated to be even more effective at reducing ammonia losses from livestock slurry and analysis of results from similar experiments using digestate (WP2.3) may show similar results. Ammonia emissions were low from the green compost and cattle FYM applications, reflecting the lower RAN content of these materials (<10% of total N applied). The use of precision application methods (i.e. a bandspreader or shallow injector) is

recommended when applying digestate to minimise ammonia losses and hence to maximise the fertiliser replacement value of the digestate.

Leaching losses
At the sandy soil site at Wensum (Norfolk), the digestate and pig slurry applications increased nitrate leaching losses above the untreated control; losses were equivalent to 15-20% of the total N applied and reflected the high RAN content of these materials (c.80% total N). In contrast, nitrate leaching losses were low (<5% of the total N applied) following the green/food compost and pig FYM applications, reflecting the lower RAN content of these materials (c.10% total N). None of the organic material applications had an effect on ammonium-N, P or *E.coli* losses in drainage waters compared with the untreated control. Where for operational reasons late summer applications of digestate need to be made, these are best targeted on oilseed rape and grassland crops that will utilise some of the applied N and reduce the risk of nitrate leaching losses over winter.

CONCLUSIONS

The results from the DC-Agri experimental programme clearly showed the potential that regular compost applications had to improve soil quality, and, in the longer term, crop quality and yield. Composts in particular, are an excellent source of OM; soils to which composts had been repeatedly applied over several years showed increases in total SOM which was associated with improvements in several other important soil properties including bulk density, shear strength, microbial biomass carbon and potentially mineralisable N. Composts have been shown to be a particularly long-lasting source of organic matter, since they contain more lignin than other commonly used forms of organic matter, such as FYM and biosolids.

Because of its low RAN content, compost applications should be seen as a means to build up long-term (organic) soil N reserves rather than as a short-term replacement for mineral fertiliser. Whole, food-based digestates are typically very good sources of RAN, but they must be used with care in order to maximise their fertiliser value and minimise their environmental impact: they should be applied during active crop growth and will use the RAN efficiently. Precision spreading equipment (e.g. a bandspreader or shallow injector) should be used to apply the digestate for optimum crop uptake and to reduce both ammonia losses and odour.

ACKNOWLEDGEMENTS
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CAN UNDERGROUND FUNGAL NETWORKS AID CROP PEST CONTROL?

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Summary: The roots of most land plants are colonised by mycorrhizal fungi that provide mineral nutrients in exchange for carbon. Here we review recent research that has discovered that these fungi can also act as a conduit for signals between bean plants *Vicia faba*, acting as an early warning system against aphid attack. Aphids cause bean plants to change their volatile production, making them repellent to aphids but attractive to aphid enemies such as parasitoid wasps. Fascinatingly, if another bean plant (without aphids) is connected to an aphid-infested plant via a fungal network, it too produces volatiles that repel aphids and attract parasitoids, while unconnected beans remain susceptible to aphids. We discuss whether reduced tillage could allow fungal network growth so crops can self-protect, reducing the need for pesticides, and we outline what research is now needed to explore the feasibility of utilising fungal-based signalling for crop pest control.

INTRODUCTION

Arbuscular mycorrhizal fungi form mutualistic symbioses with many herbaceous plants, including important crop species, they have a near global distribution and are among the most functionally important soil microorganisms (Smith & Read, 2008). Arbuscular mycorrhizal fungi often significantly improve mineral nutrient uptake (Smith & Read, 2008) and enhance tolerance to root and shoot pathogens (Whipps, 2004), nematodes (De La Peña *et al*., 2006; Vos *et al*., 2012), and drought (Smith & Read, 2008). In return, plants supply mycorrhizal fungi with carbohydrates (Johnson *et al*., 2002) that are used in part to develop extensive mycelial networks (Leake *et al*., 2004), which act as conduits for carbon (Johnson *et al*., 2002) and mineral nutrients (Johnson *et al*., 2001).

Recent research has also implicated the transport of other compounds through mycelial networks, with implications for the control of disease or competing plants: Barto *et al*. (2011) demonstrated that allelochemicals released by marigold (*Tagetes tenuifolia* Millsp) could be transported through AM fungal networks to inhibit the growth of neighbouring plants. Song *et al*. (2010a) found that inter-plant connections via common mycelial networks led to increased disease resistance, defensive enzyme activities, and defence-related gene expression in healthy tomato plants (*Lycopersicon esculentum* Mill) connected to plants infected with leaf early blight (*Alternaria solani*). This finding suggests that inter-plant transfer of pathogenic fungal disease resistance signals via these networks could be occurring.

DISCOVERY OF PLANT-PLANT SIGNALS SENT VIA FUNGI

If common mycelial networks can act as conduits for signalling compounds between plants, there clearly is considerable potential for mycorrhizal fungi to mediate plant responses to herbivorous crop pests such as aphids. Plants naturally emit volatile organic chemicals from their leaves. Sap-sucking herbivores such as aphids use these volatile organic chemicals as cues for locating host plants (Bruce *et al*., 2005). However, following the attack, the plants change the composition of the volatiles released so that they become repellent to subsequent herbivores (Bernasconi *et al*., 1998) and attractive to their natural enemies, such as parasitoid wasps (Turlings *et al*., 1995). Can mycelial connections between plants send signals that therefore induce a change in plant volatiles, thus altering pest infestations?

Indeed, recent exciting experiments have tested this (Babikova *et al*., 2013a,b). Babikova *et al*. (2013a) discovered that common mycelial networks can act as signalling conduits between crop plants and thus provide an early warning system of aphid attack. Specifically, they tested how common mycelial networks linking aphid-infested bean plants with aphid-free plants affect the attractiveness of volatiles to aphids and parasitoid wasps. They designed an experiment whereby a central bean plant acted as a 'donor' by being infested with aphids towards the end of the experiment. The donor was surrounded by four 'receiver' bean plants that never came into direct contact with aphids (Fig. 1). Two of these four receiver plants were not connected to the donor: one by means of a 0.5 µm mesh that prevented mycelial ingress and the other due to a 40 µm mesh that did enable hyphal connections but these were broken by rotating the mesh core immediately before aphids were added to the donor (after Johnson *et al.*, 2001). The two other receiver plants could form common mycelial networks with the donor plant: one grown with no barrier, allowing the intermingling of both fungal mycelium and roots with the donor, and one allowing fungal contact by means of a 40 µm mesh core that allowed fungal hyphae but not roots to grow through (Fig. 1).

After aphids were added to the donor plant, volatiles of all plants were collected (see Bruce *et al*., 2008 for details of methods) and analysed using gas chromatography (see Babikova *et al*., 2013a for details). Behavioural bioassays using a four-arm olfactometer were conducted to determine whether aphids were attracted or repelled by volatiles collected from donor and receiver plants (after Pettersson, 1970; Webster *et al*., 2010).

Crucially, they found that aphid-free receiver plants connected via mycorrhizal fungi to aphid-infested donor plants acted as if they themselves were infested, i.e. they shared similar volatile profiles and similarly repelled aphids (Fig. 2). In stark contrast, receiver plants that were not connected to the aphid-infested donors did not change their volatile profiles and thus attracted aphids (Fig. 2). This study was ground-breaking in discovering for the first time that common mycelial networks can act as an early warning system against aphid attack.

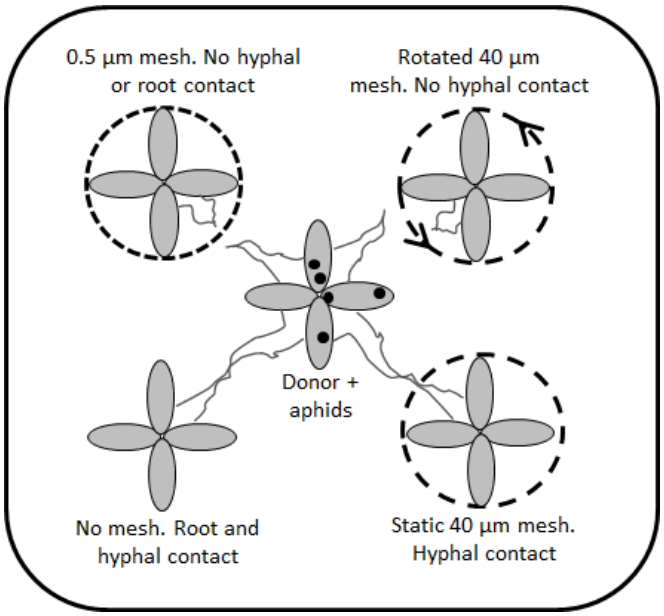

Figure 1. Experimental design used by Babikova et al. (2013) to test the hypothesis that mycorrhizal fungi transport warning signals between connected plants. Black ovals represent aphids on the donor bean plant. Wavy lines represent fungal hyphae. Dashed circles represent 40 µm mesh that allows hyphae but not roots to pass through (the arrow indicates that mesh core was rotated to break the connection). Solid circle depicts 0.5 µm mesh that denies access by fungal hyphae.

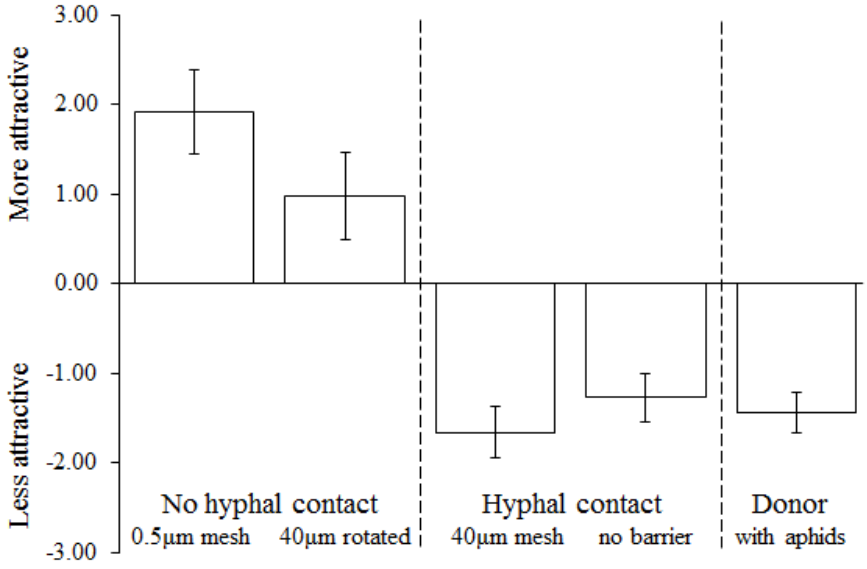

Figure 2. Attractiveness to aphids of plant volatile organic compounds from experimental bean plants (mean time in minutes spent in olfactometer arms containing volatiles minus reagent blanks ±SE). Infested donor plants and uninfested receivers that were connected to the donor were both repellent to aphids. Plants not connected to the donor remained attractive to aphids.

POTENTIAL FOR CROP PEST CONTROL?

This newly discovered signalling pathway may have potential use in crop pest control (Babikova *et al*., 2014). However, for this signalling pathway to be of real practical use in repelling aphids from neighbouring plants in the field, the signal (and the response to the signal) must be very rapid, otherwise aphids could infest before the defence response can be effective. Babikova *et al*. (2013b) showed that significant defence response was detected in receiver plants within 24 h of aphids attacking the donor bean plant. But how relevant is this to other (non-bean) crop systems and other pest types, and how long does it take for the plant to produce its maximum strength response? Johnson & Gilbert (2015) analysed data from the only available publications to address these questions (Babikova *et al*., 2013a,b for aphids; Song *et al*., 2010a,b for a necrotrophic fungus; and Song *et al*., 2015 for caterpillars). This meta-analysis showed that maximum defence responses in receiver plants were produced within 50 h of the donor plant being attacked by the aphid, caterpillar or fungal enemy, although up to 60% of maximum response could be produced within 24 h in some cases. Whether this is rapid enough for realistic crop pest control will depend on the rapidity and frequency of enemy attack on a large number of crop plants.

Babikova *et al*. (2014) outlined how this underground plant-plant signalling mechanism may have potential use in aiding crop pest control. However, there are many unanswered questions that now need to be addressed as a first step to understanding how common mycelial networks may be used for pest control (for a more in-depth discussion of the issues, see Babikova *et al*., 2014; Johnson & Gilbert, 2015; Gilbert & Johnson, 2015).

If common mycelial networks are to be used to aid in crop pest control, the most fundamental requirement is for the networks to actually exist in the arable situation, so that crop plants are connected and therefore have the opportunity to warn each other of enemy attack. However, most cropped soils are tilled to varying extents, and this is likely to break up the network. Therefore, one of the most pressing questions is: to what extent can soil be tilled while still maintaining mycelial networks that function as warning signals between plants? We are currently addressing this question through a PhD project funded through the Scottish Food Security Alliance (SFSA), a collaborative network between Aberdeen and Dundee Universities and the James Hutton Institute.

We also need to know whether these underground signals can travel long enough distances for signalling between crop plants to occur in a realistic arable context. Babikova *et al*. (2013a,b) confirmed that signals between beans can travel at least 20cm, but the further the signals can travel, the better for crop control in a field situation. In case the signals cannot travel far, it would be equally important to know whether signals can be relayed between plants, thereby greatly expanding the sphere of signalling. For example, can the nearest neighbour receiver plant also act like a donor and send the signal to the next plant along? Or can the signal operate only between the original pest-infested donor and its nearest receiving neighbours? Our SFSA-funded PhD will also design experiments to address these two crucial issues.

It may be costly for plants to send and receive the signals and respond to them (discussed by Gilbert & Johnson 2015), so we need to determine whether crop yield can be maintained if fungal network-based crop pest control is to be worthwhile. For example, it could be that pests are successfully repelled, but that crop yields suffer due to the energetic and metabolic costs to the plants in self-protecting.

Finally, if fungal network-based signalling does prove to pass these tests of distance, strength, tillage and maintaining crop yields, how can we actually tap into this signalling mechanism to protect crops? This would be the final question to address, although some ideas have already been mooted by Babikova *et al*. (2013b). For example, one idea could be to spray crops with the volatile chemicals that the plants themselves produce in their defence against pests, such as methyl salicylate, thereby using the plant's natural mechanism while lessening the need for efficient underground signalling (again, this needs experimental testing). Another idea is to use the fungal network-based signalling fully, by infesting certain individual plants with pests in a highly controlled way, so they act as sacrificial donors and send out the signals to the rest of the crop. Field trials would be needed to test this idea, and our signal distance and signal relaying experiments would be able to inform how best to conduct these potential trials.

CONCLUSION

In conclusion, this underground fungal network-based early warning system is a very recent and exciting discovery and research into the wider implications, such as use as a sustainable crop pest control method, is only just beginning. Our SFSA-funded PhD is the first step to exploring the potential of this signalling pathway as a crop pest control tool, and we encourage the arable industry to give us feedback on what may or may not be feasible in practice.

ACKNOWLEDGEMENTS

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IPM IN PRACTICE: WHAT DOES IPM LOOK LIKE ON MODERN CONVENTIONAL FARMS?

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Summary: LEAF (Linking Environment And Farming) is a leading organisation delivering more sustainable food and farming. LEAF has been pioneering Integrated Pest Management (IPM) research on all farm types since 1993 and continues to be at the forefront of developing this research into practical on-farm application. IPM offers a toolbox of techniques that can be tailored to different cropping systems, climatic conditions, pest pressures and availability of solutions. Through practicing a range of techniques, producers can make informed decisions and minimise their reliance on pesticides. Changes such as these can contribute to more sustainable farming by helping to maintain biodiversity, decrease pollution and lower the build-up of pesticide resistance. The LEAF Sustainable Farming Review provides a useful decision based framework to support farmers in adopting IFM and IPM. Another avenue for successful communication in this area is via demonstration farms and on farm research. To this end, LEAF has 36 Demonstration Farms across England and Scotland, which are able to share their experiences and expertise on how IPM works in practice within their business. Through sharing and demonstrating the success of this approach to others, the understanding and effective use of IPM can spread.

INTRODUCTION

LEAF (Linking Environment And Farming) is a leading organisation delivering more sustainable food and farming. We work with farmers, the food industry, scientists and consumers, to inspire and enable sustainable farming that is prosperous, enriches the environment and engages local communities.

LEAF was set up in 1991 to promote Integrated Crop Management and later extended its activities to encompass livestock farms, promoting Integrated Farm Management (See Figure 1 and Figure 2). Throughout this period, LEAF has been pioneering Integrated Pest Management research on all farm types and continues to be at the forefront of developing this research into practical on-farm application.

Through LEAF's network of Demonstration Farms and Innovation Centres we are able to facilitate IPM knowledge generation and exchange amongst farmers and researchers via mechanisms such as technical events and publications. In addition, our core online tool initially established in 1993, the LEAF Audit, now the LEAF Sustainable Farming Review, asks farmers to evaluate and improve all aspects of pest management within an integrated approach.

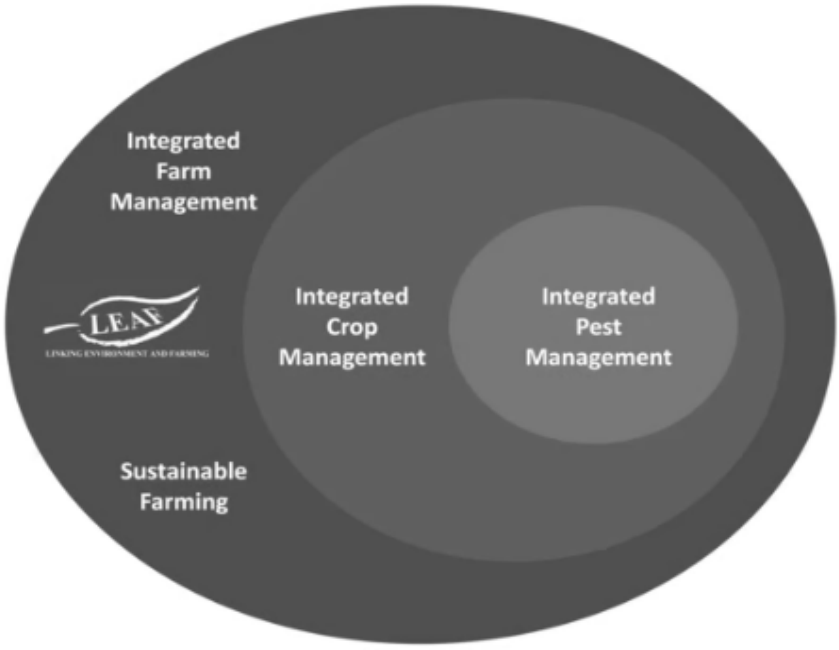

Figure 1. How IPM fits with ICM, IFM and Sustainable Farming

Figure 2. LEAF's Integrated Farm Management (IFM) Wheel

WHAT IS IPM AND WHAT DOES IT INVOLVE?

Integrated Pest Management is the careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health. IPM offers a toolbox of techniques that can be tailored to different cropping systems, climatic conditions, pest pressures and availability of solutions and consists of 8 general principles:

- Achieving prevention and suppression of harmful organisms
- Monitoring of harmful organisms
- Decisions made based on monitoring and thresholds
- Non-chemical methods
- Pesticide selection
- Reduced use of chemical pesticides
- Anti-resistance strategies
- Evaluation

HOW DOES IPM CONTRIBUTE TO SUSTAINABLE FARMING?

IPM can play a significant role in making farming more environmentally, economically and socially sustainable. Through practicing a range of techniques, producers can make informed decisions and minimise their reliance on pesticides. Changes such as these can help maintain biodiversity, decrease pollution and lower the build-up of pesticide resistance. IPM is a systems based approach where the entire systems effect is greater than the individual components. In addition, the diversity of solutions available in IPM helps ensure the long term sustainability of control measures.

In order to gain a full holistic understanding of the benefits of IPM a sound understanding of the interactions between soil, water, air and plants under the unique climatic and cropping conditions of the individual farms is required. This requirement for understanding along with ensuring strategies are within the context of sustainable production, addressing economic viability, environmental responsibility and social acceptability mean that ensuring widespread uptake of IPM has its challenges.

HOW CAN LEAF HELP ENCOURAGE THE UPTAKE OF IPM?

The LEAF Sustainable Farming Review provides a useful decision based framework to support farmers in adopting IFM and IPM measures as well as consider other options that might be available. As IFM and IPM develops LEAF will continue to communicate practical, realistic and achievable solutions, while working with others to seek new innovation and technologies to improve farm productivity, environmental enhancement and social acceptability.

The main avenue for successful communication in this area is via demonstration farms and on farm research. To this end, LEAF has 36 Demonstration Farms across England and Scotland, which are able to share their experiences, expertise and thoughts on how IPM works in practice within their business. LEAF Demonstration Farms will host visits and event which form great opportunities to exchange ideas and discuss new practices in an informal but structured setting.

Case Study 1: Morriston Farms, Ayrshire

Morriston Farm is a 650ha LEAF Demonstration Farm in Ayrshire. They are an arable farm that grows largely forage crops for neighbouring livestock farms. Strip tillage is used throughout the estate and wide grass margins are planted with wild bird seed or maintained in long term grass/wildflower mixes to increase biodiversity and for game for the estate.

Since the development of the margins, a reduced requirement for insecticides has been noticed as the margins are a haven for insects which can help with pest control. For example, ladybird populations are relied upon to control aphid populations. The ladybirds help prevent pest outbreaks and keep aphids below the threshold at which they begin to cause damage. Similarly other insects such as ground beetles encourage birds which help keep slug population low.

Case Study 2: Eric Wall Ltd. West Sussex

Eric Wall Ltd has been growing tomatoes under glass since 1977 at the nursery in Barnham, Nr Arundel, West Sussex. Biological control is an important and effective IPM technique that has been used at Eric Wall Ltd. for more than 30 years. Predatory insects are used to control pests in the glasshouses, with the most common pests being whitefly, red spider and leafminer. To control whitefly the *Encarsia* wasp is introduced; for the red spider, *Phytosseiulus* and for the leafminer, *Diglyphlus*. In addition to these a macrolophus insect is introduced which is a meat eater, to ensure populations of the predatory insects are maintained at a satisfactory level.

"The secret to our success has been the skill of the staff at our nursery – early detection of pests is essential in giving the predators every chance in controlling their numbers. There are over 60 miles of tomato plants and due to the scale of production we are reliant on the staff to feed back issues when they arise." Chris Wall, Eric Wall Ltd.

More recently Eric Wall Ltd. has been challenged with a new pest which is also new to the UK. As yet there is not a complete solution to controlling the 'Tuta Absoluta' moth without the use of some pesticides. In this instance a combination of trapping and the macrolophus insect provide good control and artificial pesticides are used to control certain hotspots when necessary.

CONCLUSIONS

LEAF Demonstration Farms such as those outlined in the case studies above are continually looking for the optimal combination of techniques to control the unique set of pest pressures faced on their farm. By combining the best of modern technology and traditional crop protection methods, alongside extensive knowledge of the issues faced on their farm, these farmers are able to effectively demonstrate the success of IPM through their innovative combinations of measures, Through sharing and demonstrating the success of this approach to others, the understanding and effective use of IPM can spread.

LEAF plays a key role in enabling the spread of IPM best practice, both through the demonstration farm network as well as offering multiple practical tools to help farmers step back and decide what would work best on their farm.

MANAGING SOIL TO PROMOTE PLANT DEFENCE AGAINST PESTS

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Summary: When a plant is attacked by an insect herbivore, volatile organic compounds (VOCs) are released, which repel insect pests and attract pest enemies. Common mycorrhizal networks (CMNs), which connect the roots of different individual plants, transfer defence signals between connected infested and uninfested plants, acting as an early warning system so that uninfested plants produce defence VOCs before attack. Here, we test the following hypotheses to establish if CMN-based signalling has potential in crop pest control. Firstly, how do tillage regimes impact CMNs and their ability to transfer defence signals? Secondly, how far can effective plant defence signals travel via a CMN? Finally, if connected to the same CMN, can different plant species transfer defence signals via CMNs, considering plant relatedness and aphid compatibility? We would appreciate feedback on if farmers could potentially utilise this information in terms of tillage practices, distance from field margins and intercropping combinations.

INTRODUCTION

Achieving food security in the 21st century and reducing the use of inorganic chemicals is a great challenge for farmers today. A possible tool is the utilisation of the plant's own defence mechanisms.

Plant-plant signalling could help achieve food security by minimising crop losses due to pests. Signalling induces a defence response in a plant when a neighbour has undergone mechanical damage (Karban et al. 2013; Song et al. 2015), is infected with fungal pathogens (Song et al. 2013) or infested with insect herbivores (Song et al. 2010; Babikova et al. 2013). A plant's induced defence response changes the emission of volatile organic compounds (VOCs), which can repel insect herbivores and attract the herbivore's natural enemies (Du et al. 1998; Bruce et al. 2008; Babikova et al. 2013), helping to regulate pest populations and reduce crop damage.

80% of land plants form mycorrhiza symbiosis, a bidirectional interaction between fungi and plants, previously understood only to transfer mineral nutrients. However, recent exciting research has shown that mycorrhizal fungi can also carry defence signals between different plants. This means that plants are warned of aphid presence and thus can produce VOCs to repel aphids and avoid an imminent attack (Babikova et al. 2013) (Figure 1.). This is possible because mycorrhizas of different individual plants fuse together (Giovannetti et al. 2001),

creating common mycorrhizal or mycelial networks (CMNs), which can transfer defence signals, although the identity of the signal(s) is unknown.

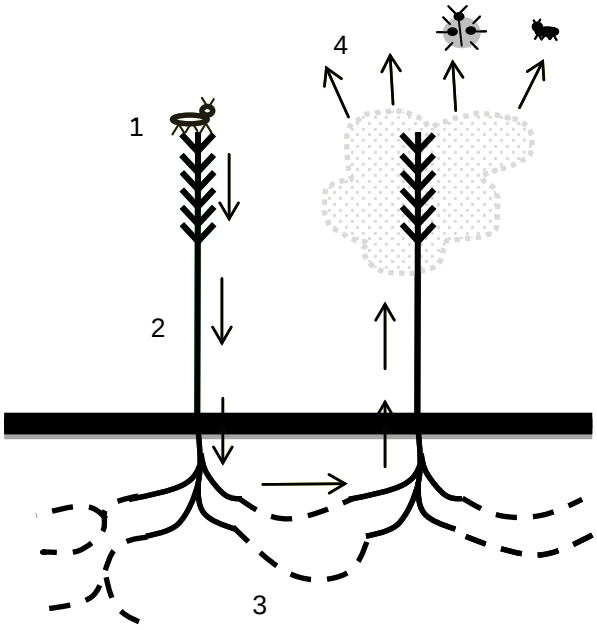

Figure 1. A diagram showing the mechanism of plant defence in response to aphid attack, using fungal networks as a conduit for warning signals, as found in Babikova et al. (2013). 1. Plant (black) suffers herbivory from aphids. 2. Plant activates defence response. 3. Fungi (dashed black line) receive signals that host plant is being attacked by aphids and induces defence signal in other host plants. 4. Other host plants emit similar VOCs to infested plant, which attract aphid natural enemies.

This PhD aims to test if the utilisation of this underground early warning system could be a useful tool for crop pest control, by answering the following three questions: 1. How do tillage regimes impact CMNs and their ability to transfer defence signals? 2. How far can effective plant defence signals travel via a CMN? 3. Can different plant species transfer defence signals via CMNs, considering plant relatedness and aphid compatibility?

HOW DO TILLAGE REGIMES IMPACT CMNS AND THEIR ABILITY TO TRANSFER DEFENCE SIGNALS?

Studies have shown that mycorrhizal colonisation of plants decreases with increasing tillage intensity (Carpenter-Boggs et al. 2003). Therefore, it is likely that CMN-based defence signal transfer could also be compromised. Firstly, tillage physically disturbs soil, which breaks up the network. Secondly, tillage could provide selective pressure for fungal species that are more tillage-tolerant, which may change the community composition of fungi (van den Bos, 2015) and might impact on their ability to transfer defence signals.

We aim to quantify and tease apart these two effects using a mesocosm experiment. Soil was sampled from two different tillage treatments, which will be used to create mesocosms and left for one year to mimic field conditions. Soil was either deeply (35cm) ploughed or minimally ploughed (shallow and non-inverted using disk ploughing). Minimal tillage was used as it was deemed a more likely soil management method for farmers to use rather than no tillage. Mesocosms will be then further tilled (disturbed) or not to form four treatments: 1. Minimal tilled soil left for one year and untilled for experiment, 2. Minimal tilled soil left for one year and tilled for experiment, 3. Tilled soil left for one year and untilled for experiment and 4. Tilled soil left for one year and tilled for experiment.

After the soil has been collected, mesocosms will be grown with barley (a crop grown in the fields from which the soil was taken) to mimic field conditions and left for one year to allow a CMN to form. Barley shoots will then be removed, leaving the root system and CMN intact. Treatments that require physical disturbance will be disturbed, and then all treatments will be planted with beans in mesh cores in the following design described in Figure 2.

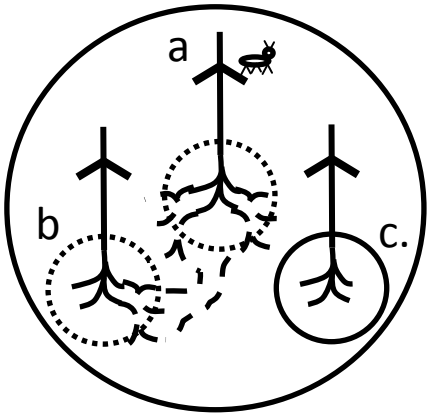

Figure 2. Mesocosm layout. Plant a. (termed 'donor' plant) will be planted in a 45µm mesh core (dotted line) to allow mycorrhizal penetration. Plant b. (termed the 'receiver') will also be planted in a 45µm mesh core to allow mycorrhizal penetration. This will allow a CMN (dashed line) to form between plants a. and b. Plant c. (termed the 'non-receiver') will be planted in a 0.5µm mesh core (solid line) to prevent mycorrhizal penetration and thus connection to the CMN so cannot receive a warning signal.

The donor plant (a.) will be infested with aphids and we predict the connected receiver plant (b.) to illicit a defence VOC response, while unconnected non-receiver plant (c.) will not change its VOCs. Under the hypothesis that tillage damages CMNs, we predict that treatments 3. and 4., that have been physically disturbed, will be less effective at transferring defence signals between plants than the undisturbed treatments 1. and 2. We predict the different fungal communities in the tilled and untilled fields will impact defence signal transfer, but in an unknown way.

To determine whether plants have undergone a defence response, VOCs will be collected from plant leaves three days after aphids were added to the donor. Pea aphids (*Acyrthosiphon pisum*) and a natural enemy parasitoid (*Aphidius ervi*) will then be placed in three-armed olfactometers

(choice chambers). We predict aphids will be repelled by the infested donor and connected recipient, and attracted to the unconnected non-recipient. The opposite effect is predicted for parasitoids. Additionally, mycorrhizal colonisation counts will be carried out, to ensure that any observed induced defence responses were due to CMNs.

HOW FAR CAN EFFECTIVE PLANT DEFENCE SIGNALS TRAVEL VIA A CMN?

Previous studies have indicated that CMNs can transfer defence signals up to 20cm (Song et al. 2010; Babikova et al. 2013). However, it is unknown if defence signals via a CMN could travel further, which have important implications in cropping densities and the distance the crop is away from a field margin. Additionally, it is unknown if a signal can be transferred via a plant connected to two different CMNs. Figure 3. explains the experimental design to address these gaps in our knowledge. All plants will be beans.

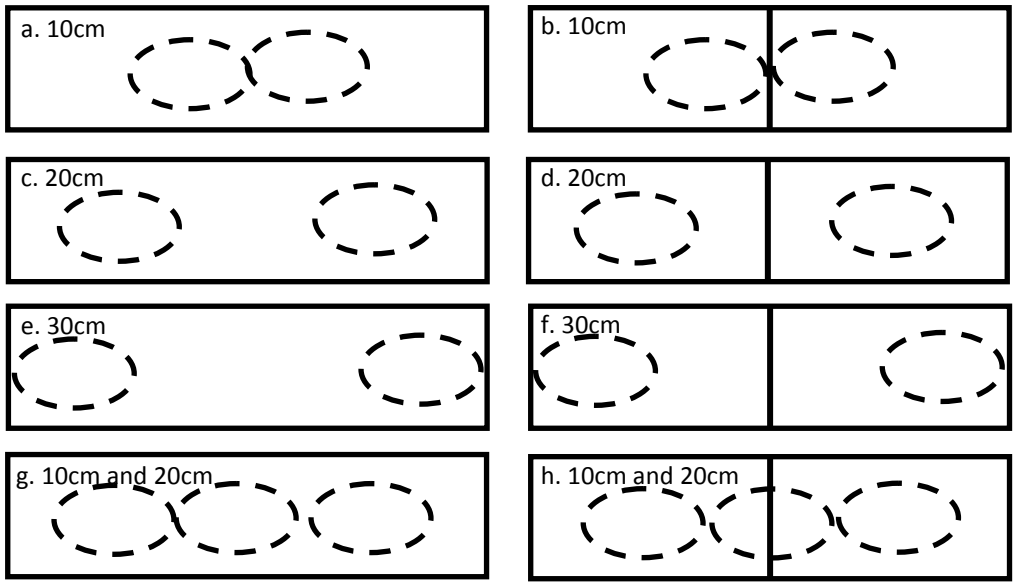

Figure 3. All plants in all treatments are in 45µm mesh cores to allow CMN formation, while not allowing the mingling of roots, so that any signals cannot be through root contact. The black line in treatments b., d., f. and h. symbolise 0.5µm mesh to prevent CMNs connecting experimental plants. Treatments a., c. and e. test if increasing distance influence defence signal effectiveness, with treatments b., d., f. and h. acting as controls. Treatment i. tests if a defence signal can be transferred via a plant connected to two different CMNs; the central plant has a split root system to test whether signals can pass from the left plant to the right plant via the central plant only (no CMN connection between the left and right plants), with g. as a control against a three-plant system.

It is predicted that with increased distance and more plants for the signal to transfer through, the defence signal will either not occur, become weaker or less effective.

Pea aphids will infest the plant on the far left of each mesocosm. Mycorrhizal colonisation counts will be undertaken, as well as oxidative enzyme abundances of harvested leaf samples, which is another indicator of a defence response. Oxidative enzyme abundance will be measured in all plants immediately prior to the addition of aphids and three days after.

CAN CMN-BASED DEFENCE SIGNALS TRANSFER BETWEEN DIFFERENT PLANT SPECIES?

To increase agricultural sustainability, crop diversification, rather than monocultures, is being encouraged. This could be beneficial for pest control, as growing a mixture of cultivars or species increases the diversity of genetic factors that alter defence compounds, like VOC emissions (Glinwood et al. 2009). This has implications for intercropping combinations and thus raises two further questions:

Is defence signalling via CMNs optimal in more related plants? The legume family host the same aphid species (pea aphids (*Acyrthosiphon pisum*)) and allows testing of the hypothesis that defence signal transfer via CMNs decreases with reduced relatedness. The same mesocosm set up will be used as described in Figure 2. All donor plants will be beans (*Vicia faba*) var. Sutton Dwarf. Both receivers will be the same as each other, except that one is connected to the donor via a CMN and one is not (Figure 2.). There will be four plant relatedness treatments to test whether signals can travel between plants: 1. The same cultivar (using beans (*Vicia faba*) var. Sutton Dwarf as receivers), testing intra-cultivar differences, 2. The same species but different cultivar (using beans (*Vicia faba*) var. Aquadulce as receivers), testing intra-species differences, 3. The same genus but different species (using vetch (*Vicia sativa* ssp *segetalis*) as receivers), testing intra-genus differences and 4. The same family but different genus (using peas (*Pisum sativum* var. Douce Provence) as receivers), testing intra-family differences.

Does sharing the same insect herbivore increase defence signal effectiveness? Legumes and cereal species host different aphid species, pea aphids and cereal aphids (*Sitobion avenae*), respectively, as they belong to different plant families. It is predicted that defence signal transfer is most efficient between plants which share the same insect herbivore species. The following treatments aim to test this: 1. Donor: Beans (*Vicia faba*), Receiver: Beans (*Vicia faba*), testing the same plant and aphid species, 2. Donor: Beans (*Vicia faba*), Receiver: Peas (*Pisum sativum*), testing a different plant species but the same aphid species, 3. Donor: Beans (*Vicia faba*), Receiver: Barley (*Hordeum vulgare*), testing a different plant and aphid species, 4. Donor: Barley (*Hordeum vulgare*), Receiver: Barley (*Hordeum vulgare*), testing the same plant and aphid species, 5. Donor: Barley (*Hordeum vulgare*), Receiver: Wheat (*Triticum aestivum*), testing a different plant species but the same aphid species and 6. Donor: Barley (*Hordeum vulgare*), Receiver: Beans (*Vicia faba*), testing a different plant and aphid species. Pea aphids and cereal aphids will be added to bean and barley donors, respectively.

As already described in previous questions, choice tests, oxidative enzymes, VOCs and mycorrhizal colonisation will be measured in these two experiments. However, for the second hypothesis, adult parasitoids and adult and larvae Coccinellidae (*Adalia bipunctata*) will be used in the choice tests, as they exhibit a different predatory style. These two studies could also help indicate which species to plant in field margins, which could act as a protective barrier around crops, although it is unknown how plant and indeed mycorrhizal diversity impacts defence signal transfer.

These experiments are the first step in testing if CMNs could be useful in crop pest control and crop and soil management. We welcome feedback on potential usefulness and feasibility.

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ESTABLISHING A PLATFORM FOR IPM IN IRISH CROPS

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Summary: Teagasc are to conduct a farmer survey to investigate perceptions of Integrated Pest Management (IPM) and quantity levels of IPM adoption across the Irish arable farming sector. This survey will provide insights into the decision processes taken at farm level regarding disease, weed and insect pest management. Once a baseline for IPM adoption has been established the goal is to use the knowledge gained to improve perception and adoption of IPM across the Ireland through targeting of research and dissemination activities. Selected IPM questions used in the survey will be asked of farmers in England and Scotland, via collaborations with Reading University and SRUC, allowing for the effects of demography and geography on perception of IPM and uptake of IPM techniques to be investigated.

INTRODUCTION

The EPIC Project

Integrated Pest Management (IPM) is the use of an optimal mix of pest (including weeds, diseases and insects) control techniques and tools, taking into account factors including profit, risk, sustainability, humans and environmental safety. EPIC (Establishing a Platform for the IPM in Irish Crops) aims to establish through detailed surveys the potential to increase adoption rates of IPM within Irish tillage and horticultural crops. This project looks to adopt, adapt and evaluate current decision support systems used in North Western Europe for a number of major cereal and horticultural pests and diseases. These specific case-studies (which focus on Ramularia leaf spot in barley, eyespot in winter wheat, late blight in potatoes and aphids in cereals and field vegetables) will determine the potential for current forecasting or risk based IPM strategies and the wider use of IPM on both tillage and horticultural crops in Ireland. These findings will be used to disseminate best practise to farm level through various mediums, including the production of crop specific IPM best practise guides. The ultimate goal of the project is to reduce the dependency of the Irish tillage sector on pesticides through the promotion of current and introduction of new IPM techniques from climatically similar areas of Europe.

MATERIALS AND METHODS

Teagasc farmer survey

In an effort to understand the current perception and adoption of IPM amongst Irish arable farmers a large survey will be conducted in 2016. The survey investigates all of the main IPM principles including a focus on pest prevention, monitoring, forecasting systems, cultural methods, anti-resistance strategies and continual evaluation of approach to pest management. This survey has two main functions; to allow for the adoption rate of specific IPM techniques to be quantified; and to provide insight into the perception of IPM amongst Irish farmers. The survey consists of approx. 40 short answer/multiple choice questions and should take 20-30mins to complete. The target of this particular survey will be broad acre crop growers (largely cereals, oilseeds and potatoes) however future sector specific surveys will target horticultural growers as well. The release of the survey will coincide with the launch of the Irish department of agriculture's new knowledge transfer discussion groups programme. The survey will also be distributed via other means including farming press websites. Data from online and hard copies of the completed survey will be collated and analysed using descriptive and parametric statistical measures. Semi-structured interviews, with selected individuals representative of the farming community, will further delve into the thought process behind IPM related decision making allowing for conclusions to be drawn on the effects of demography and geography etc. on approach to IPM.

Collaborative survey with the UK

A survey will be conducted in collaboration with research institutes and farmers groups from England and Scotland. The agreed 'core IPM questions' relate to the main IPM principles and will focus on adoption of IPM. These questions may be included in a larger survey or as a complete survey by themselves. All data will be collated and analysed together. The goal of this collaboration is to improve perception and adoption of IPM across the British Isles.

ACKNOWLEDGEMENTS

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WHERE HAVE ALL THE RABBITS GONE?

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Summary: Wild rabbits have been an integral part of the wildlife in the UK for many years and been both a pest and a source of food for a range of our iconic predators. This paper gives case studies of a decline in wild rabbit numbers in recent years which mirror a similar decline in a national trend that have seen numbers in parts of Scotland decimated and coincides with the introduction of Rabbit Haemorrhagic Disease (RHDV) in 1994. The implications of the reduction in numbers, gained from the previous outbreak of myxomatosis, are that numbers of predators will decrease e.g. buzzards and that rabbits will become less of an agricultural pest. However evidence from both Australia and New Zealand would suggest that resistance to RHDV will increase and rabbit numbers will again increase.

INTRODUCTION

The European wild rabbit (*Oryctolagus cuniculus*) was probably introduced into Britain about 1000 years ago and kept in warrens for food (Sheail, 1971). These rabbits escaped and became a serious pest in many part of Britain including Scotland (Kolb, 1994) where it was estimated to have caused damage worth an estimated £38m. Myxomatosis became established in southern England in the autumn of 1953 and rapidly spread to other parts of Britain in 1954 where it reportedly killed 99% of naïve rabbits (Thomson & Worden, 1956). However the myxoma virus soon became a less virulent virus (Hudson & Mansi, 1955) and rabbits developed resistance (Ross & Sanders, 1987) so that numbers increased to an estimated third of pre-myxomatosis levels by the early 1990s (Flowerdew *et al*., 1992). A more recent recent study of the epidemiology of the myxomatosis showed it did not now re-occur on an annual basis and that its impact on rabbit numbers were likely to be minimal (Boag *et al*., 2013). In 1997 rabbit numbers in Scotland were estimated to have reached a peak of 9,500,000 (Battersby, 2005). However in 1994 a new rabbit virus, Rabbit Haemorrhagic Disease Virus (RHDV), became established in Britain (Chasey *et al.,* 1997) and spread patchily across the country possibly influenced by the presence of a non-pathogenic strain which could impart immunity (Forrester *et al*., 2006) and could be implicated in reducing rabbit numbers especially in Scotland (Battersby 2005). The possible protection imparted by the presence of a non-pathogenic strain of RHDV may now have been compromised by the introduction of a new strain of pathogenic RHDV (Bailey *et al*., 2014) which could explain the reduction in rabbit numbers in Scotland from 1995 to 2009 of -93 % in intermediate upland/islands and -84% and -76% in upland and lowlands respectively (Aebischer *et al*., 2012). After the

introduction of myxomatosis into Great Britain predators e.g. stoats and buzzards declined (Sumption & Flowerdew, 1985) while brown hare numbers increased (Flowerdew *et al.,* 1992).

This paper highlights the reduction of rabbit numbers across five sites in Scotland and northern England and compares these with the national trends in rabbit numbers and farms where rabbits were considered a pest. The implications of significantly fewer rabbits on agricultural production and wildlife are also discussed.

MATERIAL AND METHODS

The data given in this paper is based on either the number of rabbits published by the Game Conservation Trust in their Gamebag Census and those mainly shot by the senior author when undertaking a project into the population dynamics of rabbit parasites and the interactions between parasites and other diseases (Boag, 1972; Boag *et al*., 2013; Lello *et al*., 2004).

The rabbits at Pitroddie were collected monthly as part of a 38 year long term study (Boag *et al.,* 2013). A census of rabbit numbers at this farm was undertaken on 11 occasions by lamping rabbits c. one hour after dusk (Greenwood, 1996) and the data collected from the census was used to confirm the trends in numbers of rabbits shot.
Gamebag records have also been shown to relatively accurately reflect actual populations size (Boag , 1987; Tapper, 1977) and used in this paper to highlight trends in rabbit numbers at the other sites. They are also the basis upon which the Game Conservancy Trust published the trends in rabbit numbers in Scotland (Aebischer *et al*., 2012).

RESULTS

Rabbit populations have not in the past been subject to annual, objective, focussed monitoring schemes as are many animals are e.g. bats probably because they, as a species, are not under threat and are considered a pest. Results from the 5 sites shown in Table 1 all show a decline in rabbit numbers even though the hunting effort was relatively constant at each site. The decline occurred at different dates at different sites e.g. on the Isle of Coll it happened in 2006 while at Pitroddie farm it started in 2013.

Further analysis of the data from Pitroddie farm (Table 2), where animals were collected monthly, shows that there was a similar marked decline in the months where rabbits were counted by lamping and those shot.

The data from the Game Conservancy Trust, which only goes up to 2009, shows statistically significant declines in rabbit numbers in all three regions of Scotland. However numbers were still higher in 2009 than 1961 which was only 7 years after myxomatosis became widespread in Scotland.

Data from farmers included in a survey for the Science and Advice for Scotland Agriculture (SASA) indicate that in 2000 36.1% farmers ranked rabbits as their most serious pest but in 2010 only 17.4% considered them as their most serious pest.

Table 1. Number of rabbits collected annually at five sites in Scotland and Northern England.

Isle of Coll		Hutton Institute		Pairney Farm		Coldstone Farm		Pitroddie Farm	
Year	Number	Year	Number	Year	Number	Year	Number	Year	Number
1985	42	1990	39	2000	20	2004	20	2003	185
1999	51	2008	34	2008	13	2005	12	2004	233
2001	50	2009	133	2009	2	2006	28	2005	256
2002	21	2010	44	2010	0	2007	56	2006	258
2004	40	2011	28			2008	11	2007	266
2006	15	2012	30			2009	9	2008	297
2007	9	2013	18			2010	6	2009	312
2008	10	2014	3			2011	1	2010	207
2010	9	2015	3			2012	0	2011	176
2011	9					2013	3	2012	143
2012	3					2014	0	2013	88
2013	15							2014	45
2014	5							2015	15

Table 2. Number of rabbits shot and counted by lamping at Pitroddie farm.

Month/Year	Dec 2007- Mar, Jun, Sept 2008	Dec 2008 – Mar, Jun, Sept 2009	Dec 2009 – Jun, Sept 2010	Sept, Dec 2014 – Mar, Jun 2015
Rabbit numbers shot	100	100	65	9
Rabbit numbers lamped	613	542	540	17

DISCUSSION

Tests have yet to be undertaken to ascertain if the new stain of RHDV (variant2) reported by Bailey *et al*., in 2014 has caused the recent decline at Pitroddie farm. It is interesting to note that reports show resistance to RHDV occurs in young rabbits less than two months old (Abrantes *et al*., 2012) but at Pitroddie farm only one of the 17 rabbits collected up till September in 2015 was born in 2015, the rest were born in 2014 or earlier. The reason for this is unclear but increased predator pressure focused on the few young rabbits born in 2015 from the existing large population of predators which existed when rabbits were more plentiful may explain the low numbers of young rabbits in 2015. Data from New Zealand, where RHDV was illegally introduced in 1997, has shown that although the disease was an effective biocontrol

agent its efficacy has waned possibly due to changes in immunity to the disease (Parkes *et al*., 2002).

Table 3. Game Conservancy Trust data on the percentage changes in rabbit numbers over time in Scotland

Area	Sites	Changes (%) 1961-2009	Changes (%) 1995-2009
Lowlands	102	+1428*	-76*
Intermediate uplands/islands	56	+97	-93*
True uplands	383	+343*	-84*

*Significant at P< 0.05

Since RHDV was introduced into Australia in 1996 it has also been shown to adapt and persist (Schwensow *et al*., 2014) and, unlike myxomatosis, the virulence of the disease has been reported to be increasing as the genetic resistance in the rabbit population has also increased (Elsworth *et al*., 2014).

Rabbits have been an integral part of the countryside in much of Britain for a long time having initially been a source of food for humans, then becoming a pest and a source of food for a number of our iconic wildlife species e.g. buzzard, fox, stoats etc. (Sheail, 1971). They also prefer and maintain short swards (Bhadresa, 1977; Iason *et al*., 2002) which had benefited some rare wild plants and insects e.g. the Adonis blue butterfly (*Polyommatus bellagus*) (O'Connor *et al*., 2014) . However heavily grazed grass can be replaced by moss and lichens (Fenton, 1940) which makes the soil prone to erosion (van Nierop & van der Meijden, 1984). The demise of rabbits after myxomatosis led to the increase a range of plants including mouse eared hawkweed (*Hieracium pilosella*) (Bishop & Davy, 1984) and juniper (*Juniperus communis*) (Thomas, 1960).

The recent decline in rabbit numbers could have a significant impact on both wildlife and agricultural productivity in Scotland. Aebischer *et al*., (2012) estimated the numbers of rabbits in Scotland to be 9,500,000 in 1995. If the trends in the decline of numbers seen between 1995-2009 (Table 3) continued to 2015 then rabbit numbers in Scotland will now probably be well below 1,000,000. The impact of such a decline could be similar to that seen after myxomatosis where predator numbers e.g. buzzard (*Buteo buteo*) stoat (*Mustela erminea*) and fox (*Vulpes vulpes*) fell (Sumption & Flowerdew, 1985) and brown hare (*Lepus europeaus*) numbers increased (Barnes & Tapper, 1986). There is evidence that the recent decrease in rabbit numbers may have already led to a downward trend in predators in Scotland as buzzard numbers have decreased in Scotland since 1998 especially between 2003 and 2008 when numbers fell by 17% (Baille *et al*., 2010). The beneficial impact of fewer rabbits on agricultural production was seen after myxomatosis (Thomas, 1960) but by 1986 Mills (1986) estimated that rabbits in the UK were back to 20% of pre-myxomatosis numbers and costing farmers over £100m. The costs in Scotland of rabbit damage to crops and of controlling them is

unknown but probably runs into £ms. The fact that the percentage of farmers reporting rabbits as a major pest had halved between 2000 and 2010 also suggests that the damage caused by rabbits has fallen significantly.

In the future it is likely that resistance to RHDV will again lead to an increase in rabbit populations but experience from Australia and New Zealand would indicate this may take many years. Meanwhile farmers who have had a serious rabbit problem in the past may be wise to exploit the present low numbers by continuing control programmes so that numbers do not, as predicted, rise in the future to damaging proportions.

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HOW DIVERSE CAN CROP MIXTURES BE IN AN INTEGRATED PEST MANAGEMENT CONTEXT?

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Summary: Mixtures can range from near-isogenic lines, through cultivars to similar species (e.g. cereals) to very different species such as cereals and legumes. Components can be of very different proportions and contribute different benefits to the overall crop. Disease control is only one of these and rarely determines the final choice. Components that can be included depend on the end-use and the end-user. We give some examples of current work looking at this diversity spectrum and deployment in an IPM context.

INTRODUCTION

In our work on mixtures in the context of northern Britain, we first looked at crop mixtures of spring barley cultivars for malting, demonstrating yield gains, disease reductions and maintenance of good malting quality (Newton *et al*., 2009). Whilst maltsters will not accept mixtures even with such evidence, in conjunction with SRUC, spring barley mixtures have been demonstrated to have continued relevance in the small feed market. We demonstrated winter wheat mixtures for distilling, again showing small yield benefits, disease reduction and maintenance of quality, and these were used commercially for a few years. Winter barley mixtures show greater benefits in yield increase and disease reduction and we have previously reported their robustness over different fertiliser inputs, cultivation methods, component numbers and homogeneity of mixing. However, all these have been mixtures of equal proportions of mostly elite cultivars within species or crop types available to farmers such as malting 2-row spring barleys. The similarity of these cultivars imposes strong constraints on the synergistic interactions that mixtures exploit to the benefit of the whole crop. We know from ecology that the more diverse the traits of vegetation, the greater the opportunities for complementarity and facilitation between plant types (Brooker *et al*., 2015). The net result is greater ecosystem productivity, i.e. more biomass. How much can we apply this to crops?

We report examples of how we have started to investigate this from both theoretical and practical perspectives. Firstly, using spring barley we have examined: 1) how particularly small proportions of component cultivars contribute to the overall effect of the mixture; 2) highly contrasting Scots bere accessions mixed with elite spring barley; 3) different canopy types mixtures to look at resource capture; and 4) the effect of mixtures on other species in the

canopy, i.e. weeds. Secondly, with winter barley we investigated 5) the interaction of different straw biomass types on both grain and straw production. Finally, we recently started investigating: 6) how different crop species interact, for either biomass (for anaerobic digestion) or silage use, and 7) for different grain uses following separation of components in cereal-legume interactions. Finally, 8) we discuss the potential uptake, benefits and constraints of such mixtures in the context of integrated Pest Management (IPM).

MATERIALS AND METHODS

Field trials were carried out using standard agronomic protocols wherever practical and compromise treatments were used, where mixture components had different requirements such as for nitrogen or herbicides. Sowing rates were tailored to specific experimental aims as were harvest methods and assessments. Likewise appropriate design and analysis protocols were applied to each experiment detailed in relevant publications for each. Brief details are indicated as appropriate by the respective representative results below.

RESULTS

1) Proportions of component cultivars in mixture

Three series of spring barley mixtures pairs were sown where component 1 comprised 0, 5, 10, 20, 30, 50, 70, 80, 90, 95 and 100% by seed number and component 2 made up the missing proportion to 100%. The trial comprised three replicates and standard disease assessments and showed disproportionate disease reduction at component proportion extremes (Fig 1).

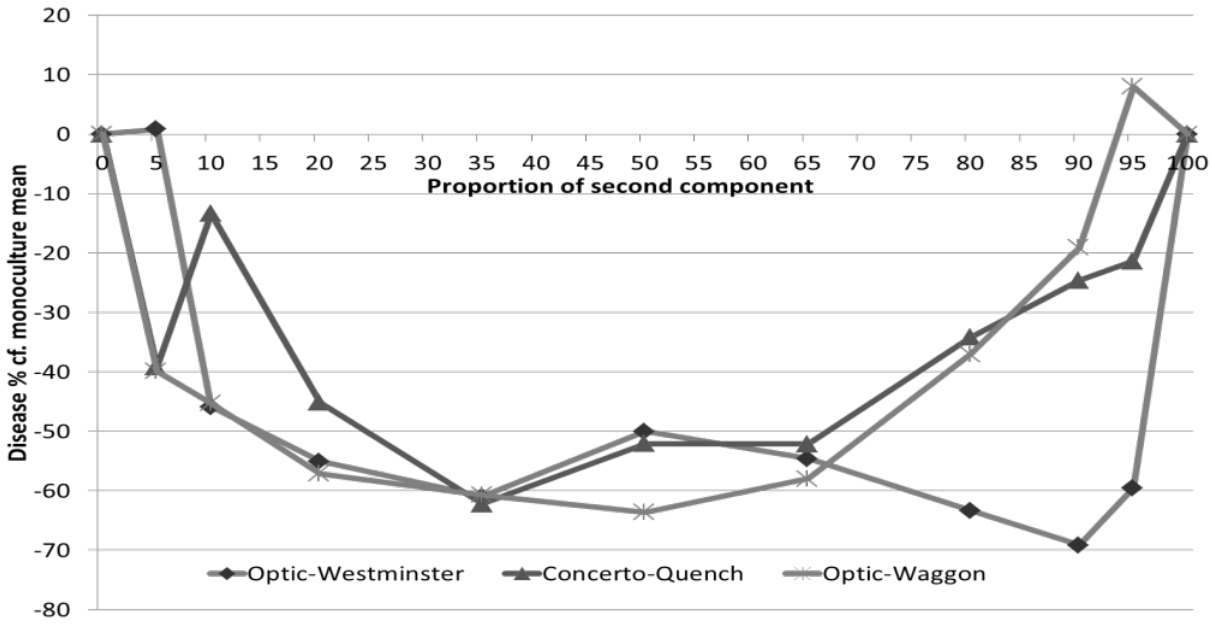

Figure 1. Disease (Rhynchosporium) reduction compared with monoculture mean in mixtures of pairs of spring barley cultivars in a range of proportions.

2) Scots bere - elite spring barley mixtures

Some single genotype lines from Scots bere populations were mixed in equal proportions with elite spring barley lines in three replicate trials at two contrasting sites, one a continuous barley site at Mylnefield, and the other at Balruddery (Fig 2). The bere lines were tall and matured about three weeks earlier than the elite lines and ear shedding and lodging were problematic with several lines. Where lodging did not occur (e.g. bere112) the yield was above the mean of the components but the amount showed interaction between the site and the elite genotype. Where lodging was a problem (e.g. bere119) the mixtures were not beneficial.

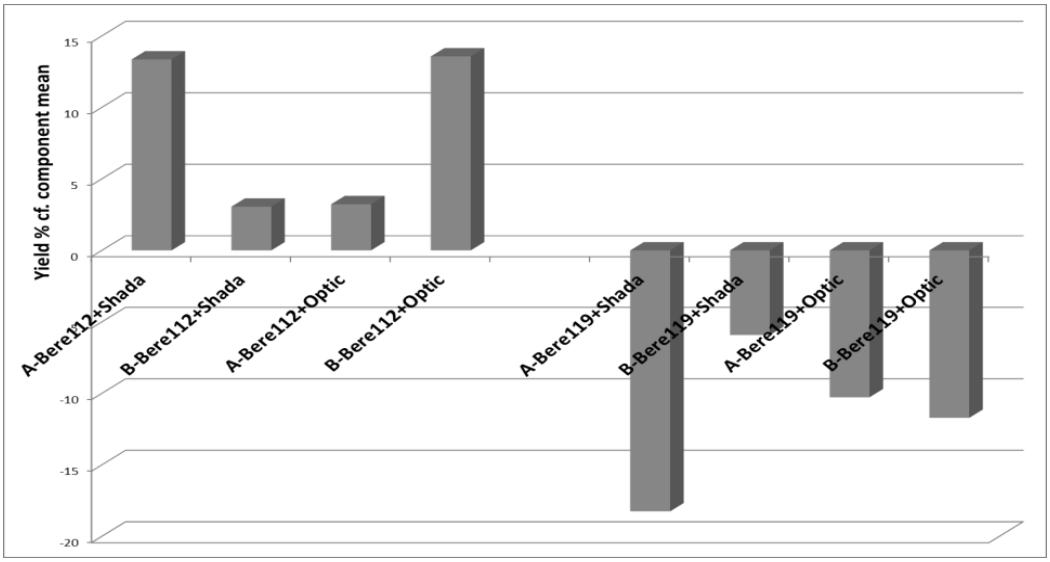

Figure 2. Yield of mixtures of Scots bere (lines 112 and 119) and elite barley (Shada or Optic) compared with component means. A and B indicate different field sites.

3) Canopy type mixtures

Two series of contrasting canopy types were selected from a bi-parental mapping population segregating for two semi-dwarfing genes: dwarf (D) expressing both genes, semi-prostrate (S) expressing the *sdw1/denso* gene, erectoid (E) expressing the *ari-e.GP* gene and tall (T) expressing neither gene in 2-, 3- and 4-component mixtures in a three replicate trial and assessed for disease and yield. The mean of the 2- and 3-component groups was greater than the mean of the components grown in monoculture (Fig 3) although the only disease, powdery mildew, was not significantly reduced in this trial.

4) Effect of mixtures on weeds

A three replicate trial of five cultivars of spring barley, a mixture of all five, and no barley was sown at three densities, 120, 220 and 320 seed m^{-1} but otherwise standard conditions. In both early and late July the plots were all assessed for weed composition. One of the rarer arable weeds , *Valerianella rimosa*, showed differential behaviour, as more individuals grew in the plots with barley than those without, particularly at low barley density, and at this density the

mixture had most *V. rimosa* plants (Fig 4). However, plants were smaller and had less total biomass in the crop (data not shown).

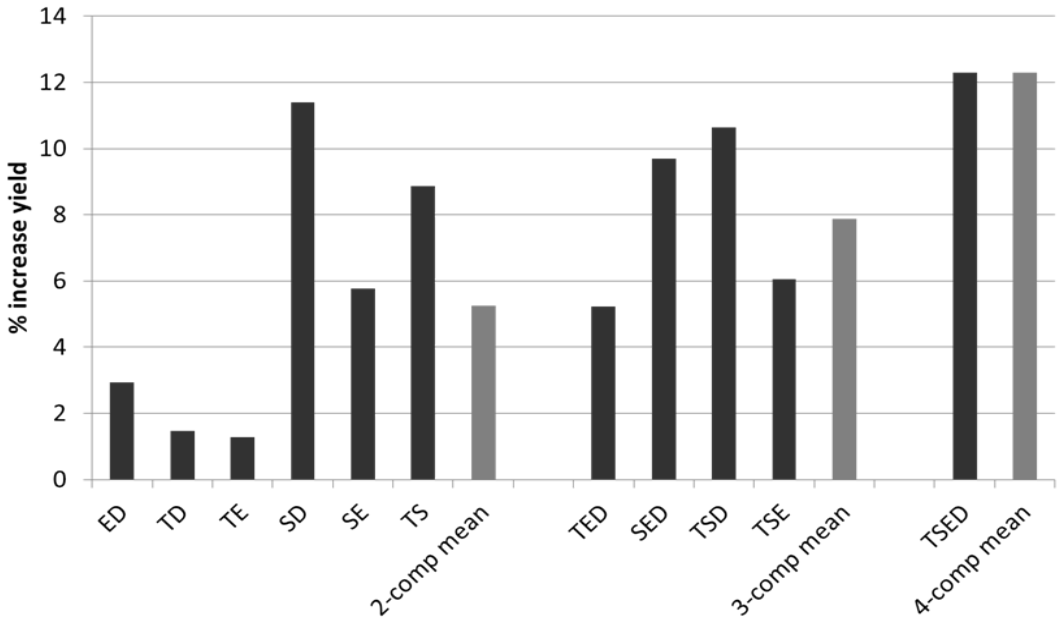

Figure 3. Effect of mixing spring barley cultivars with contrasting canopy types: dwarf (D), semi-prostrate (S), erect (E) and tall (T) in 2-, 3- and a 4-component mixtures on yield.

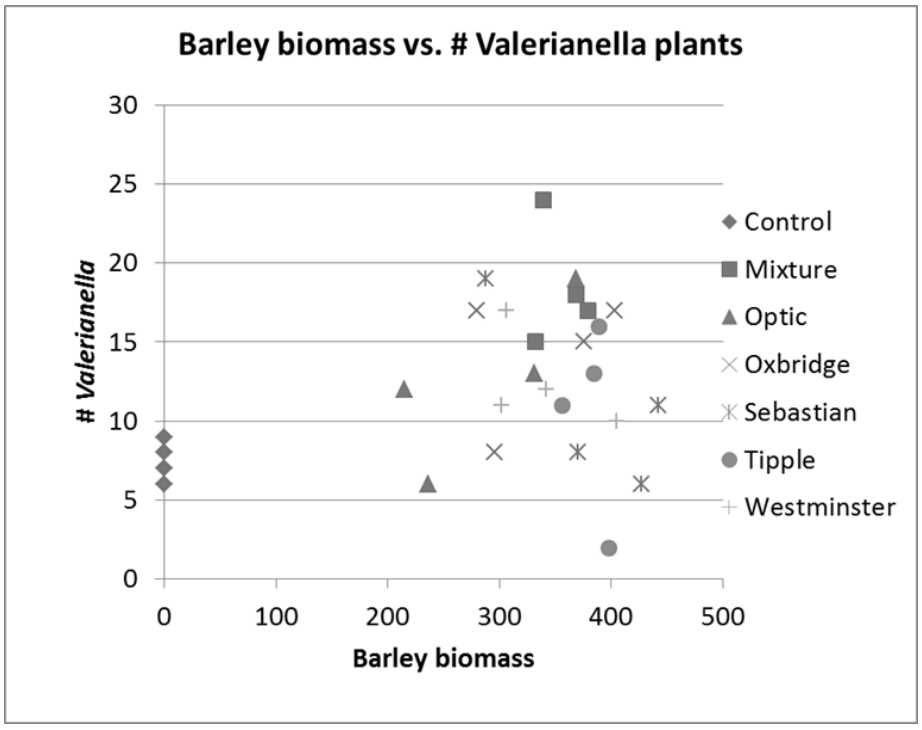

Figure 4. Effect of spring barley cultivar and a mixture on growth of a rare weed, *Valerianella rimosa*.

5) Interaction of different straw biomass types on both grain and straw production

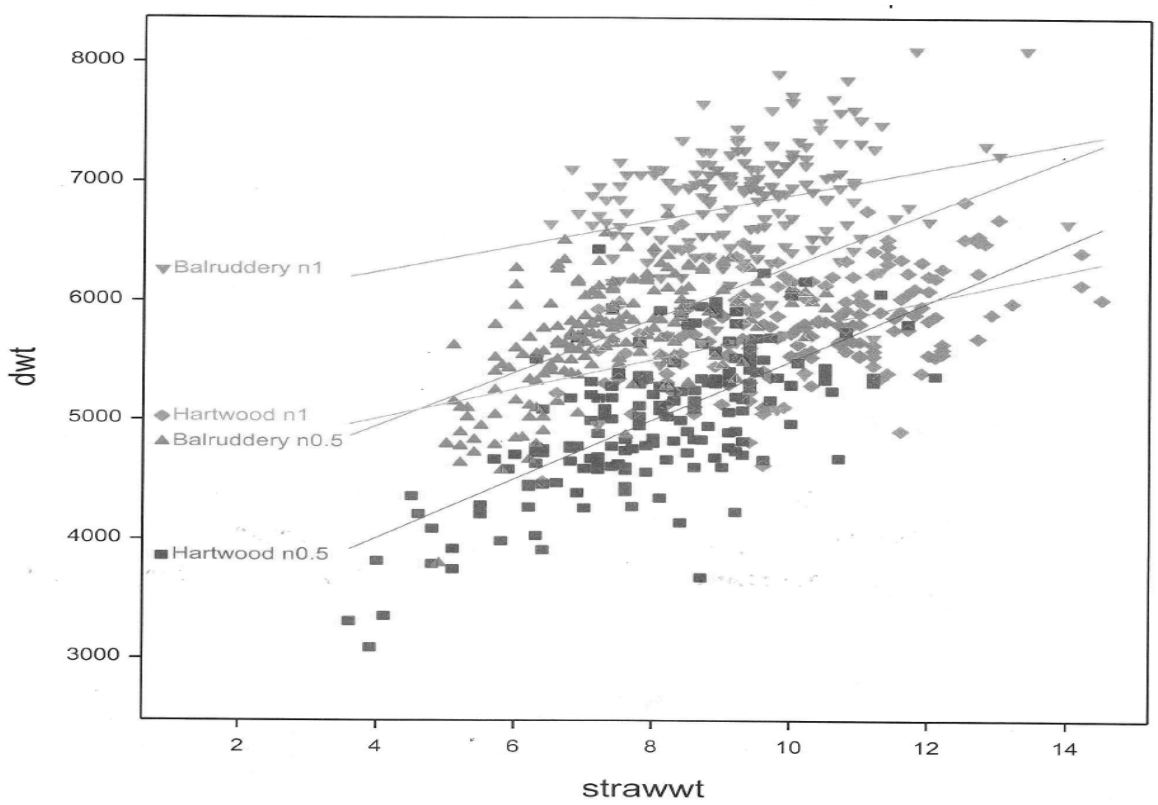

Figure 5. Relationship between grain weight (dwt) and straw weight (straw wt) in winter barley mixtures and their interaction with site (Balruddery or Hartwood) and nitrogen (n0.5 or n1).

Six winter barley cultivars selected for their contrasting amounts of straw production were grown singly and in all 2-, 3-, 4- and 5-component equal proportion combinations at a standard arable site, Balruddery Farm in Perthshire, and a wetter predominantly grassland site at Hartwood Home Farm in Lanarkshire. Two nitrogen fertiliser rates were used, half (n0.5) and full (n1) with respect to the normal rates for winter barley agronomy. Disease was reduced and grain weight increased in mixtures compared with monocultures as expected from previous work. Overall straw weight was not significantly increased in mixtures and analysis of different straw production type combinations is in progress. However, overall the effect of higher nitrogen rate was to increase grain more than straw yield at low total straw weights whilst making little difference at high straw weights (Fig 5).

6) Different crop species mixtures for biomass

Wheat, barley, oats, rye and triticale were grown singly and in various combinations either by themselves or with peas or beans at approximately 10%, grown as winter crops and cut and baled for use as silage. These were grown at a standard arable site, Balruddery Farm in Perthshire, and a wetter predominantly grassland site at Hartwood Home Farm in Lanarkshire. Three nitrogen fertiliser rates were used, zero (N0), half (N0.5) and full (N1) with respect to the normal rates for winter barley agronomy. The peas established very poorly, especially at Hartwood where few survived, but the beans grew well. Whilst fresh weights of crops of varying composition are difficult to analyse, some interactions can be noted. The Balruddery site appeared to be more responsive than Hartwood to the high nitrogen treatment and the

legumes were less responsive at Hartwood at the half-rate nitrogen (Fig 6), probably confounded by their poor establishment.

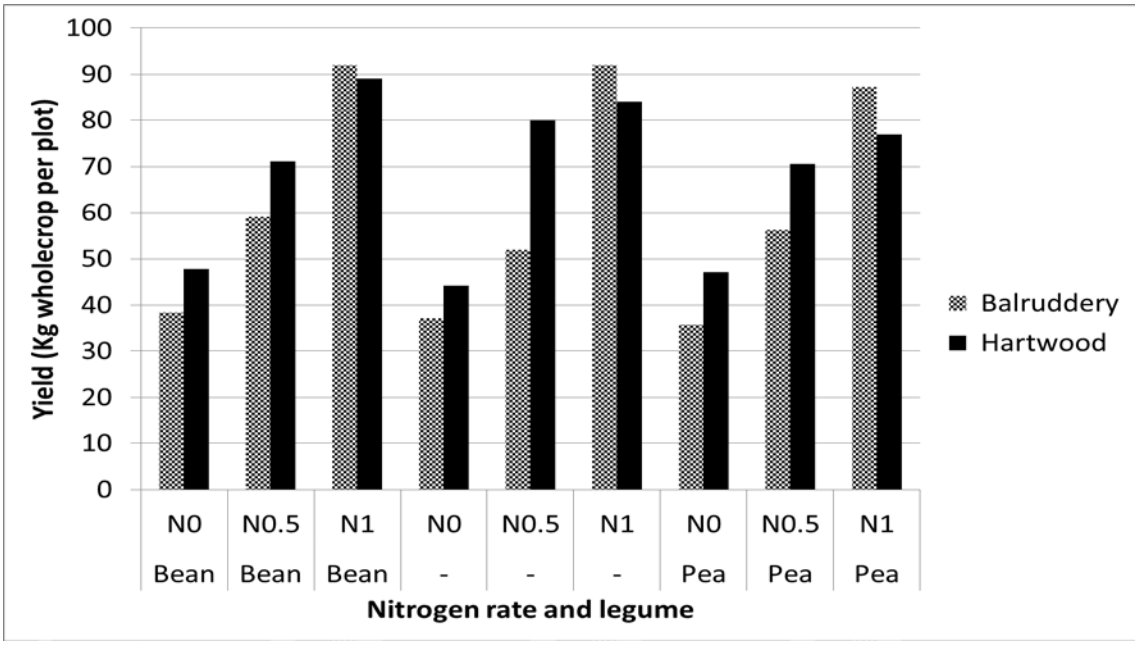

Figure 6. Mean whole-crop fresh weight biomass for cereal-cereal and cereal-bean or cereal-pea mixtures at different nitrogen rates at two sites, Balruddery and Hartwood.

7) Different crop species mixtures for grain

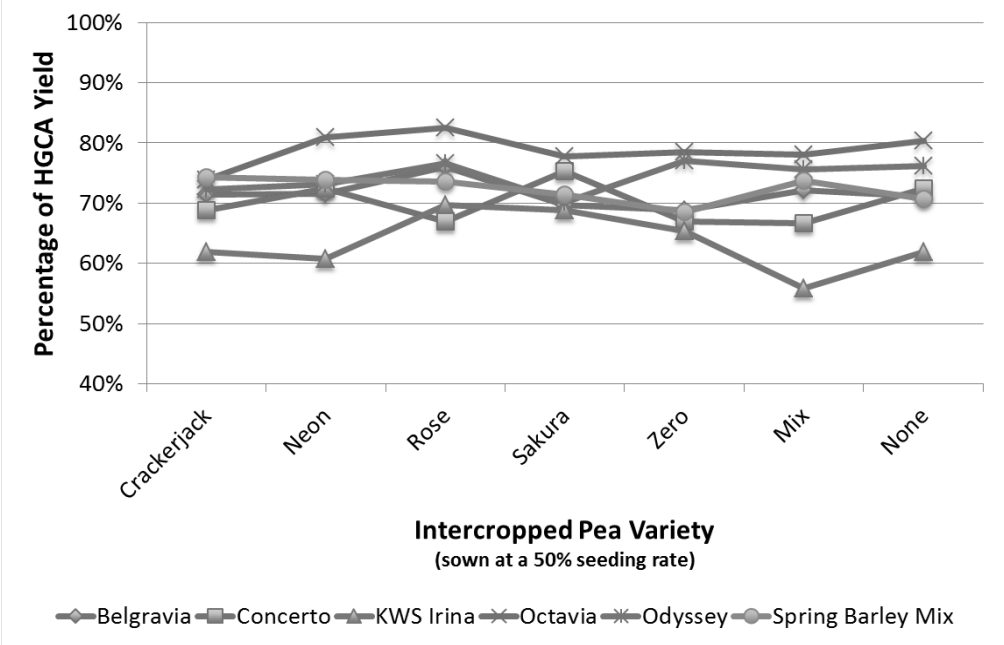

Figure 7. Yield performance of different spring barley cultivars mixed with different pea cultivars at 50% sowing rate and no nitrogen fertiliser compared with untreated AHDB published yields (100% seeding rate).

Peas and spring barley were mixed, harvested at maturity and the seed separated by size. Five different pea cultivars, five different barley cultivars and a five component mixture of each were sown in mixtures using 50% of their normal sowing rate and no nitrogen fertiliser. The Land Equivalent Ratio (LER), cost and profit from production were calculated assuming malting quality for the barley. Clearly some spring barley cultivars were more suitable than others for mixing with peas at this density, and similarly pea cultivars interacted differentially (Fig 7). On average both LER and profitability were reduced by 21% compared with equivalent monocultures.

8) Uptake, benefits and constraints of the use of mixtures in integrated Pest Management

For a survey we recently listed 35 IPM options for barley, classified as seed bed, mechanical/growing medium, cropping/biological control, management, and input efficiency. "Use of mixtures where markets allow" was just one of these and it did not feature amongst the options surveyed amongst growers and people in the barley supply chain. This is probably because of the resistance of some markets, the prevalence of highest yield as a cultivar choice driver and the perceived difficulties of their use on farm. However, from basic principles and on-going experimentation we show that for many of the 35 option, alone they may have a small gain but in the context of IPM these compound to increase efficiency and resilience of the whole crop. Furthermore, combination of many of these options needs to take into account inter-dependency. In the case of mixtures this could influence component choice, for example facilitating use of particularly advantageous resistance or weed-competitive trait in small proportions where the cultivar expressing the resistance has other less desirable traits. They may facilitate reduced dose fungicides as disease builds-up at a reduced rate in mixtures and they are more effective at reduced disease pressures. They may not be compatible with some elicitor-based crop protectants but this has yet to be demonstrated. Both cereal species and cereal-legume mixtures present several agronomic challenges especially with compatible agrochemicals, but tackled in an IPM context there are synergies to be exploited especially in the weed competition and nutrition areas.

DISCUSSION

There are many advantages to be exploited from diverse mixtures, but also many practical issues limiting their use in practice. The proportions trial demonstrated that disproportionately large disease reduction can be gained from including small proportions of component cultivars, showing the potential for reducing disease in otherwise preferred cultivars without reducing their dominance in a mixture (Fig 1). This effect is supported by theory in modelling mixtures. However, whilst very diverse components can be successfully mixed to give benefits sometimes, some traits such as lodging or too wide a maturity window can be too problematic (Fig 2). Other diverse traits such as canopy morphology offer more potential to both yield (Fig 3) and powdery mildew control (not shown). Mixtures can also have potential benefits to wider ecosystem services such as biodiversity as the survival of more individuals of a rare weed species in the barley (Fig 4) shows how the crop itself is important for maintaining biodiversity of a wide range of functional types of weed, a balance that would not be achieved in just the crop field margins. That the mixture seemed to perform best in this respect at low density is likely to be due to properties of the combined canopy architecture, indicating the potential for designing mixtures as part of a biodiversity strategy. Mixtures may be beneficial also for contrasting crop uses on some sites (Fig 5, 6, 7). There is potential to optimise straw as well as

grain yield in mixtures but to draw robust conclusions from this and the whole-crop biomass mixtures more analysis needs to be done and establishment of the legumes in all mixtures and sites needs to be improved. Nevertheless, there appears to be good response to nitrogen inputs and potential for optimising the interaction of fertiliser and legume-derived nitrogen. For seed / grain use the pea-barley for grain/seed mixtures were still very profitable when grown under no nitrogen inputs but both profitability and LER were reduced compared with the mean of their monoculture components. Quality assessments are still in progress so agronomic changes may have to be made to achieve market specification. However, there was variation in species compatibility for mixing and varying both sowing rates and nitrogen inputs have considerable potential for optimising the interaction. This will facilitate crop design for low input and organic situations where nitrogen additions need to be reduced. The potential for diverse design in mixtures for different purposes is therefore great.

Crop mixtures will not replace use of single cultivars and will often not compare favourably with the best elite cultivar in any one year and location. This is similar to novel crop protection approaches using resistance elicitors that do not have the same efficacy as fungicides but can be used very effectively in an IPM context. Mixtures offer stability or resilience as well as performance that normally exceeds the mean of the component species or cultivars. There is considerable unrealised potential for optimising their agronomy and reducing inputs. Good choice of these component cultivars is the key to consistent benefits, but so is combination with the many other options that comprise a good IPM strategy. Mixture design tools and principles are needed in this context and work is continuing to derive these in international collaborations with research groups, breeders and extension services.

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GROWING CROPS FOR ANAEROBIC DIGESTION – NEW OPPORTUNITIES AND NEW CROPS FOR ARABLE FARMERS

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Summary: SRUC present novel crop trials for Anaerobic Digestion (AD). Arable farmers seek new crops and markets to maintain viability. World grain prices are depressed while CAP subsidy payments are falling and CAP greening requires greater crop diversity. SRUC are currently trialing novel crops for use in AD on a range of sites across Scotland supported by the Scottish Biofuels Programme and Scottish Government. Alongside assessing yield, agronomy, biogas yields, costs and returns of the different crops SRUC are also researching the practicalities of fitting these new crops in to existing rotations. SRUC trials demonstrate that winter rye has high and consistent dry matter yields, energy beet has been less consistent while grass and legume mixtures offer lower input and lower output potential for less favourable sites. Energy beet and winter rye performed well at low nitrogen levels. Future work will explore legumes ability to reduce nitrogen fertiliser use and improve sustainability.

INTRODUCTION

Scottish arable farmers badly need to find new crops and markets to maintain viability. World grain prices are depressed following three large harvests while changes to CAP subsidy payments are set to lower subsidy income. According to a Scottish Government Direct Payments Update in February 2014 cereal farmers in Scotland will lose €19m per year under the new CAP scheme which commenced in 1 January 2015. CAP greening rules also require farmers to widen their range of crops to boost biodiversity. Arable farmers are also increasingly seeking ways to increase organic matter and improve nutrient recycling. At the same time the UK and Scotland are supporting increases in renewable heat and electricity production supported by Feed in Tariffs for electricity and Renewable Heat Incentive payments for renewable heat and biomethane injection in to the gas grid. Since October 2015, to be eligible for incentive payments feedstocks used in AD production must meet sustainability requirements including limits on carbon emissions. Renewable incentives have spurred the development of a growing number of crop based AD plants across Scotland creating a growing (local) market for energy crops.

ESSENTIALS OF ANAEROBIC DIGESTION

Anaerobic digestion (AD) uses microbes to convert organic matter into methane and carbon dioxide (referred to as "biogas"), in the absence of oxygen. The biogas can be used to provide heat, generate electricity or upgraded to biomethane (carbon dioxide is removed) for injection into the gas grid. In addition AD can provide other benefits; utilising wastes, replacing synthetic fertiliser with recycled nutrients in digestate, reducing emissions of methane, cutting odours and enhancing water quality.

Feedstock can be any biodegradable non woody plant, animal matter (manures/slurry), energy crops (such as grass silage, rye or maize silage and energy beet) and food waste. Whatever the feedstock used, it is important that the mix is kept relatively consistent and that the balance of carbon to nitrogen is suitable for the microbes. Extensive testing of different crop feedstocks in Germany reveals that in terms of total energy yield per hectare selection of crops can be made almost entirely on dry matter production. When it comes to determining the optimal mix for a specific AD plant a much wider range of characteristics must be considered in candidate crops. First amongst these are residence time; the length of time that the crop remains undigested in the primary digestion tank. It also essential to keep the carbon: nitrogen balance in the feedstock at around 30 which can limit the use of legumes in the feedstock mix.

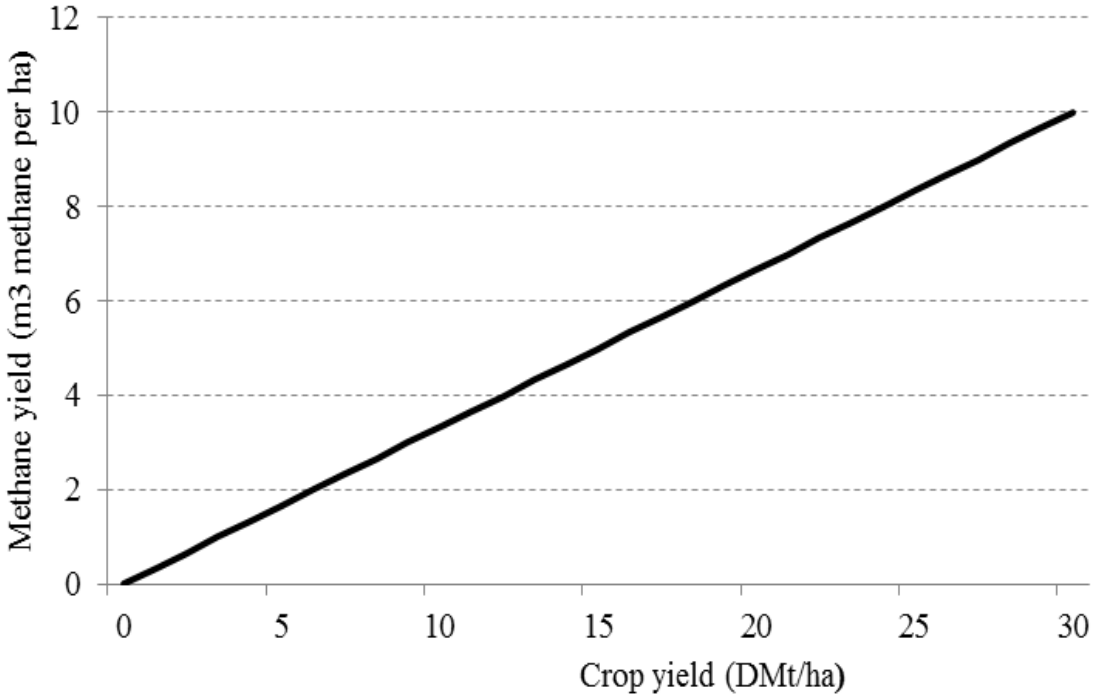

Figure 1. Energy crop dry matter yield and methane production.
Source: SRUC, KWS

CROPS FOR AD

Crop selection

In choosing crops for anaerobic digestion a number of considerations need to be made with regard to likely crop yield per hectare, methane yield per tonne of harvested crop, capabilities for crop harvesting and processing, and storage. Other agronomic factors such as fertiliser and pesticide requirement will also need to be taken into account. These factors will have a major impact on the financial viability of the operation.

SRUC has completed two years of trials for AD crops where winter rye has demonstrated high and consistent dry matter yields, energy beet has shown high but variable energy yields while grass and legume mixtures offered lower energy yields with reduced inputs and on less favourable land.

Table 1. Harvested dry matter values at different nitrogen fertiliser rates.

	Total DM yield: kg/ha			
	Nitrogen rate kg N/ha			Mean for crop
Crop	50	100	150	
Maize	10.9	15.0	15.4	13.8
Spring oats	8.6	7.9	8.0	8.2
Hybrid winter rye	17.8	17.5	17.1	17.5
Triticale	7.2	9.0	8.7	8.3
Energy beet	3.5	6.1	5.1	4.9
Mean for N rate	9.6	11.1	10.9	
LSD:- Crop: 1.44 ($P<0.05$) ; N rate: 1.12 ($P<0.05$): Crop/N rate: 2.50 ($P<0.1$)				

The results indicate that there was no response to nitrogen application for all crops above an application rate of 100 kg/ha of N and in the spring oats and winter rye there was no response above 50 kg/ha. Inorganic nitrogen fertiliser input to the crop is a major factor in the overall environmental sustainability of the system and the aim should be to minimise its use. The recycling of nutrients from the application of digestate will play a significant part in reducing the requirement for inorganic fertiliser. Energy beet yields were considerably lower than would normally be expected indicating that, under Scottish conditions, its yield may be inconsistent.

Methane yield per hectare

For many of the higher yielding agricultural crops a considerable amount of research has been carried out to measure the amounts of methane produced under anaerobic digestion. These are often carried out under varying conditions of temperature, vessel size, and bacterial inoculant. This has led to a wide range in methane potential reported for any one particular crop. Indeed in large scale operations there is likely to be variation among systems and even within a particular AD unit with regard to methane production.

Potential methane productivity can also be measured from the proportions plant constituents such as sugar, fibre, protein and oil content of the crop and provides a useful means of direct comparison.

Table 2. Potential crop methane yields from biochemical analysis andtypical crop yields.

Crop	Methane (m^3/dry t)	Crop yield (dry t/ha)	Methane (m^3/ha)
Dedicated AD crops			
Maize	298	11.4	3397
Hybrid winter rye	280	13.6	3808
Energy beet	265	14.3	3710
Conventional crops			
Winter barley	276	12	3312
Winter wheat	277	11	3047
Oats	273	11	3003
Potatoes	276	14	3864
Perennial ryegrass	269	10	2690
P.ryegrass silage	272	9	2448

Source: SRUC

The potential yield of methane per tonne does not actually vary greatly across crops. The main effect on methane production per hectare is derived from the crop yield. Taking this into account it can be seen from the table above that the highest methane yields per hectare are derived from maize, hybrid winter rye and energy beet. In general crops are managed to produce greatest yield, but stage of growth does have an impact on methane productivity. For instance methane yield from pasture grasses decreases in the later stages of growth.

Future work is planned to explore the potential for legumes in arable and perennial forage mixtures to reduce nitrogen fertiliser use, improve sustainability and enhance energy output. Further research is also needed to investigate the optimum harvest time for each crop with regard to maximising methane output and fitting in with crop rotations where a number of crops are being used as AD feedstocks.

ECONOMICS OF AD CROPS

Crop costs

SRUC has prepared indicative estimates of the costs of producing some of the main energy crops based on experience from trials and discussions with existing growers. These are an indication only as wide variations in crop yield, machinery and labour costs and other factors will have a large bearing on performance at the individual farm level. Only two years of trial data have been obtained for some of the newer crops such as winter hybrid rye and energy beet under relatively favourable growing conditions. Therefore when budgeting future returns a conservative approach to yields has been taken to account for likely more challenging seasons ahead.

Table 3. Energy crops cost of production estimates

	Energy beet	Hybrid rye	Grass silage	Maize
Yield: fresh (t/ha)	65	40	36	38
Dry matter (%)	22%	34%	29%	30%
Yield : dry matter (t/ha)	14.3	13.6	10.4	11.4
Methane yield (m^3/dry matter t)	265	280	272	298
Methane yield (m^3/fresh t)	58	95	79	89
Energy (MWhr TH*/fresh t)	0.7	1.1	0.7	1
Costs				£/ha
Seeds	194	154	20	167
Fertiliser	160	224	364	192
Sprays	249	80	8	78
Contract cultivation	224	195	51	176
Contract harvesting	320	154	273	157
Total cost (£/ha) standing	827	653	443	613
Total cost (£/ fresh t) standing	12.7	16.3	12.3	16.1
Total cost (£/ha) delivered	1,147	807	716	770
Total cost (£/ fresh t) delivered	17.6	20.2	19.9	20.3
Total cost (£/MWhr TH*) delivered	27	18.3	26.6	20.2

*Source: SRUC, * MWhr TH; Mega Watt hours of Thermal energy*

Assumptions

These costs exclude charges such as land rental, interest, management time and any margin requirements. Yields are based on crops grown in southern and central Scotland assuming average weather conditions. Actual yields will vary widely and are much less certain in the north with maize not recommended out with south west Scotland. Several more years of trial results are required to build greater confidence in the yields of energy beet, hybrid rye and maize. Harvest of winter hybrid rye is typically around the middle to end of July with maize and energy beet harvests in late October. Certified seed has been assumed for all crops and hybrid seed for rye and maize. Full rates of artificial fertiliser have been calculated to match crop offtake. Where digestate from an AD plant is applied fertiliser rates should be adjusted accordingly. In practice digestate use is likely to reduce but not entirely replace the requirement for artificial fertiliser due to mismatches in nutrient availability and timing between crops.

Energy crops offer the potential to spread the workload, reducing farm labour and machinery costs. Whole crop cereals enable an earlier harvest (mid July), taking the pressure off combine

and grain drying capacity, and allowing early entry for cultivation and sowing of winter crops particularly oilseed rape. Moving to perennial forage grasses and legumes in place of conventional arable crops reduces the workload at spring or autumn sowing periods

Crop returns and contracts

The price and conditions of supply for AD feedstock vary widely from plant to plant. The contract price will reflect the basis of the sale; standing crop or delivered plant. It will also reflect the method agreed to share other costs such as specialist machinery and the value and costs assigned to any digestate applied to the land. AD feedstock prices currently vary from £65 to £85/dry t standing or £85 to £105/ dry t delivered. There are also options to simply lease the land for the AD operator to then complete all the cultivation and harvesting.

DISCUSSION

AD crops bring new options for farmers in support of widely differing business strategies. For many producers energy crops may currently appear a good alternative to second cereals and winter barley due to low grain prices. In an uncertain world AD plant operators are unique in offering such long contracts to farmers. However it must be recognised that the future situation could be very different if grain prices were to rise significantly. For most growers a mix of enterprises and contract durations will deliver the most balanced income stream. Retaining conventional cereal crops will provide flexibility to benefit from any commodity price rises whilst adding a proportion of AD crops will help buffer the business from further price falls. For illustration two contrasting strategies have been outlined below but ultimately it is down to each farmer to find a strategy that suits their situation best;

Simplify - 100% grass and winter rye - Low input, low labour system, may suit farmers looking to reduce their workload as they move towards semi-retirement or farmers seeking to free up time to devote to diversification or off-farm employment.

- *Diversify - AD crop as part of a mix* - Integrate AD crops as part of an ongoing arable business to gain benefits of income security, spreading workload, building organic matter and diversifying the cropping mix and rotation. This still enables land to be devoted to mainstream arable crops such as first wheat and spring malting barley enabling part of any upside to commodity prices to be captured.

ENVIRONMENTAL AND OTHER FACTORS AFFECTING THE QUALITY OF WHEAT SEED PRODUCED IN SCOTLAND

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Summary: Records held by the Official Seed Testing Station on aspects of seed quality are a currently underutilised source of information on the quality of seed crops produced in Scotland. Preliminary analysis of this data has shown that the variation in seed viability (germination and tetrazolium tests) can largely be explained by season of production and variety. Seasonal differences are mainly a result of differences in weather, and results indicate that conditions during the later stages of seed maturation and at harvest may be most important for the development of maximum seed quality. In seed lots produced in a single year, with similar levels of germination, there can be considerable differences in vigour. These differences have been explored by ageing seed lots and looking at survival curve parameters.

INTRODUCTION

It is well documented that the environment during plant growth affects seed development, seed dormancy and seed quality (Bewley *et al*., 2013; Sanhewe *et al*., 1996). At the Official Seed Testing Station (OSTS) for Scotland we have observed trends in poorer and more variable seed testing results in years when environmental conditions are worse during seed development and the harvest period.

It is now acknowledged that the climate is changing (Jenkins *et al*., 2009). In areas such as Scotland, where some crops grow at the edge of their range, the impacts of climate change on crop growth and seed development may be the most severe. Crops such as wheat, which show greater susceptibility to extreme weather events, may be particularly vulnerable. There is a requirement to look into the likely impact that predicted future weather patterns will have on crop production, in order to inform growers whether certain crops will remain viable options for growing in Scotland.

When investigating the quality of a seed lot there are limitations to germination and tetrazolium tests. A seed lot may have high viability, but low vigour, which would not be picked up in a standard viability test. To gain additional information about seed quality and vigour it is possible to construct seed survival curves, which can be obtained by raising the temperature and moisture content at which seeds are stored at to speed up their deterioration and then monitoring their survival over time. This gives an idea of seed vigour – how well seeds will store and how well they will perform in the field in adverse conditions.

The OSTS has records of seed testing results from the past 15 years. This valuable and currently underutilised resource could provide a lot of information about seed quality. In order to investigate some of these areas we have analysed seed testing results for wheat. In addition to this we selected seed samples from 2014 to investigate quality in greater detail.

METHODS

Seed testing results for wheat samples tested from 1999 – 2015 for both certification and advisory purposes were collated and analysed. To supplement this data, 19 wheat accessions received for testing in 2014 were selected for further analysis. A range of varieties from different growing areas were selected, in order to analyse the effect of both factors. Seed moisture content was adjusted by placing seeds in a sealed plastic electrical box (Ensto, Finland) at 20°C over a non-saturated solution of Lithium Chloride (LiCl) giving 47% RH (Hay *et al*., 2008). After 10 d equilibration, seeds were transferred to experimental storage conditions of 45°C and 60% RH (in a second sealed plastic box over a different concentration of non-saturated LiCl solution). Seed moisture content should be similar for the two environments, thus on transfer to 45°C and 60% RH the seeds only have to equilibrate to the higher temperature. Sub-samples of seeds were removed at 7 d intervals and tested for ability to germinate. Germination tests were carried out on one replicate of 50 seeds. Seeds were sown on wet paper towels and incubated at 20°C for 12d. Germination was assessed according to the Rules of the International Seed Testing Association (ISTA, 2015).

Logistic regression analysis was carried out to investigate the effect of different factors on seed quality. Probit analysis was carried out to estimate p_{50} (the time taken for viability to fall to 50%) and fit seed survival curves to the data using the equation:

$$v = K_i - p/\sigma$$

where v is the viability [normal equivalent deviates (NED)] of a seed lot stored for period p (d), K_i is initial seed lot viability (NED) and σ is the standard deviation of the distribution of seed deaths in time (d) (Ellis and Roberts, 1980). Thus, p_{50} is the product of K_i and σ. Data was analysed using GenStat for Windows 14th Edition (VSN International Ltd, UK).

RESULTS

We looked at the proportion of results with viability greater or equal to 85% (the standard for certified seed), and the proportion greater than 90%, as an indication of a better quality seed lot. Initial analysis showed clear differences in the viability of seeds produced in different growing seasons (Figure 1), and also varietal differences (Figure 2). Differences between seeds grown in different areas were not as apparent. Both germination and tetrazolium data was analysed, and similar patterns were seen for both tests. Over the period of study, germination was particularly high in the years 2003, 2006 and 2014; with over 90% of samples achieving a germination of at least 85%. In these years summer temperatures were higher than average, and rainfall was low. 2008 and 2012 were poorer years, with the percentage of samples achieving the certification standard being 60 and 39% respectively. These lower germination results corresponded with years in which temperatures were lower and rainfall higher (Figure 3).

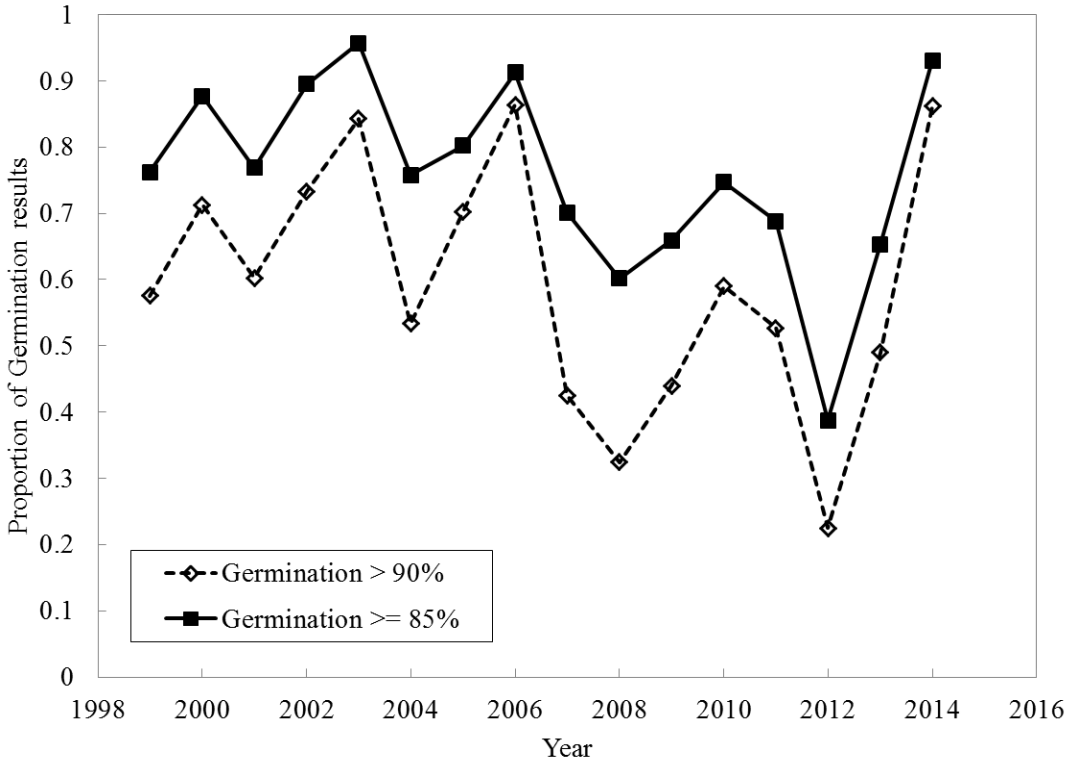

Figure 1. The proportion of germination results of seed tested between 1999 and 2014 ≥85% (solid symbols and lines) or >90% (hollow symbols and dashed lines).

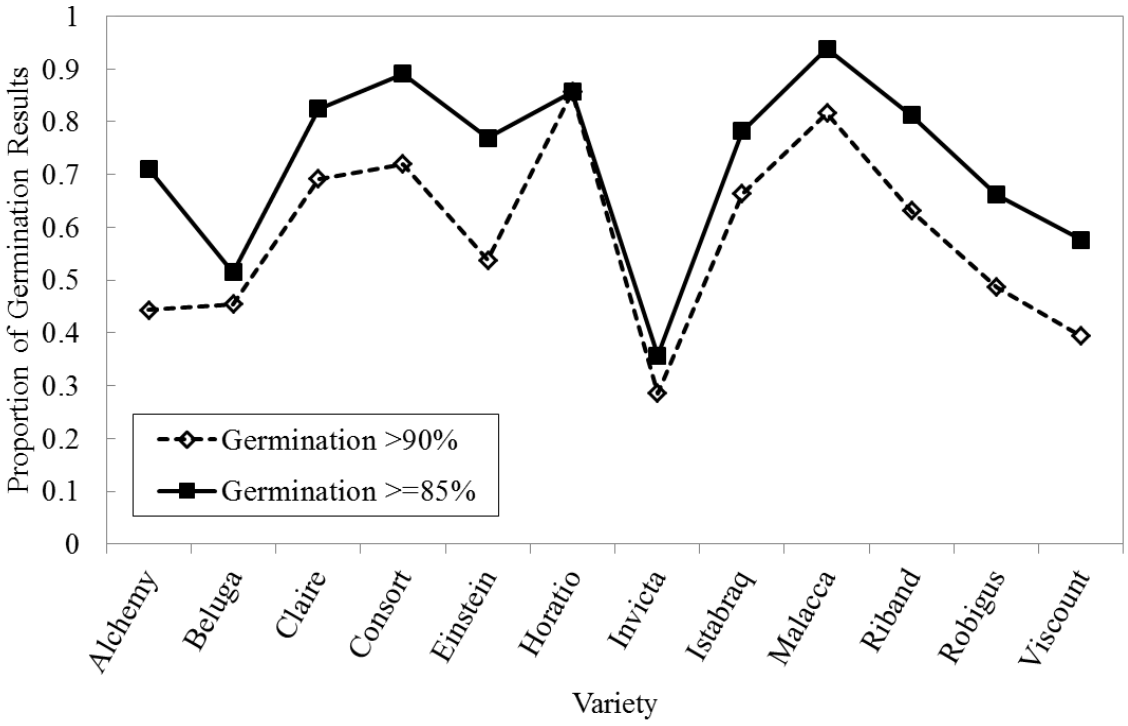

Figure 2. The proportion of germination results of different varieties of seed tested at the OSTS that are ≥85% (solid symbols and lines) or >90% (hollow symbols and dashed lines).

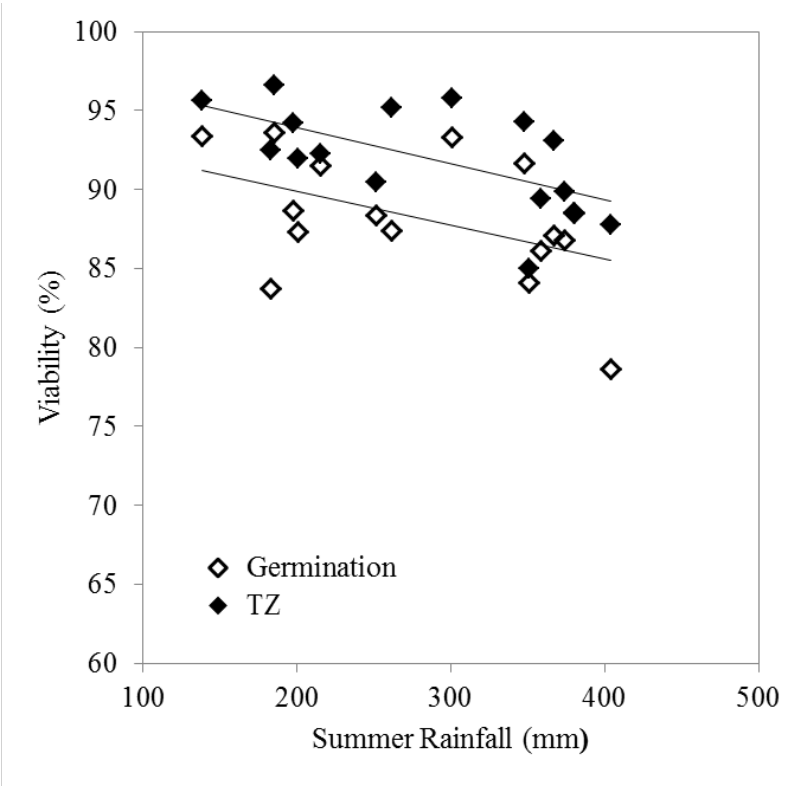

Figure 3. Mean germination (hollow symbols) and tetrazolium (solid symbols) results of seed tested at the OSTS between 1999 and 2014 plotted against summer rainfall in Eastern Scotland.

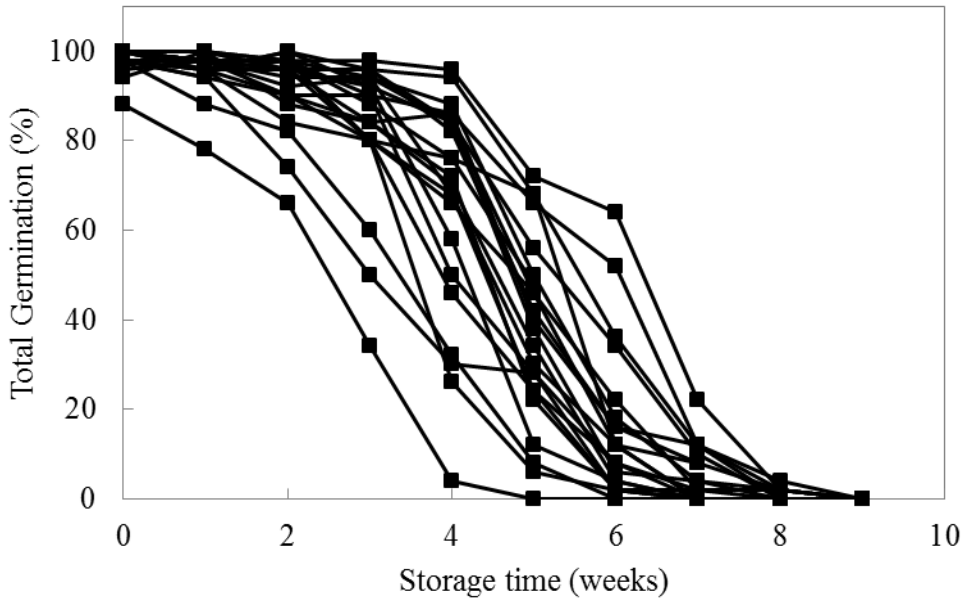

Figure 4. Survival curves for 19 wheat seed lots grown in Scotland in 2014 and stored at 45°C and 60% RH.

Logistic regression analysis showed significant effects of season (p-value < 0.001) and variety (p-value < 0.001) on the proportion of germination results > 90%. The effect of region was not significant (p-value = 0.39). The interaction between variety and region was also found to be

significant (p-value = 0.02). Climate is closely linked to season, and further investigation found that weather conditions over summer months (June to August) better explained variation in germination than either annual or spring weather conditions, with rainfall explaining slightly more variation than mean temperature, though both annual and summer rainfall and temperatures had significant effects (all p-values < 0.001). There is a trend that wetter summers lead to lower seed viability. Of the 12 most common varieties tested the proportion of seeds with a germination of at least 85% ranged from 94% for Malacca, to 36% for Invicta.

Analysis of wheat seed lots from 2014 by constructing seed survival curves (Figure 4) showed significant differences in seed longevity between seed lots. Time for viability to fall to 50% (p_{50}) varied between 13.2 and 38.9 days. There was a general trend that seed grown further south had greater initial viability (K_i), and more uniform viability loss (lower σ) (Table 1).

Table 1. Survival curve parameters for wheat seed samples grown in 2014

Area	Variety	K_i (NED)	σ (d)	p_{50} (d)
Borders	Alchemy	3.22	9.43	30.3 ± 0.88
Central	Alchemy	2.60	10.00	26.0 ± 0.90
Central	Alchemy	2.65	12.66	33.4 ± 1.02
Borders	Horatio	2.97	10.42	31.0 ± 0.92
Borders	Horatio	2.55	12.05	30.7 ± 0.99
Central	Horatio	3.13	10.31	32.3 ± 0.92
Central	Horatio	3.12	11.24	35.0 ± 0.96
Borders	Istabraq	3.26	9.43	28.7 ± 0.85
Borders	Istabraq	3.53	8.62	30.4 ± 0.84
Central	Istabraq	2.83	10.10	28.5 ± 0.91
Grampian	Istabraq	2.71	12.50	34.1 ± 1.01
Central	Leeds	3.06	9.26	28.4 ± 0.88
Central	Leeds	1.99	10.75	21.3 ± 0.94
Grampian	Leeds	1.74	12.35	21.3 ± 1.02
Borders	Viscount	3.84	6.67	25.5 ± 0.75
Central	Viscount	3.20	12.20	38.9 ± 1.00
Central	Viscount	2.19	10.53	23.0 ± 0.93
Grampian	Viscount	1.64	12.50	20.5 ± 1.03
Grampian	Viscount	1.27	10.42	13.2 ± 0.98

DISCUSSION

The main factor affecting the variation in viability results from the past 15 years of seed testing data is season. Some years produce better quality seed than others. This is likely to be largely due to climate differences between years. Previous research has shown that weather conditions during early stages of development affect seed yield in wheat (Spink *et al*., 2000). Our data showed that in terms of seed quality summer weather is more influential than spring weather. This suggests that conditions during later stages, seed maturation and harvest, have a greater effect on seed quality. Both rainfall and temperature significantly affect seed viability, with

rainfall having a slightly greater affect. It is likely that high rainfall during seed maturation and at harvest prevents drying, inhibiting maturation events that take place during later stages of seed development. Lower temperatures may also slow down maturation processes (Sanhewe *et al*., 1996). These problems may be specific to Scotland; a wetter, colder period during seed maturation is likely to lead to a longer seed development period. Other areas of the UK may be dealing with shorter growing seasons as a result of climate change. Weather conditions alone however cannot explain the variation in seed viability; there also is a significant effect of variety, showing that genetics is also important in determining final quality.

When looking at individual seed lots from a single year, there is clearly a degree of variation, as evidenced by the range of p_{50} values. Higher K_i (initial quality) values in samples grown in the Borders region may be due to these samples experiencing better conditions during seed development. These results also highlight the fact that even among seed lots with high germination there can be considerable differences in the quality. In some cases, for example if the seed is to be stored or sown in adverse conditions, then vigour testing could be valuable. Conditions during 2014 favoured seed production, and it will be interesting to investigate differences in seed lots produced under less favourable growing conditions.

The factors affecting seed quality are complex. The significant effect of the interaction between variety and region is an indication of this – it is possible, for example, that certain varieties are more robust and able to cope with adverse weather conditions during development. Through further analysis we hope to better understand variation in seed quality, in order to provide the best advice on how wheat growers can respond to a changing climate.

ACKNOWLEDGEMENTS

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THE USE OF A RAMULARIA DISEASE FORECAST IN INTEGRATED PEST MANAGEMENT SCHEMES

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Summary: Ramularia leaf spot is major disease of barley crops across Northern Britain and control relies on timely fungicide application. Symptoms generally appear post-flowering so thresholds cannot be used to guide fungicide use. A number of factors have been implicated in determining the severity of disease epidemics. These factors can be biotic or environmental. A new project is underway to investigate the different influences on disease epidemics and determine the potential of developing a robust UK wide forecast. This presentation will describe how a forecast could be used in planning fungicide programmes.

INTRODUCTION

Barley is the second most important cereal crop grown in the UK in 2015, 7.28 million tonnes of barley were grown with a market value of over £1 billion. Ramularia leaf spot (RLS) is now a major disease of barley crops in the UK. RLS has also been observed in a number of countries across the temperate regions of the world (Havis *et al.*, 2015). Losses due to RLS have been estimated at anything from 20% to 70% of total yield and in the UK, losses average around 0.5 t/ha. The reduction in grain quality due to RLS also leads to a further economic cost to growers. This is due to an increase in the number of thin grains at harvest. Currently, there are no fully resistant varieties available and control depends on the use of effective fungicides. The vast majority of symptoms appear in the crop post flowering. However, at this point in crop development, no fungicides applications are permitted. Therefore, the use of normal disease thresholds to predict economic damage and to trigger crop protection sprays is not possible. Research from Norway indicated a strong relationship between high levels of relative humidity in spring barley in early June and final disease severity (Salamati & Reitan, 2006). This observation was used to formulate a risk forecast for winter and spring barley crops in Scotland based on leaf wetness in barley crops at stem extension (early April and June respectively) (Havis *et al.*, 2013). A project was established to investigate the potential of developing a robust risk forecast model for RLS across the UK. The aims of the project are: i) to refine the initial model by comparing the dates for stem extension over years and sites with disease levels in the crop, ii) to extend the forecast to the rest of the UK by using information from the meteorological network funded by AHDB and disease scores from RL and other trials, and iii) to gather information on disease levels across the UK and quantify levels of fungal DNA in seed and plant samples using a real time PCR.

Classic Integrated Pest Management schemes to control disease problems would involve a number of aspects: i) determining acceptable disease levels, ii) preventative cultural practices, iii) monitoring, iv) mechanical control, v) biological controls, and vi) responsible use of fungicides. Not all of these aspects have been shown to influence RLS. No mechanical control measures have been demonstrated and biological control has not given consistent control (Brown *et al.,* 2014). Monitoring of visual symptoms would not be useful with RLS as symptoms appear late in the season after the last potential fungicide application date. The effect of disease levels on yield has been demonstrated previously (Havis *et al*., 2014). The risk forecast under development would contribute to a more responsible use of fungicides as their use would be based on risk rather than a prophylactic treatment.

MATERIALS AND METHODS

Control of Ramularia leaf spot

A number of field trials were conducted at SRUC trial sites in 2014 and 2105 at Bush Estate, Midlothian, Drumablin, Lanarkshire and Gilchriston, East Lothian investigating the control of RLS in winter and spring barley. Winter barley trials were conducted with cv. Saffron in both years. In 2014 cv. Optic and cv. Overture were used and a range of T2 sprays and timings were tested. A number of fungicides were tested, including prothioconazole (pro) (Proline 275®), pyraclostrobin (pyr) (Comet®), chlorothalonil (chlor) (Bravo®), bixafen+prothioconazole (bix+pro), Siltra X Pro®, isopyrazam+cyprodinil (iso+cyp)Bontima®, fluxapyroxad+epoxyconazole (flux+epo) Adexar®. 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 Version11.1 (VSN International Ltd, Hemel Hempstead, UK).

Ramularia leaf spot monitoring

In order to assess the risk of RLS, untreated plots in AHDB winter and spring barley trials across the UK were scored for visual symptoms on the F-1 leaf layer between GS 75-85. Meteorological data was collected from adjoining and nearby stations. Surface wetness figures were calculated for the two week period following GS30 and sites ranked as high, medium or low risk. High risk was classed as more than 7,500 mins of surface wetness, medium risk was defined as between 7,500 and 4,000 mins of surface wetness and low risk as less than 4,000 mins of surface wetness.

RESULTS

Results show that winter barley programmes with higher rates of fungicides are more effective against RLS. They also gave the biggest response in terms of yield response. However, much of the disease response was due control of Rhychosporium in the trials (data not shown).

Table 1 RLS and yield in winter barley trials (2014/15)

GS 24	GS 31	GS 45	GS 53	RLS (audpc)	Yield (t/ha)
Untreated	Untreated	Untreated		49.7	7.12
	pro 0.18 l/ha + pyr 0.25 l/ha		pro 0.18 l/ha + chlor 0.5 l/ha	36.4	8.47
	pro 0.36 l/ha + pyr 0.5 l/ha		pro 0.36 l/ha + chlor 1.0 l/ha	23.7	8.69
	pro 0.18 l/ha + pyr 0.25 l/ha	pro 0.18 l/ha + chlor 0.5 l/ha	chlor 1.0 l/ha	41.9	8.63
	pro 0.36 l/ha + pyr 0.5 l/ha	pro 0.36 l/ha + chlor 1.0 l/ha	chlor 1.0 l/ha	21.1	8.62
F ratio				0.08	0.005
LSD (P=0.05)				20.38	1.03

Table 2 RLS and yield in two spring barley trials 2014

GS 25	GS 45	GS 53	RLS (audpc)	Yield (t/ha)
Untreated	Untreated	Untreated	30.2	7.797
pro 0.36 l/ha + pyr 0.5 l/ha		pro 0.36 l/ha	12.4	8.377
pro 0.36 l/ha + pyr 0.5 l/ha		bix+pro 0.5 l/ha	6.9	8.325
pro 0.36 l/ha + pyr 0.5 l/ha		iso+cyp 1.0 l/ha	4.8	8.369
pro 0.36 l/ha + pyr 0.25 l/ha		flux+epo 0.5 l/ha	8.2	8.272
pro 0.36 l/ha + pyr 0.5 l/ha		pro 0.36 l/ha + chlor 1.0 l/ha	9.6	8.555
pro 0.36 l/ha + pyr 0.5 l/ha	pro 0.36 l/ha + chlor 1.0 l/ha		5.5	8.49
			0.144	0.49
			16.44	0.616

Risk assessment 2015

Surface wetness figures were lower than has been seen in previous years, reflecting the good growing conditions in April. However, the weather turned much cooler at the start of May and this will have affected crop growth and development.

There was considerable variation in levels of surface wetness in May and June across sites, reflecting a dry early summer in many parts. Levels in East Lothian were exceptionally low.

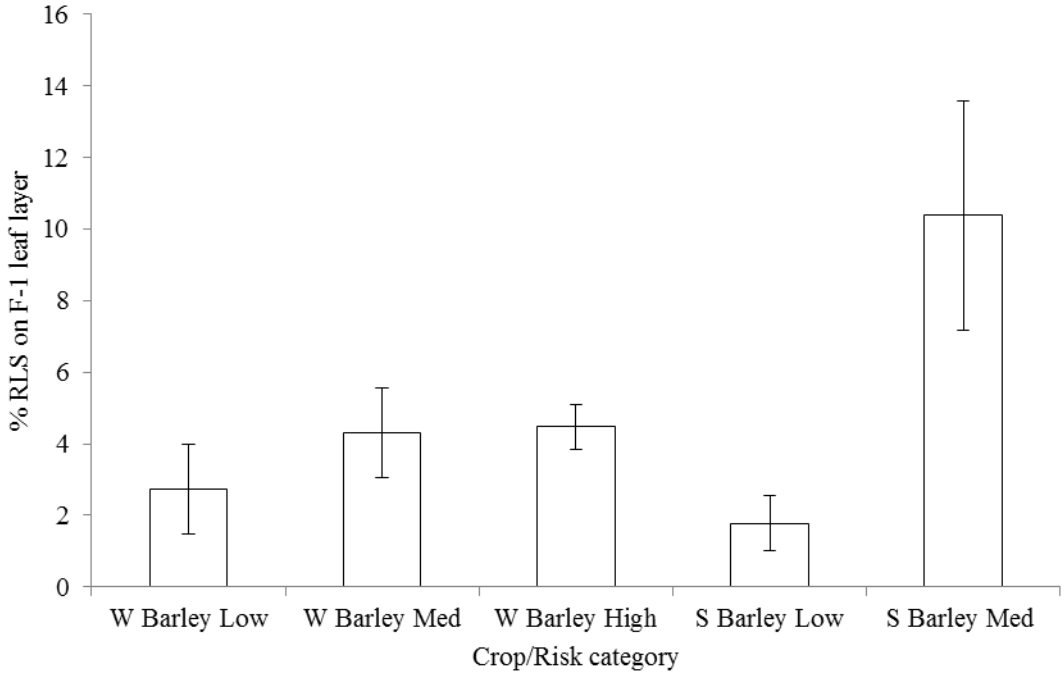

Figure 1. RLS levels in AHDB trials 2015.

Comparison of the disease scores and risk categories indicated a weak relationship in winter barley crops. There was considerable variation within categories (reflected in the high S.E. values). No sites were ranked as High risk for spring barley.

DISCUSSION

In the absence of complete varietal resistance control relies on an effective late fungicide application. The new spring barley ratings suggest there is no significant difference between varieties on the spring barley recommended list. Previous recommendations from SRUC on controlling RLS focussed on a T2 timing at booting stage (GS45-49). However, the introduction of Succinate dehydrogenase inhibitor fungicides allowed greater flexibility with this timing. Results from spring barley trials indicated that SDHI fungicides at GS 53 gave superior control of RLS compared to pro and pro+chlor programmes (Table 2). However, differences in disease control were not significantly different in 2014 across the sites. Interestingly the control of RLS achieved by SDHI fungicides (bix+pro, iso+cyp,flux+epo) did not lead to increased yield compared to other programmes. Comparing timings for the pro+chlor sprays showed that the GS45 timing gave lower disease levels than the GS53 timing.

However, the yield results were reversed with the latter spray giving a slightly higher yield. Further trials are required to compare the most effective T2 timing for RLS control.

Winter barley programmes show that increasing the fungicide dose at GS 53 reduces RLS levels and increases yield (Table 1). Reduction was significant compared to the untreated control. Increased reduction in RLS levels were achieved by pro+chlor at GS45 and then chlor at GS53. Paveley *et al.* (2003) reported that splitting fungicide doses gave increased control of *Zymoseptoria tritici*. These results suggest that splitting late chlor applications may give improved control of RLS compared to a single application.

Results from the risk forecast for UK sites shows that the variation in disease levels cannot be explained solely by differences in surface wetness at stem extension. The crude classification of sites into High, Medium and Low risk needs to be underpinned by robust analysis of the underlying relationship between the two variables. The correlation observed in individual seasons previously (Havis *et al.*, 2012) does not appear when multiple years are analysed together (data not shown). Correlation analysis for the data from 2015 shows a weak overall relationship for northern sites but a stronger one for southern sites (data not shown). A stronger relationship was seen in spring barley results (data not shown). The influence of environmental conditions on RLS has been reported previously and the UK wide data set will include crops grown under very different conditions. A number of risk factors associated with RLS in both crops were identified previously e.g. seed infection, varietal choice, cultivation system, sowing date and the presence of spores in the environment (Havis *et al.*, 2014). Others may include rate of crop development and presence of other foliar disease. In order to produce a robust model to include into IPM control programmes further examination of other contributory factors will be required. Other risk models e.g. eyespot risk are based on the additive contribution of a number of factors (Burnett & Hughes, 2004).

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SURVEY OF THE IMPACT OF THE NEONICOTINOID RESTRICTIONS ON SCOTTISH OILSEED RAPE CROPS[1] AND RECOMMENDATIONS FOR ALTERNATIVE CROP PROTECTION STRATEGIES[2]

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Summary: Oilseed rape is an important break crop in Scottish arable rotations and use of insecticidal seed treatments has been an integral component of crop protection. A survey of 16% of the Scottish crop sown in 2014, the first winter rape crops since EU restrictions on neonicotinoid insecticides were imposed, was conducted. The survey found that more autumn insecticide sprays, almost exclusively pyrethroids, were applied to crops than in the previous year. Some of these additional sprays were precautionary. Whilst overall crop damage was rated as low by the majority of growers, 1% of the crop area was redrilled due to autumn cabbage stem flea beetle damage. A further 1% of the crop was redrilled due to non-insect related issues. Average yield in 2015 was very similar to that reported in 2014. Recommendations for alternative crop protection strategies are presented.

INTRODUCTION

In December 2013 the European Commission implemented restrictions on the use of three neonicotinoid insecticides (clothianidin, imidacloprid and thiamethoxam) on crops considered to be attractive to bees and on spring-grown cereals due to concerns about impacts on pollinators. In Scotland the main effect of these restrictions is the loss of insecticidal seed treatments for oilseed rape.

Oilseed rape is an important break crop in Scottish arable agriculture and approximately 35,000 hectares are grown each year, of which 98% are winter-sown varieties (Anon, 2015). Use of insecticidal seed treatments to protect crops from insect damage during emergence and establishment has always been an integral part of Scottish oilseed rape production (Hughes *et al*., 2014). The aim of this survey of Scottish growers was to gather information about the impact of the neonicotinoid restrictions on oilseed rape cultivation. The second part of this paper consists of SRUC recommendations for alternative crop protection strategies in the absence of neonicotinoid seed treatments.

MATERIALS AND METHODS

A random sample of arable farms, stratified by farm size and geographic region, was selected as part of SASA's statutory post-approval monitoring programme for pesticide use on arable

crops harvested in 2014 (Monie *et al*., 2015). During this survey farmers were asked whether they were willing to participate in a voluntary survey relating to their 2014/15 oilseed rape cultivation. Crops sown in 2014 were the first winter oilseed rape crops planted since the neonicotinoid restrictions were implemented. Ninety-six growers, collectively cultivating 5,465 ha of winter oilseed rape were recruited. These crops were grown on more than 100 different holdings and represented 16% of the 2014/15 Scottish crop area.

Growers were contacted twice for information, once during the winter of 2014 and once in October 2015. At the first data collection point information was collected about crop sowing and cultivation. Growers were also asked about perceived pest pressure of those species usually controlled by insecticidal seed treatments (the aphid *Myzus persicae* and flea beetles *Psylliodes chrysocephala* and *Phyllotreta* spp.) and autumn insect damage incurred. Data was also collected about autumn foliar insecticide use to protect crops during establishment and emergence. At the second data collection point information was collected about crop yield and incidence of Turnips Yellow Virus (TuYV), which is transmitted by *Myzus persicae*.

RESULTS

Crop Cultivation

More than 80% of the winter oilseed rape surveyed was sown in August 2014, mostly in the last two weeks of the month, with the remainder drilled by the end of September. Seed rate was dependant on variety with a mean of 3.1 kg/ha (range 1.2 to 6 kg/ha). Some growers (26% of those surveyed) made pre-emptive operational changes to crop cultivation to attempt to mitigate for the absence of insecticidal seed treatments. The most common adaptation, adopted by 11% of growers surveyed, was to alter seed rates to alleviate potential plant loss from insect damage. In addition, 9% of growers amended seed bed cultivations (such as implementing minimum tillage, strip tillage or direct drilling in place of ploughing) to allow a quicker turnaround from harvest of the previous crop and earlier oilseed rape establishment. Some growers also altered drilling dates (5%) and used the TuYV resistant variety Amelie (2%) to try to reduce the risk of insect damage.

Perception of pest pressure

Growers rated their perception of aphid and flea beetle populations during crop emergence and establishment; the period when crops are usually protected by a systemic seed treatment. The majority of growers reported that aphid populations were either low or not seen (70%) with only 5% ranked as moderate to high or high (Figure 1). The majority of growers also reported flea beetle populations to be low or not seen (62%), 25% ranked levels as low to moderate or moderate and 10% as moderate to high or high. Of those growers who reported seeing flea beetles on their crops 51% identified them as cabbage stem flea beetle (CSFB, *Pyslliodes chrysocephala*), 3% as both CSFB and *Phyllotreta spp*. flea beetles and 4% as *Phyllotreta spp*. only. The remaining 42% did not know which species of beetle were present in their crops.

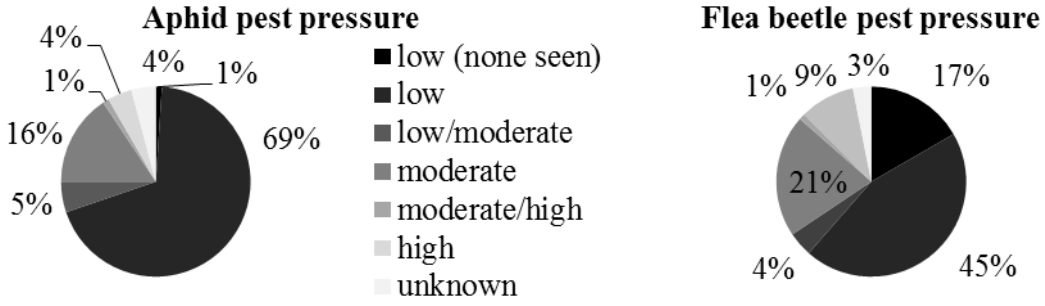

Figure 1. Farmer perception of insect presence in crops in autumn 2014.

Insecticide Treatments

The growers surveyed made more autumn foliar insecticide applications to their winter oilseed rape crops in autumn 2014 than they did in autumn 2013. In 2013, when crops had been grown from neonicotinoid treated seed just over half of growers did not apply an autumn insecticide spray. In 2014, 38% did not use a foliar insecticide. The majority of growers (59%) applied the same number of sprays as they did in the previous year, 8% applied fewer sprays and 31% applied more sprays. Overall, the total number of autumn insecticide sprays applied by the growers surveyed was 45 and 67 in 2013 and 2014 respectively, representing an increase of almost 50%. However, growers were also asked what information was used when making a decision to spray and seven of the 67 sprays applied in 2014 were identified as precautionary, therefore the magnitude of increase in sprays may not be related directly to increased pest numbers or damage but also influenced by concerns about crop protection due to the lack of a seed treatment. Growers reported basing their decision to spray on a number of factors including: agronomist advice, crop walking, evidence of damage, exceedance of thresholds and information from technical bulletins and from the media.

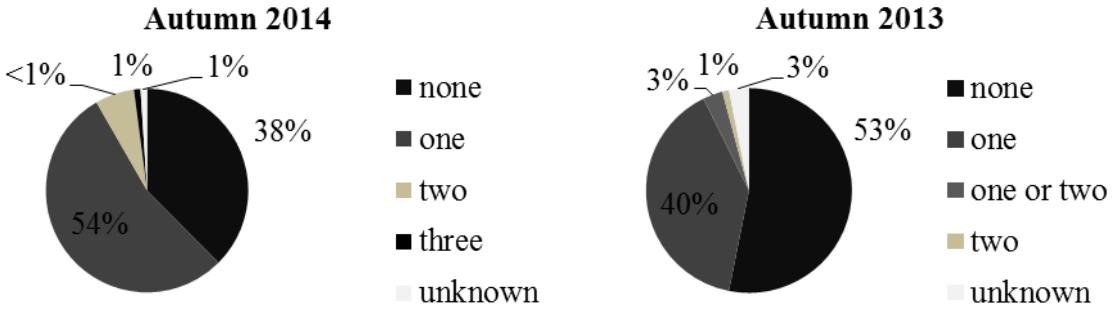

Figure 2. Number of autumn insecticide applications in 2014 and 2013.

The main targets (67% of sprays) of autumn insecticide use in 2014 were flea beetles (CSFB and *Phyllotreta spp*.). Aphids were the focus of 4% of sprays and rape winter stem weevil (***Ceutorhynchus*** *picitarsis*), which is not an approved target of neonicotinoid seed treatments, was the reason for 22%. All but one of the sprays encountered (97%) were pyrethroid insecticides. Of the other insecticides which were approved in 2014, or given emergency or extension of use approval, only acetamiprid was recorded. Autumn use of indoxacarb, pymetrozine and thiacloprid was not encountered. Despite concern about the pyrethroid resistance status of both *Myzus persicae* and CSFB 74% of growers did not report problems controlling pests with sprays and those that did predominately cited operational issues with foliar control (e.g. weather conditions, lack of time to spray, lag between pest detection and

spraying). Only two growers (3% of those applying sprays) reported that they could not achieve sufficient efficacy with foliar insecticides applications.

Insect Damage and Crop Yield

The level of insect damage to the crop reported during the crop emergence and establishment period was rated as low by 63% growers, low/moderate or moderate by 28% and high by 6% (unknown for the remaining 3%). Where the pest responsible for the damage was identified by the grower it was attributed primarily to flea beetle feeding on the crop (88%) with stem weevil and aphid damage being cited in 13 and 6% of cases respectively.

Of the 96 growers and 5,465 ha surveyed, eight growers collectively redrilled 131 ha (2.4%) of failed crop. Of that area, 60 ha (1.1% of sample), grown by two farmers, were re-drilled due to damage from cabbage stem flea beetle; these crops were located in Lothian and Tweed Valley. The remaining 71 ha (1.3% of sample) was lost to a variety of non-insect related issues including weather at drilling, poor seed vigour and pests such as slugs, rabbits and pigeons.

At the second data collection point growers were asked about incidence of TuYV, a virus spread by *Myzus persicae*. Infected plants can exhibit a variety of symptoms but may also be symptomless or resemble other conditions such as nutritional deficiency. TuYV can only be definitively detected by seriological testing (Stevens *et al*., 2008). Of the growers who responded (n=87) the majority (79%) monitored their crops for symptoms of TuYV. Of those who checked their crops 4% saw symptoms of the virus. However, only one of the growers surveyed had their crops tested for TuYV and virus presence was confirmed in that crop.

Overall, there was very little difference in crop yield on the farms surveyed between 2015 and 2014 (average yield 4.3 and 4.2 t/ha respectively). This corresponds with 2015 Scottish yield estimate of 4.2 t/ha reported by the Scottish Government (Anon, 2015). There was some variation in yield between years at grower level, 31% of growers reported a decreased yield (>5%), 22% no change ($\pm$5%) and 47% an increased yield (>5%). Growers were asked what factors contributed to differences in yield between 2014 and 2015. Two growers cited CSFB damage as the main factor for decreased yield. The remainder of growers cited a number of reasons for yield variation including weather conditions, differences in variety and seed bed cultivations and incidence of non-insect pests such as slugs and pigeons. In relation to TuYV incidence, of the two growers who reported symptoms in their crops one reported a yield increase and the other a yield decrease (14 and 18% respectively). The single grower whose crop tested positive for TuYV reported a yield increase (11%). When yield data was split by region, average yields only decreased in Grampian where poor weather conditions, very wet weather at drilling and frosts at flowering, were reported by a number of growers.

Farmer attitudes

Growers were asked at the end of the season if they would grow oilseed rape in future if the neonicotinoid restrictions continued. The majority (82%) said that they would grow again in future, 13% were less likely, 1% more likely and 4% were unsure. Farmers were invited to provide reasons for their response and the majority of those that were less likely or not sure based this on the need to consider future market price and economic return. Two of the respondents (3% sample), both of whom had to re-drill due to CSFB damage said they would be less likely to grow the crop in future due to the lack of neonicotinoid seed treatments.

DISCUSSION

This survey found that, for some growers, it was necessary to apply additional insecticidal sprays to combat damage that has been controlled in the past by systemic seed treatments. However, it was acknowledged that some sprays were precautionary rather than reactive. CSFB damage necessitated the redrilling of 1% of the crop area, and a similar area was redrilled in response to non-insect related issues. Average yield in 2015 was very similar to the year before when neonicotinoid seed treatments were available, although this has to be balanced with the increased economic cost associated with additional sprays.

Overall, the impact of the restrictions appears to be less severe than has been encountered in some areas of south east England (Scott & Bilsborrow, 2014). However, with the exception of growers in Grampian, most reported that the 2014/15 season was favourable for oilseed rape cultivation with a dry autumn for both sowing and application of foliar sprays, a mild winter and a cool extended growing season. This, coupled with reports of low autumn pest pressure resulted in a favourable year for oilseed rape cultivation in this first year of the restrictions. A second survey is being conducted currently to allow collection of comparable data during the second year of the restrictions. Seventy per cent of the farmers in this survey have agreed to take part to allow comparison across years and a supplementary sample will be recruited. This will allow the Scottish Government to continue to monitor the impact of the restrictions on Scottish oilseed rape growers.

However, whilst the restrictions are in place there are alternative crop protection strategies that can be adopted. As discussed earlier the key autumn pests previously controlled by seed treatments are *Phyllotreta spp*. flea beetles, cabbage stem flea beetle and peach-potato aphid as vectors of TuYV

Flea beetle damage is seen as small holes in the cotyledons and first true leaves of the emerging crop. The beetles are mainly active during dry soil conditions, so growers should be prepared to apply a pyrethroid insecticide if feeding punctures are present on germinating plants. Once three leaves have emerged, there is no need for treatment.

Cabbage stem flea beetles also cause shot-holing of leaves, but in addition they lay eggs near plants and the larvae burrow into the stem which can lead to winter kill, no stem elongation or lodging in spring. In Scottish crops the adult beetle feeding damage tends to be worse than the larval damage. One way to assess the risk from cabbage stem flea beetle is to look at the trailers during the winter oilseed rape harvest – the beetles will be caught up in the harvest and can be found on the trailer and in the harvested seed. They will cause no harm to the seed if taken back into the store, but their presence is a ‘heads up’ that the beetle poses a threat on the farm to the next winter rape crop.

As with the smaller flea beetles, pyrethroid insecticides can be applied if the damage thresholds for cabbage stem flea beetle have been exceeded. Note that there is some concern that cabbage stem flea beetles have become resistant to pyrethroid insecticides, and in the absence of the neonicotinoid seed treatment an insecticide treatment should only be used if these damage thresholds have been exceeded, bearing in mind that they may not give the level of control expected due to potential resistance. An alternative option is acetamiprid which was granted an emergency approval (20151968) specifically for use against cabbage stem flea beetle on winter oilseed rape up to the five leaf stage with latest date of application of 25th November 2015.

There are caveats associated with the use of acetamiprid for this purpose - no more than two foliar applications of any neonicotinoid insecticide (e.g. acetamiprid or thiacloprid) can be applied to the crop: one application in the autumn and one application in the spring (for pollen beetle management).

Peach-potato aphids may well be carrying TuYV. Peach-potato aphids are resistant to pyrethroid insecticides so will not be controlled by the pyrethroid insecticides used against flea beetles. Growers should look for aphids on the leaves (including the underside of the leaves) and if aphid colonies are present there are several options available to reduce the threat from TuYV: pymetrozine, thiacloprid and an emergency authorisation for the use of flonicamid up to 8 leaves of the crop or 3rd February 2016.

The SRUC Crop Report, SRUC Crop Clinic website (www.sruc.ac.uk/cropclinic), AHDB Aphid News, aphid trapping data (aphmon.fera.defra.gov.uk/) and the UK network of aphid suction traps: www.rothamsted.ac.uk/insect-survey/bulletins and www.sasa.gov.uk/aphid-bulletins-current-year can all provide information on the flights and presence of peach-potato aphids at the time of crop emergence. Using crop intelligence information to decide whether insecticide treatments are required will be a key decision making tool for oilseed rape growers in the absence of the neonicotinoids.

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IDENTIFICATION OF THE *MICRODOCHIUM* SPECIES CAUSING REDUCED SEEDLING EMERGENCE IN SPRING OAT AND BARLEY SEED

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Summary: A range of oat and barley seed lots with high *Microdochium* infection levels (*M. nivale* and *M. majus*) and low level *Microdochium* infection controls were assessed for emergence. Dead seeds and both abnormal and normal seedlings were then tested for the presence of these *Microdochium* species. A correlation between seedling emergence and total % *Microdochium* species was established for both crops (oat seed, R^2=0.635; barley seed, R^2=0.393). Using *Microdochium* species specific PCR primers, colonies of *Microdochium* arising from infected dead seeds and seedlings were identified. All *Microdochium* colonies isolated from affected oats were identified as *M. nivale*. 26% of the oat seeds and seedlings described as dead and abnormal tested positive for *M. nivale.* Barley abnormal seedlings were infected with only *M. majus*, no dead seeds were found to be infected. On barley normal seedlings, *M. nivale* was found only on 0.7%, compared to 4.5% for *M. majus*.

INTRODUCTION

Microdochium species can be a significant cause of poor emergence but the relationship between species type, seed infection and crop loss is unclear. Field experiments conducted between 2011 and 2013 by the Official Seed Testing Station (OSTS) at SASA showed no clear relationship between seed infection with *Microdochium* species (*M. nivale* and *M. majus*) and seedling loss in spring barley seed, but they did show a higher risk of seedling loss in spring oats particularly when seed bed temperatures were low (McNeil *et al*, 2014).

McNeil *et al* (2014) examined *Microdochium* species colonies from agar plate tests of spring barley and oat seed lots in 2011 and 2013, using *M. nivale* and *M. majus* specific PCR primers (Glynn *et al*, 2005). A clear preference was shown for *M. nivale* to colonise oat seed (90%), and barley seed was colonised more equally by both *M. nivale* and *M. majus* (40%:60%). These results provided some evidence for *Microdochium* host specificity on seeds, but it was not clear whether *M. nivale* or *M. majus* or both pathogens were responsible for the seedling losses observed in field experiments.

This paper reports the results of laboratory experiments to define which of the two *Microdochium* species is responsible for seedling loss on spring oat and barley seed.

MATERIALS AND METHODS

Seed lots

Ten seed lots each of untreated spring barley and oats, eight with high levels of *Microdochium* infection (range shown in Table 1), and two with low infection for use as controls (with 1% or nil infection) were selected from samples submitted in 2012 and 2014 for testing to the OSTS, SASA. Details of the seed lots are given in Table 1.

Table 1. Year, total no. of samples, total % *Microdochium* species and % germination for the seed lots selected.

	2012	2014
No. of oat lots	1	9
No. of barley lots	4	6
Oat % infection	48	0-59
Barley % infection	34-70	1-63
Oat % germination	89	67**-98
Barley % germination	71-94*	92-97

*tetrazolium test showed germination potential of 96%, glyphosate treatment had reduced germination.
**tetrazolium test showed 16% sprouted seed, although *Microdochium* infection 56%.

Agar plate and Germination tests

Agar plate tests to ascertain the level of *Microdochium* spp. (*M. nivale* and *M. majus*) on the seed lots were conducted in accordance with the International Seed Testing Association (ISTA) method 7-022.

Germination tests were conducted in accordance with ISTA Rules 2015, using the rolled-paper towel method or organic growing medium if appropriate.

Seedling emergence experiment

For each lot, 50 seeds were planted in each of two seed trays with Levingtons F2 plus sand. Seed trays were placed at 3^{o}C in the dark for 10 days then held at 15^{o}C with 12 hours light for a further 14 days. Trays were examined daily for seedling emergence.

Colony identification of *M. nivale* or *M. majus*

After 14 days growth at 15^{o}C seedlings were assessed according to the ISTA rules and described as normal, abnormal or dead. The presence of disease symptoms or other abnormalities on seedlings was also recorded. Every seedling once assessed was placed into a sealable bag and labelled with a reference number and stored at -20^{o}C for further testing.

All dead seeds and abnormal seedlings, and 20 normal seedlings from each sample were plated onto potato dextrose agar and emerging fungal colonies were recorded. Fungal colonies identified as *Microdochium* species, were isolated into pure culture and simple DNA

extractions carried out. These *Microdochium* colonies were identified as either *M. nivale* or *M. majus* using the PCR primers developed by Glynn *et al* (2005).

RESULTS

Seedling emergence

For both oat and barley seedlings, first emergence occurred between one and four days after commencing incubation at 15°C, and full emergence was achieved between seven and eleven days. At four days 66% of oats had emerged, increasing to 76% after seven days. Barley showed 83% emergence at four days. The total percentage of emerged and non-emerged seedlings is illustrated in Figures 1 and 2. Seed lots 9 and 10 are the low infection controls.

The relationship between the number of seeds emerged and total percentage *Microdochium* species for oats was R^2=0.635 and for barley was R^2=0.393. The four control oat and barley seed lots all showed good emergence and had zero or 1% *Microdochium* species infection.

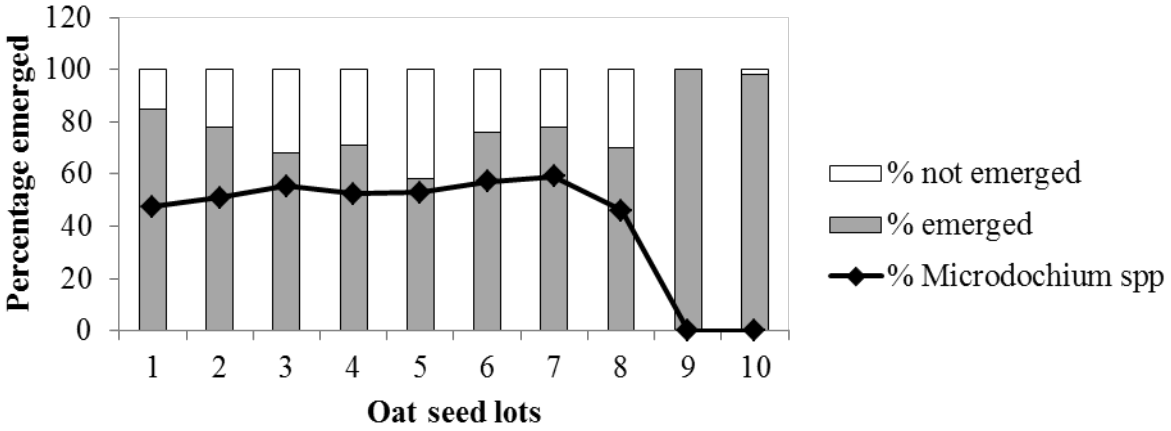

Figure 1. Percentage emerged/not emerged and percentage total *Microdochium* seed infection for each oat seed lot.

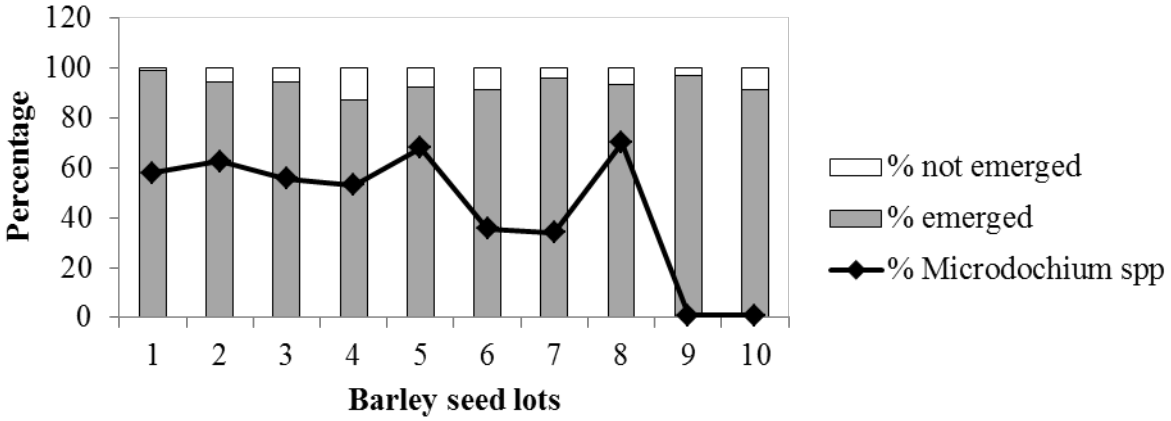

Figure 2. Percentage emerged/not emerged and percentage total *Microdochium* seed infection for each barley seed lot.

Identification of *Microdochium* species causing seedling loss

The percentages of dead seeds and both abnormal and normal seedlings confirmed by PCR as infected by either *M. nivale* or *M. majus* are shown in Table 2 (oats) & 3 (barley). No *M. majus* was detected on any of the oat dead seed or abnormal and normal seedlings. *M. nivale* was detected on 9 out of 10 oat seed lots. Lot 5 with the lowest emergence had 23% dead, 36% abnormal and 7% normal infected with *M. nivale*. On barley, *M. nivale* was only detected on normal seedlings in lot three. *M. majus* was detected on eight of the ten lots on abnormal and normal seedlings but not on dead seeds.

Table 2. Percentages of dead seeds, abnormal and normal seedlings from oat lots confirmed infected with *Microdochium nivale* or *majus*.

	Dead		*Abnormal*		*Normal*	
Sample	*M. nivale*	*M. majus*	*M. nivale*	*M. majus*	*M. nivale*	*M. majus*
1	0.0	0	16.7	0	25.0	0
2	15.0	0	0.0	0	13.3	0
3	6.3	0	100.0	0	9.5	0
4	8.0	0	0.0	0	19.0	0
5	22.9	0	36.4	0	6.9	0
6	47.6	0	45.5	0	17.1	0
7	55.0	0	40.0	0	8.7	0
8	42.9	0	41.7	0	4.5	0
9	0.0	0	0.0	0	2.5	0
10	0.0	0	0.0	0	0	0

Table 3. Percentages of dead seeds, abnormal and normal seedlings from barley lots confirmed infected with *Microdochium nivale* or *majus*.

	Dead		*Abnormal*		*Normal*	
Sample	*M. nivale*	*M. majus*	*M. nivale*	*M. majus*	*M. nivale*	*M. majus*
1	0	0	0	16.7	0	0.0
2	0	0	0	11.1	0	12.5
3	0	0	0	25.0	7.0	18.5
4	0	0	0	16.7	0	4.3
5	0	0	0	25.0	0	0.0
6	0	0	0	0.0	0	7.9
7	0	0	0	0.0	0	9.5
8	0	0	0	0.0	0	0.0
9	0	0	0	0.0	0	0.0

10	0	0	0	6.7	0	0.0

The total percentage of dead and abnormal confirmed as infected with *Microdochium* species compared to percentage *Microdochium* species infection on seed lots are shown in Figures 3 and 4.

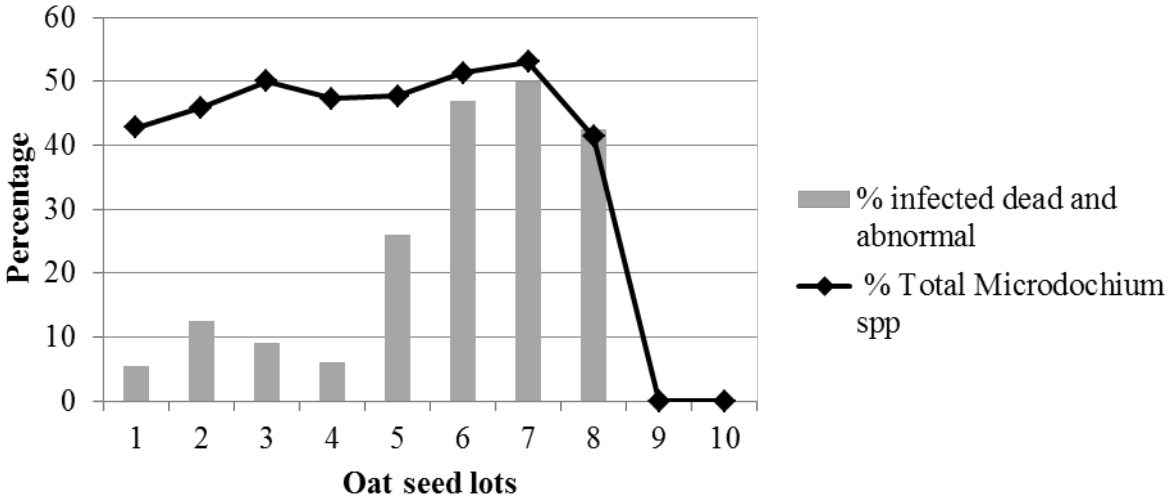

Figure 3. Percentage dead seed and abnormal oat seedlings and total percentage *Microdochium* species seed infection of each lot.

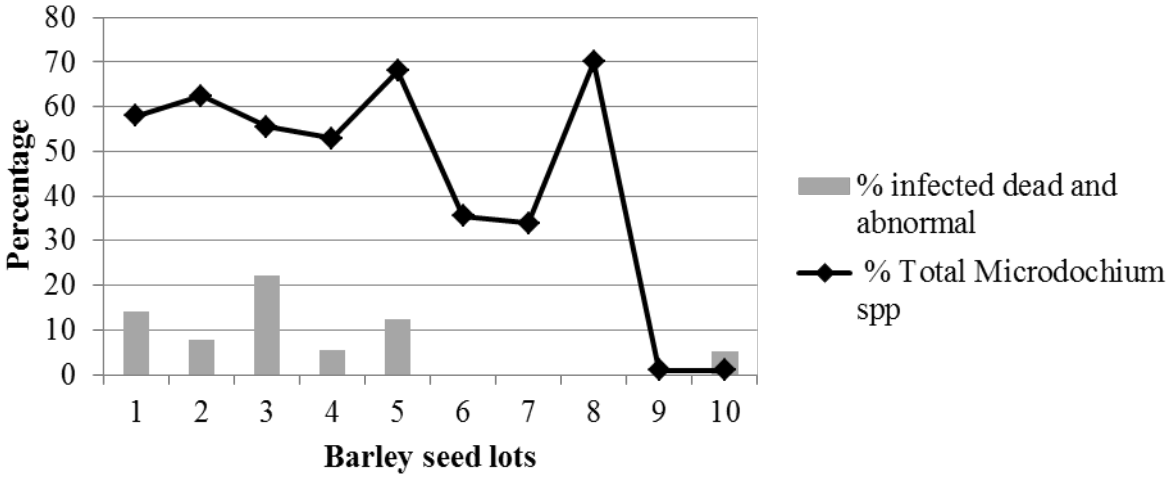

Figure 4. Percentage dead seed and abnormal barley seedlings and total percentage *Microdochium* species seed infection of each lot.

DISCUSSION

The relationship between the number of seeds emerged and the total percentage *Microdochium* species for oats gave an R^2=0.635 and for barley R^2=0.393 (i.e. *Microdochium* species accounts for approximately 64% of the emergence variability in oats and only 39% in barley). This corroborates the findings of McNeil *et al* (2014) where maximum R-squared values in

their field experiments were R^2=0.911 for oats and R^2=0.462 for barley. This confirms that a stronger relationship exists between *Microdochium* species seed infection and seedling loss in spring oats than in spring barley.

This experiment has confirmed the host specificity of previous experiments (McNeil *et al*, 2014) showing that only *M. nivale* was detected on the dead and abnormal seedlings in all oat seed lots and that *M. majus* infected the abnormal barley seedlings with *M. nivale* only being detected on barley normal seedlings in lot 3.

In these laboratory experiments, *M. nivale* was responsible on average for 26% of seedling loss in oats. In seed lot 7, *M. nivale* accounted for 55% of dead seeds (seedling loss), a similar percentage to the total *Microdochium* seed infection of 59% recorded in the agar plate test.

In barley 7% of seedling losses were due to *M. majus*. *M. nivale* was only detected in normal seedlings which occasionally showed signs of disease but it has not shown itself responsible for seedling loss. No dead seeds were found to be infected with *Microdochium* species, but 4.5% of normal seedlings were found to carry *M. majus* and only 0.7% *M. nivale*.

Perry (1986) found that *Microdochium* species caused no reductions in plant populations of spring barley and suggested that *Microdochium* species exist as persistent endophytes within symptomless plants and the soil-borne element may explain their common occurrence on seed. The presence of this pathogen as an endophyte may explain the high levels of infection found on spring barley seed and the low levels of seedling loss encountered.

Glynn *et al* (2008) found no difference in pathogenicity of *M. nivale* and *M. majus*. However, within this laboratory experiment we have shown that there does appear to be a difference, as *M. majus* has not been implicated or associated with seedling loss in the oat seed lots.

ACKNOWLEDGEMENTS

These experiments were carried out with the assistance of the OSTS analysts, with special mention to Sam Borrowman for all the *Microdochium* species colony identifications. Thanks go also to Adrian Roberts, BioSS, for statistical advice.

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QUANTIFYING THE REAL THREAT OF ALS-RESISTANT BROAD-LEAVED WEEDS IN UK ARABLE CROPPING SYSTEMS AND DEVELOPING EFFECTIVE MANAGEMENT STRATEGIES

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Summary: The number of populations of broad-leaved weeds tested annually and confirmed as ALS-resistant in the UK remains low. Over a four-year period less than 50 'suspect' UK populations of broad-leaved weeds were tested of which 22 common poppy (*Papaver rhoeas*), 8 common chickweed (*Stellaria media*) and 7 scentless mayweed (*Tripleurospermum inodorum*) were confirmed as ALS-resistant. The collection of broad-leaved weed seed is more complicated than for grass weeds which may be inhibiting the number tested. The use of ALS-inhibiting herbicides remains high across the rotation and so there is a risk of resistance developing. However, trial results show that ALS-resistant populations of common poppy can be effectively controlled by herbicides with alternative modes of action. Non-chemical options are limited and therefore it is essential that a wide range of herbicide actives remain available to farmers to enable effective broad-leaved weed management and reduce an over reliance on ALS-inhibitor herbicides.

INTRODUCTION

The current state of herbicide resistance in broad-leaved weeds in the UK was summarised by Hull *et al*., (2014) reporting acetolactate synthase (ALS)-resistant common poppy (*Papaver rhoeas*) on over 40 farms in nine counties, common chickweed (*Stellaria media)* on more than 50 farms in 13 counties and scentless mayweed (*Tripleurospermum inodorum)* on five farms in three counties (two England and one Scotland). In the UK the main cases of broad-leaved weed resistance are target site resistance (Marshall *et al*., 2010) to ALS-inhibiting herbicides, however cases of mecoprop-resistant chickweed were reported by Lutman & Snow (1987), but this now appears to be quite isolated with no further cases in nearly 30 years. Across Europe broad-leaved weeds have shown resistance to other herbicide modes of action (Torra *et al*., 2010). The risk of broad-leaved weed resistance increasing rapidly in the UK is potentially high as there is an increased use of ALS herbicides (Heap, 2015) and a lack of herbicides available with alternative modes of action. Broad-leaved weeds generally produce a high number of seeds, and are long-lived in the seedbank, particularly in the case of common poppy, which are high risk for developing resistance (Tatnell *et al*., 2007). However, in reality there

has not been a rapid increase in reported cases of resistance over the last 15 years since the first report of broad-leaved weed resistance, which is contrary to what may have been expected.

Herbicide resistance in UK grass weeds is now widespread (Hull *et al*., 2014, Moss *et al*., 2011) and by learning the lessons from black-grass resistance in particular, and applying the knowledge gained for its management or prevention, it is hoped that broad-leaved weed resistance will not develop to the same extent as the grass weeds.

Broad-leaved weed resistance is being monitored closely and data from ADAS and crop protection company resistance testing from the last four years have been amalgamated to quantify the current extent of broad-leaved weed resistance in the UK. A current research project, in its final year, aims to develop practical solutions to prevent a wide-scale increase in ALS resistant broad-leaved weeds, focussing on common poppy, through effective management in a cereal/oilseed rape crop rotation (Tatnell *et al*., 2014). Results from the broad-leaved weed seed testing for herbicide resistance are presented in this paper, along with guidelines for effective management of broad-leaved weeds from the research project field experimental data.

MATERIALS AND METHODS

Seed testing of selected broad-leaved weeds

Populations of broad-leaved weeds, including common poppy, common chickweed and mayweed were identified in UK field sites where control levels had been poor for more than two cropping seasons. Seed collected by ADAS in July 2014 were tested for resistance to a range of herbicides using a standard glasshouse pot test method detailed below (crop protection company test methods may vary). Plastic plant pots measuring 9cm diameter were filled with Kettering loam 'weed' mix (80% sterilised loam + 20% grit + 2kg Osmacote slow release fertiliser) to 2 cm below the pot rim and placed in trays on the glasshouse bench and watered to field capacity over a period of 24 hours before sowing seed. Pots were labelled and weed seeds were hand sown with six replicates per weed population.

Pots were placed in a glasshouse with a temperature/light regime of 18^{o}C for 14 hours with lights and 12^{o}C for 10 hours no lights. Weeds were thinned to three plants/pot at the 1-2 leaf (BBCH 11-12) stage.

In the ADAS tests weeds were sprayed at the 4-6 true leaf stage (BBCH 14-16) using the treatments shown in Table 1, an untreated control was included for each weed population. Herbicides were applied in 200 l/ha water using a Mardrive automated pot sprayer, with two F110 nozzles at 2 bar. Plants were assessed 4 weeks after treatment with a visual score of the plants using a 0-10 rating (where 10 = live/healthy plants and 0= dead plants) and a foliage fresh weight (g) of plants per pot. A total of 12 poppy, two chickweed and six mayweed samples were tested by ADAS in 2015.

Table 1. Herbicide treatments and dose used against broad-leaved weeds tested by ADAS.

Herbicide treatments		Weed species		
Active ingredient	Dose g a.i./ha	Poppy	Chickweed	Mayweed
Metsulfuron-methyl	6g	✓	✓	✓
MCPA	1000g	✓		
Fluroxypyr	200g		✓	
Mecoprop-p	1380g		✓	
Florasulam	0.25g		✓	✓
Clopyralid	100g			✓

Crop protection companies also tested a number of broad-leaved weed populations from sites where resistance was 'suspected', so it is important to note that these were not random samples, but they had been identified as sites with control issues. It was not possible to determine whether there was any overlap between the ADAS and company samples, however the chance of more than one test from the same field site was considered negligible. In total, less than 10 populations of each weed species were tested annually by the companies between 2012 and 2015.

Field experimentation on common poppy

A three-year field experiment was established on a site with known ALS-resistant common poppy in Cambridgeshire in 2012. Four simple treatments were tested 1) untreated control, 2) ALS-inhibitor alone, 3) ALS-inhibitor + non-ALS and 4) non-ALS herbicide, which were replicated four times and the specific herbicides were selected depending on the crop present. Plots measured 12m x 12m, with buffer strips between each replicate block to minimise pollen transfer. The plots remained in the same position each year to ensure the resistance pressures remained constant and the crop rotation included wheat (2013), wheat and oilseed rape (2014) and wheat (2015). Poppy heads were counted in the June of each season to assess the level of weed control for each treatment. After the first two experimental years a decision was taken to remove the ALS-inhibitor herbicide treatments and to manage the weed population with a non-ALS herbicide only, due to the very high weed numbers and lack of control from any ALS-inhibitors on this known resistant population. Results are therefore presented for two experimental years.

RESULTS

Seed testing

There were a relatively small number (20) of broad-leaved weed populations available for seed collection in 2014, despite a large effort to find populations where control had been poor over the previous few seasons. Of the seed tested by ADAS eight common poppy (all from England) and four mayweed populations (one Scotland, three England) were confirmed ALS-resistant (tested with metsulfuron-methyl), however both chickweed samples (from England) tested were fully controlled by metsulfuron-methyl (89% and 94% control) and greater than 97% control was achieved by the other three herbicides actives tested (Table 2).

Table 2. Number of broad-leaved weeds tested and level of control from herbicides.

Herbicide active ingredient	Species (Populations tested)					
	Poppy (12)		Chickweed (2)		Mayweed (6)	
	Resistant populations	Range % control	Resistant populations	Range % control	Resistant populations	Range % control
Metsulfuron-methyl	8	0-45	0	89-94	4	44-57
MCPA	0	88-98	-	-	-	-
Fluroxypyr	-	-	0	97-98	-	-
Mecoprop-P	-	-	0	97-99	-	-
Florasulam	-	-	0	97-99	0	100
Clopyralid	-	-	-	-	0	98-99

- *Not tested*

The level of control in all non-resistant populations was greater than 81%.

A total of 25 populations were confirmed to have ALS-resistance by crop protection companies over the four-year period. These included 14 common poppy, 8 chickweed and 3 mayweed populations. All common poppy populations were from England. All chickweed populations, except one were from Scotland and two out of the three mayweed populations were also from Scotland. There were a total of nine populations collected in 2015 which are currently being tested.

Field experimentation results

Data from the two-year poppy field experiment are shown in Figure 1. An ALS-inhibitor herbicide alone achieved no control of this resistant poppy population compared to the untreated controls. However, a non-ALS herbicide alone gave good levels of control (mean 89%) compared to the untreated controls of this ALS-resistant population.

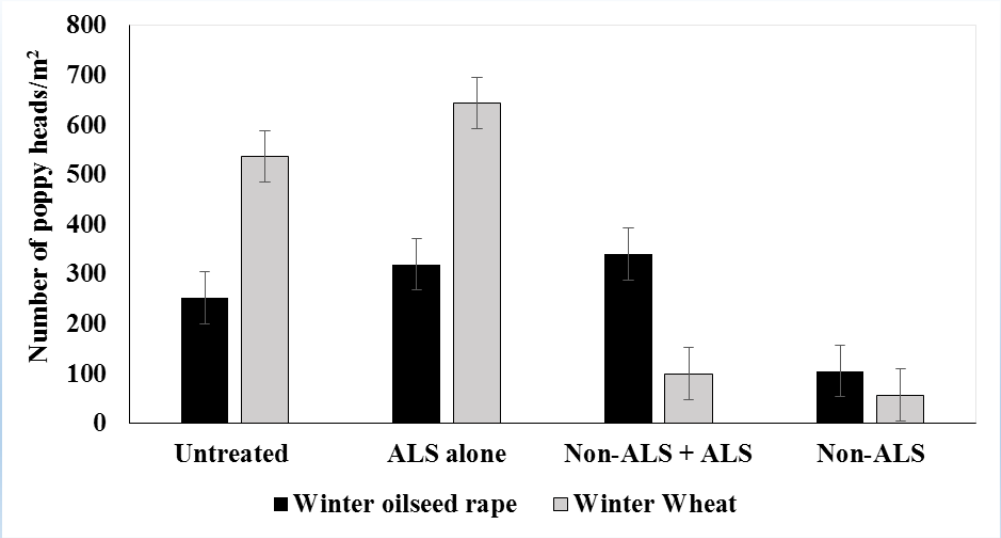

Figure 1. Mean number of common poppy heads per m^2 for different crops and herbicide treatments.

DISCUSSION

The number of populations of broad-leaved weeds tested annually in the UK for resistance remains low and there are three possible reasons for this. 1) Broad-leaved weeds seed collecting is much more difficult than for grass weeds due to their biology. Except for common poppy, where capsules above the crop canopy ripen together making collection simple. For mayweed and chickweed seeds mature over an extended time period and are often below the crop canopy. 2) The mechanism of resistance identified to date in UK broad-leaved weeds is target site, whereas the resistant grasses also have enhanced metabolism resistance. Therefore finding resistant individuals and selecting those in broad-leaved weed populations is less likely than if enhanced metabolism mechanisms were involved. However, this might change if enhanced metabolism resistance is detected in UK populations. 3) Control levels remain good due to availability of alternative modes of action and this results in only limited, often seasonal, poor control concerns. Confirmed ALS-resistant populations now include 12 mayweed, more than 70 poppy and more than 40 chickweed in the UK.

In addition to the low numbers of 'suspected' resistant seed tested, only a small proportion of broad-leaved weed populations have confirmed resistance, despite an increasing reliance on the high risk mode of action ALS-inhibiting herbicides. This is also likely to be because although there is a reduced number of herbicides available, of those remaining there are still many options to effectively control broad-leaved weeds in an arable rotation (Marshall *et al*., 2010). This is illustrated by the results of the two-year rotational poppy field trial, where a highly ALS-resistant population was well controlled by a robust herbicide programme, including pre-emergence and post-emergence non-ALS herbicides. However, if legislation or other changes remove non-ALS herbicides the effective management of key broad-leaved weed such as common poppy will be threatened. In addition to product availability, the timing of a post-emergence herbicide for effective broad-leaved weed control must be at the correct weed growth stage, otherwise control will be reduced and false resistance identified. Seasonal weather variations will affect the weed growth in the spring and so the timing of the post-emergence herbicide must be tailored to ensure maximum herbicide efficacy. Non-chemical control is not as effective for weed management of broad-leaved weeds compared to the grasses due to their high seed production and seed longevity.

Resistant broad-leaved weeds are currently manageable in the UK if a robust herbicide programme including alternative modes of action are available and applied at the correct timings. However, any loss of active substances and the increased reliance on a smaller group of herbicides, in particular the ALS-inhibitors, will lead to increased resistance development. As early identification is essential to reduce risks broad-leaved weed patches must be monitored closely and appropriate management, such as the removal of small patches with a non-selective herbicide or by hand, should be administered to combat the spread of resistance.

ACKNOWLEDGEMENTS

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HALAUXIFEN-METHYL A NEW HERBICIDE FOR THE CONTROL OF BROADLEAF WEEDS IN CEREALS

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Summary: Arylex [TM 1] active, common name halauxifen-methyl, is a new active substance discovered by Dow AgroSciences for the cereal market. It is a post-emergence HRAC Group O herbicide which belongs to the new family of 6-arylpicolinate herbicides. Halauxifen-methyl is a foliar systemic herbicide with efficacy maintained even under cool temperature conditions and it can be used from 1st September to 30th June. GF-2644 (halauxifen-methyl + florasulam 6+5 g a.e.-a.s/litre, OD) was applied in the autumn and spring to seven key broadleaf weed species and GF-2819 (halauxifen-methyl + fluroxypyr-meptyl, 12+280 g a.e/litre, EC) was applied in the spring to four key broadleaf weed species. Control of all species, including ALS-resistant *P. rhoeas* and *S. media*, was equivalent or better than the standards. Halauxifen-methyl shows broad spectrum activity, with robust and consistent efficacy of broad leaf weeds even during periods of poor / variable weed growth, providing the grower with flexibility on application timing.

[1]Trade mark of Dow AgroSciences Ltd

INTRODUCTION

Halauxifen-methyl is a new active substance discovered by Dow AgroSciences for the global cereal market. It is a post-emergence HRAC Group O herbicide (synthetic auxin) which belongs to the new family of 6-arylpicolinate herbicides. It is a foliar systemic herbicide with limited residual activity for use in winter and spring cereals from 1st September to 30th June. It is translocated through the phloem and xylem to the meristematic areas and induces a phenotypic response on sensitive plant species similar to the natural/synthetic auxin herbicides. Efficacy is expressed independent of variable weather conditions. Therefore it offers a wide window of application and more opportunity for application. Furthermore, as a member of the HRAC Group O herbicides, halauxifen-methyl is considered to be a low risk herbicide for resistance and can be used as a resistance management tool. Applied at low use rates (< 7.5 g a.e/ha), halauxifen-methyl offers broad spectrum broadleaf weed control of commercially important weed species including; *Chenopodium album, Galeopsis tetrahit, Galium aparine, Fumaria officinalis, Lamium species, Polygonum convolvulus, Papaver rhoeas, Stellaria media* and small *Veronica* species. This paper introduces two formulations designed for the cereal market which both contain the new active substance halauxifen-methyl.

Efficacy of halauxifen-methyl (6 g a.e/litre) + florasulam (5 g a.i/litre), (OD formulation coded GF-2644) and halauxifen-methyl (12 g a.e./litre) + fluroxypyr-meptyl (280 g a.e/litre) (EC formulation coded GF-2819) on commercially important weed species including ALS-resistant

weeds was determined. The formulations were applied in the autumn or spring to winter and spring cereals.

MATERIALS AND METHODS

Trial design

Fourteen replicated field trials were conducted to GEP standards in the UK, Germany and Ireland over a 4 year period spanning 2010 to 2014. All trials were designed as randomized complete blocks with 4 replicated plots per treatment and a minimum plot size of 2 x 6 m. The growth stages of all test species at application were recorded (Table 1). All applications were sprayed post-emergence to the crop and weeds using a precision small plot sprayer calibrated to deliver 150 to 250 litre/ha.

Crop safety assessment

Trials were assessed for any visual symptoms of crop injury. Parameters for assessment were % visual chlorosis, growth inhibition (stunting) and vigour reduction. Assessments were typically made at 7, 14, 28 and 56 days after application.

Efficacy assessment

Assessments for % visual control relative to the untreated plots were made at regular intervals and the final assessment data are presented in Tables 2-8 and in Figure 1. Control was assessed on a linear scale, where 0% represents no control and 100% represents plant death.

Application timing

Autumn applications were made to winter wheat at Zadoks 11-22 and winter barley at Zadoks 12-14 (Tables 2 to 4). Spring applications were made to winter wheat at Zadoks 22-30 (Tables 5, 8 & Figure 1) and to spring wheat & barley at Zadoks 23-32 (Tables 6-8). Weed growth stages and densities were as follows:

Table 1. Weed growth stages and densities at time of application.

Species	Growth stage	Weed density/m2	Weed height/diameter (cm)
Stellaria media	BBCH 21-22	20	0.5 -10
Papaver rhoeas	BBCH 11	11	2.5
Matricaria chamomilla	BBCH12-13	40	2
Matricaria inodora	BBCH 12-14	11	5
Chenopodium album	BBCH 26-27	9	7
Fumaria officinalis	BBCH 13-32	22-49	13-32
Polygonum convolvulus	BBCH 23-32	10-47	2-5
Stelleria media	BBCH 30 -31	15-18	3-20
Papaver rhoeas	BBCH 22-24	174	8-12

RESULTS

Autumn applications

Four trials were conducted in UK and Germany. In both countries, *Matricaria species, S. media* and *P. rhoeas* are important autumn germinating weeds in cereals and ALS-resistant populations have been confirmed in the UK. GF-2644 was applied at 0.75 litre/ha and compared to Boxer (florasulam 50 g a.i/litre SC), Picona (pendimethalin + picolinafen 320 + 16 g a.i/litre, SC), Spitfire (florasulam + fluroxypyr-meptyl, 5 + 100 g a.s -a.e/litre SE) and Quartz 50 (diflufenican 50 g a.i/litre SC). Crop injury assessments were conducted but no phytotoxicity was observed from any treatment and no data are presented.

Table 2. Mean % visual control of M. inodora and M. chamomilla 165 -167 days after herbicide application in UK 2010 and Germany 2012 in winter wheat

Species	Treatment	Rate	% Visual control
M. inodora (UK)	Untreated	0	0
	GF-2644	0.75 L/ha	99
	florasulam	75 ml /ha	97
	pendimethalin+picolinafen	3 L/ha	76
	diflufenican	100 ml/ha	76
Data from 1 trial			(P=0.05, LSD=8)
M. chamomilla (Germany)			
.	Untreated	0	0
	GF-2644	0.75 L/ha	100
	florasulam	75 ml /ha	99
	pendimethalin+picolinafen	3 L/ha	96
Data from 1 trial			(P=0.05, LSD=6)

Table 3. Mean % visual control of S. media 86 days after herbicide application UK 2010 in winter wheat

Treatment	Rate	% Visual control
Untreated	0	0
GF-2644	0.75 L/ha	99
Florasulam	75 ml/ha	100
Pendimethalin+picloinafen	3 L/ha	83
Diflufenican	100 ml/ha	34
Data from 1 trial		(P=0.05, LSD=11)

Table 4. Mean % visual control of P. rhoeas 86 days after application in UK 2012 in winter wheat

Treatment	Rate	% Visual control
Untreated	0	0
GF-2644	0.75 L/ha	100
Florasulam	75 ml/ha	5
Florasulam+fluroxypyr	0.75 L/ha	5
Pendimethalin+picolinafen	3 L/ha	100
Data from 1 trial		(P=0.05, LSD=11)

Spring applications

A total of nine trials were conducted in the UK, Germany and Ireland. In the UK *F. officinalis, C. album, P. convolvulus* and *G. tetrahit* are important spring germinating weed species in cereals. GF-2644 was applied at 1 litre/ha and GF-2819 was applied at 0.5 litre/ha. Comparisons were made to florasulam, florasulam+fluroxypyr, Ally SX (metsulfuron-methyl 200 g a.i/ kg), Ally Max SX (metsulfuron-methyl + tribenuron-methyl 14.3 + 14.3 % w/w SG), and Duplosan KV (mecoprop-P 600 g a.e./litre, SL). Crop injury assessments were conducted but no phytotoxicity was observed from any treatment and no data are presented.

Table 5. Mean % visual control of F. officinalis 55 - 84 days after herbicide application in UK and Germany 2012 – 2013 in winter wheat.

Treatment	Rate	% Visual control
Untreated		0
GF-2644	1.0 L/ha	100
GF-2819	0.5 L/ha	99
Mecoprop-P	2 L/ha	99
		(P=0.05, LSD=4)
Data from three trials (UK 2, GER 1)		

Table 6. Mean % visual control of C. album 55 days after herbicide application in UK 2013 in spring barley.

Treatment	Rate	% Visual control
Untreated	0	0
GF-2644	1 L/ha	99
GF-2819	0.5 L/ha	99
Florasulam+fluroxypyr	1.0 L/ha	40
Mecoprop-P + metsulfuron-methyl+tribenuron-methyl	0.5 L/ha +30 g/ha	93
Data from 1 trial		(P=0.05, LSD=12)

Table 7. Mean % visual control of P.convolvulus 60-70 days after herbicide application in UK 2012 - 2013 in spring wheat and barley.

Treatment	Rate	% Visual control
Untreated	0	0
GF-2644	1 L/ha	91
GF-2819	0.5 L/ha	96
Florasulam+fluroxypyr	1.0 L/ha	95
Florasulam	125 ml /ha	74
		(P=0.05, LSD=10)
Data from three trials		

Control of ALS resistant P. rhoeas and S. media populations

Three trials were conducted in England (ALS-resistant P. rhoeas) and Ireland / Scotland (ALS-resistant S. media). In the UK ALS resistance and reports of difficult to control populations of both species are on the increase. GF-2644 was applied at 1 litre/ha to P. rhoeas and GF-2819 was applied at 0.5 litre/ha to S. media (Fig 1 and Table 8) Comparisons were made to florasulam, florasulam +fluroxypyr, metsulfuron-methyl Starane Hi Load HL (fluroxypyr-meptyl 333 g a.e/litre, EC) and Pointer SX (tribenuron-methyl 500 g a.i/kg, WG). Crop injury assessments were conducted but no phytotoxicity was observed for any treatment and no data are presented.

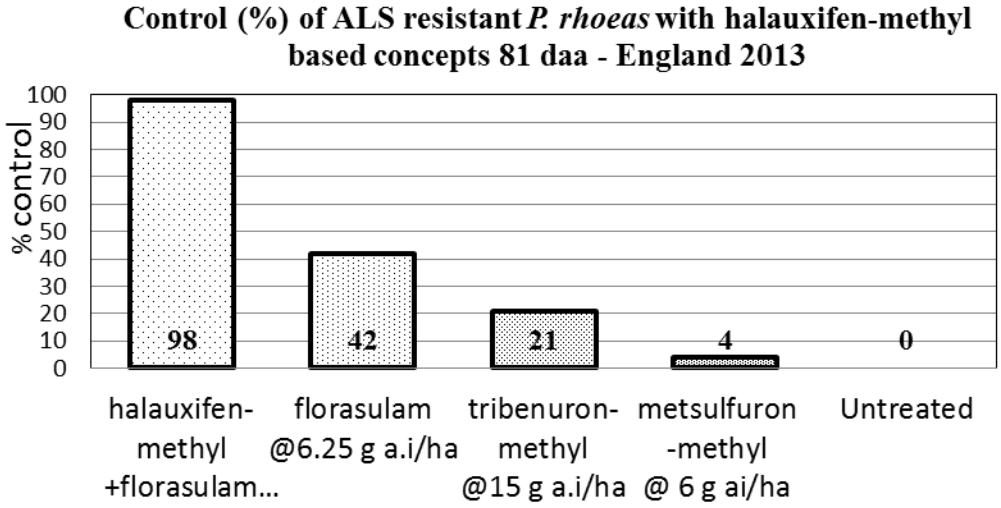

Figure 1. Mean % visual control of ALS resistant P. rhoeas, data from one trial.

Table 8. Mean % visual control of S. media 84 days after herbicide application in one Scottish and one Irish trial applied in 2014, and established in winter & spring wheat

Treatment	Location Ireland	Rate	% Visual control
Untreated		0	0
GF-2819		0.5 L/ha	95
Florasulam+fluroxypyr		1.0 L/ha	95
Metsulfuron-methyl+tribenuron-methyl		30 g pr/ha	0
Data from one trial			
	Scotland		
Untreated		0	0.0
GF-2819		0.5 L/ha	100
Florasulam+fluroxypyr		1.0 L/ha	100
Florasulam		125 ml/ha	100
Fluroxypyr		0.6 L/ha	100
Metsulfuron-methyl+tribenuron-methyl		30 g pr/ha	38
Data from one trial			(P=0.05, LSD=2.01)

DISCUSSION

GF-2644 applied in autumn provided excellent control (> 99 %) of *M. inodora, M. chamonilla* and *S. Media* (Tables 2-3). Efficacy was equivalent to florasulam and equivalent or significantly (P=0,05, LSD) better than pendimethalin+picolinafen and diflufenican. The weather conditions for the *P. rhoeas* trial (Table 4) at application were good, with mild temperatures and dry conditions following a wet autumn. Within a day of application the weather turned cold with temperatures below freezing and snow cover up to 20 days after application. Under these conditions the standards, florasulam (75 ml/ha) and florasulam + fluroxypyr (0.75 litre/ha), were not effective, providing only 5 % control 86 days after application. The adverse weather conditions did not impact the efficacy of either GF-2644 or pendimethalin+picolinafen with both herbicides achieving 100 % control of P. rhoeas.

GF-2644 and GF-2819 applied in the spring provided high levels of control of *C. album, F. officinalis* and *P convolvulus* in winter and spring cereals (Tables 5-7), with both formulations providing > 99 % control of *F. officinalis* and *C. album*. This level of control was equivalent to that of mecoprop-P (1200 g a.e/ha) and duplosan + metsulfuron-methyl + tribenuron-methyl (0.5 litre/ha + 30 g/ha). Control of *P. convolvulus* was also high with both GF-2644 and GF-2819 achieving > 91 %, which was equivalent to florasulam +fluroxypyr applied at 1 litre/ha which provided 95 % control of *P. convolvulus.*

With the increase in cases of ALS-resistant and difficult to control populations of *S. media* and *P .rhoeas*, it is important that any new herbicide provides effective control of these populations with no cross resistance to the ALS chemistry. There was no cross-resistance between GF-2644 and GF-2819 and the ALS herbicides (Table 8 and Figure 1). GF-2644 applied at 1 litre /ha provided 98 % control of ALS-resistant *P. rhoeas* compared to 42 % with florasulam (125 g a.i/ha), 21 % with tribenuron-methyl (15 g a.i/ha) and 0 % with metsulfuron-methyl (6 g a.i/ha). GF-2819 applied at 0.5 litre/ha and florasulam +fluroxypyr-meptyl at 1 litre/ha provided 95 - 100 % control of ALS-resistant S. media compared to 0 – 30 % with metsulfuron-methyl applied at 6 g a.i/ha. This high efficacy supports the use of halauxifen-methyl as a new resistance management tool for ALS resistant populations. In addition to species discussed in this paper other trials work conducted throughout Europe on halauxifen-methyl containing formulations demonstrated robust efficacy on: *Anagallis arvensis*; many *Brassica* species including *Capsella bursa-pastoris*; *Geranium* species; *Galium aparine*; *Lamium* species; *Myosotis arvensis, Senecio vulgaris; Veronica* species (2-4 leaves) and volunteer legumes (Becker et al, 2015). Halauxifen-methyl containing formulations controlled autumn and spring germinating weeds including ALS-resistant biotypes, even under adverse weather conditions. In conclusion Halauxifen-methyl is a new flexible broad spectrum herbicide that can be used in winter and spring cereals from 1st September to 30th June, offering a wide window of application and more “spray days”.

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CAN WE EVER GET ENOUGH? THE NEED FOR INCREASED PRODUCTIVITY AND IMPROVED DISEASE RESISTANCE, SPECIFICALLY IN WHEAT

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Summary: During the last decade there has been much debate about the need to sustain and increase the productivity of British Agriculture to help meet the challenges of global population growth and climate change. These issues have been discussed in great depth elsewhere, including the Foresight 'Future of Food and Farming' Report (2011) and the Royal Society publication 'Reaping the Benefits' (2009). As well as the imperative of feeding humankind, it is also my belief that increasing productivity will be essential if in the future land is to be spared for other environmental benefits, such as nature reserves and on farm stewardship schemes. In addition I believe that the areas of the farmed environment designated for crop production must be managed intensively to ensure efficient crop production and to make the most of the benign UK environment, which is ideally suited to produce high yields of the staple arable crops. Plant breeding will be essential to help achieve these goals. Plant breeders must provide high yielding, disease resistant varieties. Using the example of winter wheat I believe that current genetic yield gain can be maintained, although for the longer term it may be insufficient to meet national and global demand. As important will be the use of enhanced phenotypic selection, molecular markers, genomics and other technologies to improve selection for disease resistance, including the selection of 'durable' resistance components and recombining 'stacks' of resistance genes. This will be of increasing relevance if reductions in fungicide efficacy continue and new legislation removes useful pesticide active ingredients. That said, even with improved selection methods and access to new sources of resistance it is clear that new varieties will require rational agronomic packages, designed to maximise the genetic potential of the variety and to protect harvestable yield.

CONTEXT

Breeding winter wheat varieties for increased yield potential in the UK has a long and successful history and has made a significant contribution to crop production and economic output (DTZ report, 2010; Mackay *et al*, 2011). Although there are differences between the quality classes of wheat, yield gains have averaged around 0.5t/ha per decade since 1950 (Clarke *et al*, 2012).

It is possible to estimate future yield trends by extrapolation of data from HGCA Recommended List Trials (RLT) (figure 1) and I am confident that such yield gains will continue to be possible with the application of new breeding technologies, particularly molecular marker assisted selection (MAS) and genomics (including genomic selection, GS, and genome wide association studies, GWAS). However, even if such genetic yield advance is

maintained it is clear that it will fall some way short of the 30% to 50% increase needed over the next 20 to 30 years (Foresight Report 'Future of Food and Farming', 2011; Royal Society 'Reaping the Benefits' Report, 2009). Thus for the longer term yield increase must be accelerated. An efficient hybrid seed system for winter wheat could help in this regard but I believe that increasing photosynthetic efficiency may be the only sustainable solution.

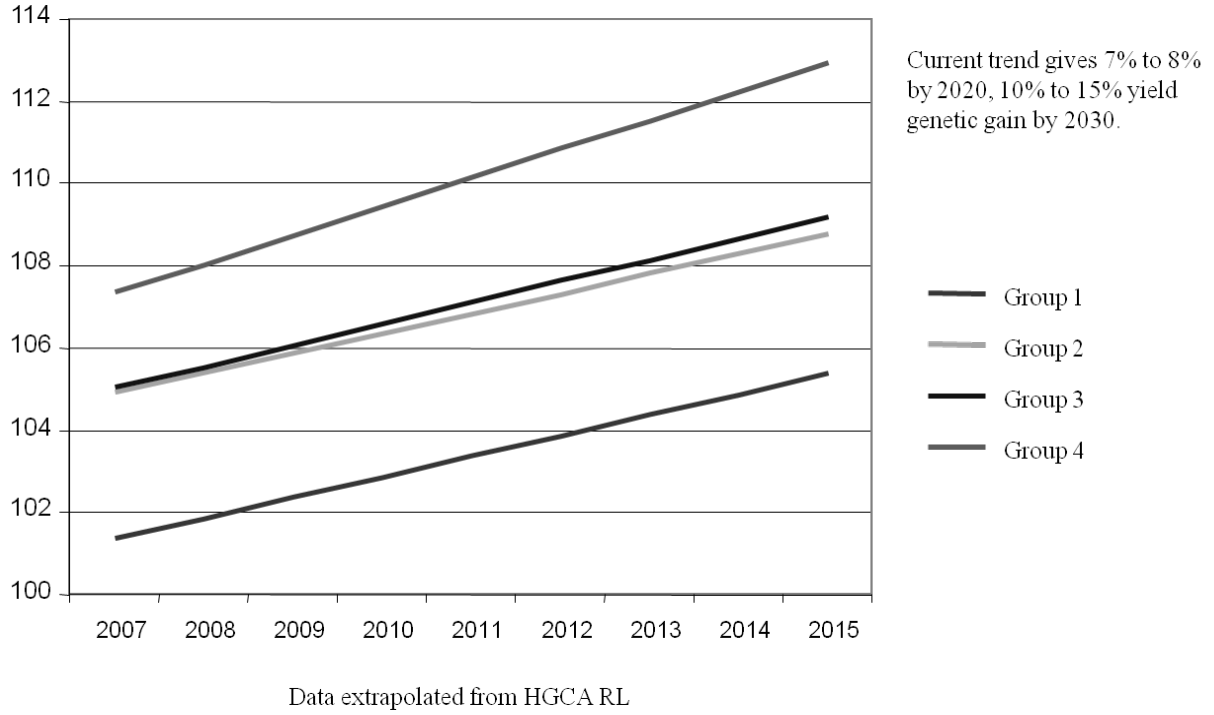

Figure 1 Trend for increasing UK winter wheat yields, by quality class

In the shorter term it is clear that the yield potential of new varieties must not only be fully exploited by relevant on farm agronomy but also be protected by adequate genetic disease resistance bred into the varieties. Genetic resistance is the most efficient way to protect a variety's yield potential but in my opinion will not remove the need for rational pesticide use in the foreseeable future. However breeding varieties that economically require only a single fungicide spray at full flag leaf or ear emergence would be a justified target.

The current disease resistance ratings on the HGCA RL demonstrate breeders have had some success in breeding for resistance and the reduction in fungicide response over the last 10 years (Figure 2) would also appear to support this success.

However, another important reason for the reduction in fungicide response is the erosion of the efficacy of important groups of systemic fungicides due to pathogen adaptation rather than increasing varietal resistance.

As shown in figure 3 there is an overall reduction of fungicide response between 2004/05 and 2014/15 that does not always appear to be related to the disease resistance profile of the variety. For example the variety Claire, which in general had an adequate disease resistance profile, gives a larger fungicide response in 2004/05 than in 2014/15. This apparent reduction in fungicide efficacy only serves to increase the importance of breeding stable genetic resistance.

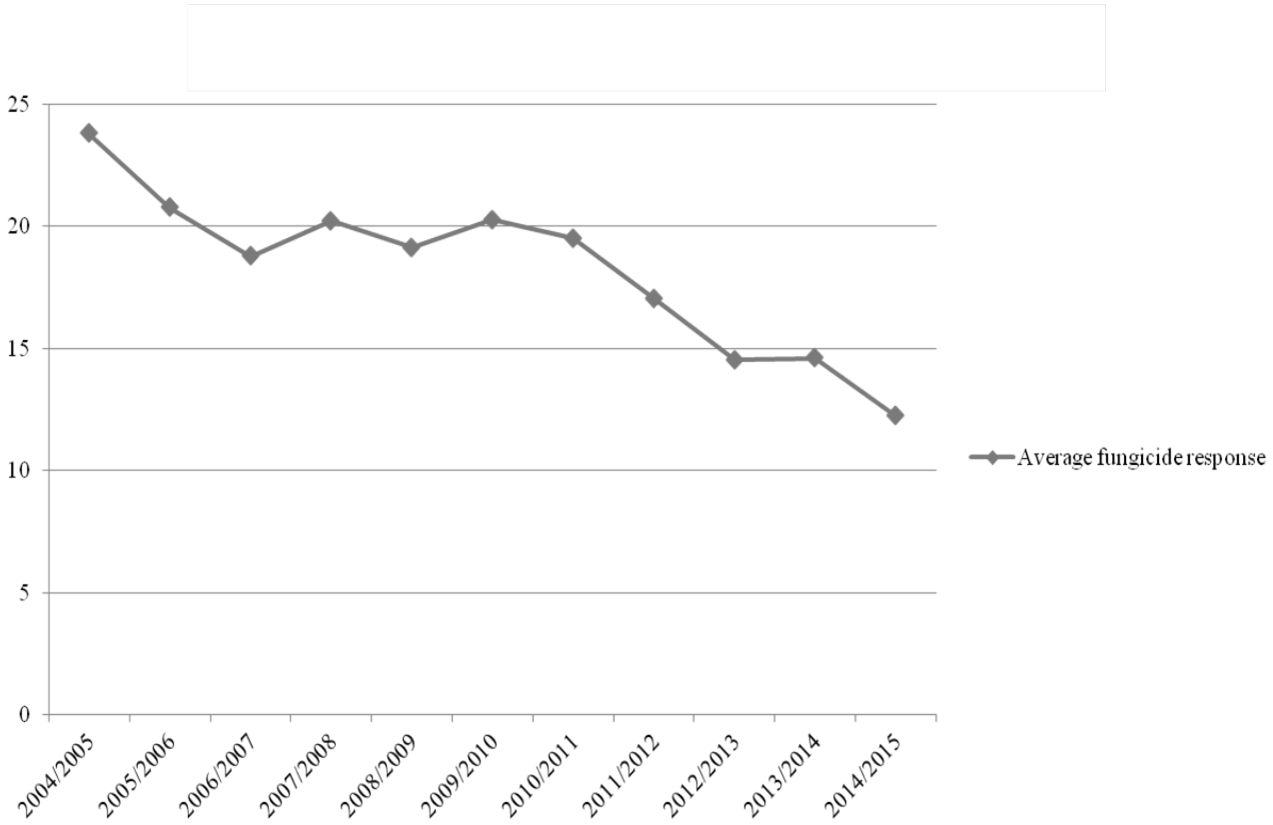

Figure 2 Average fungicide yield response in winter wheat HGCA RLT, 2005 to 2015

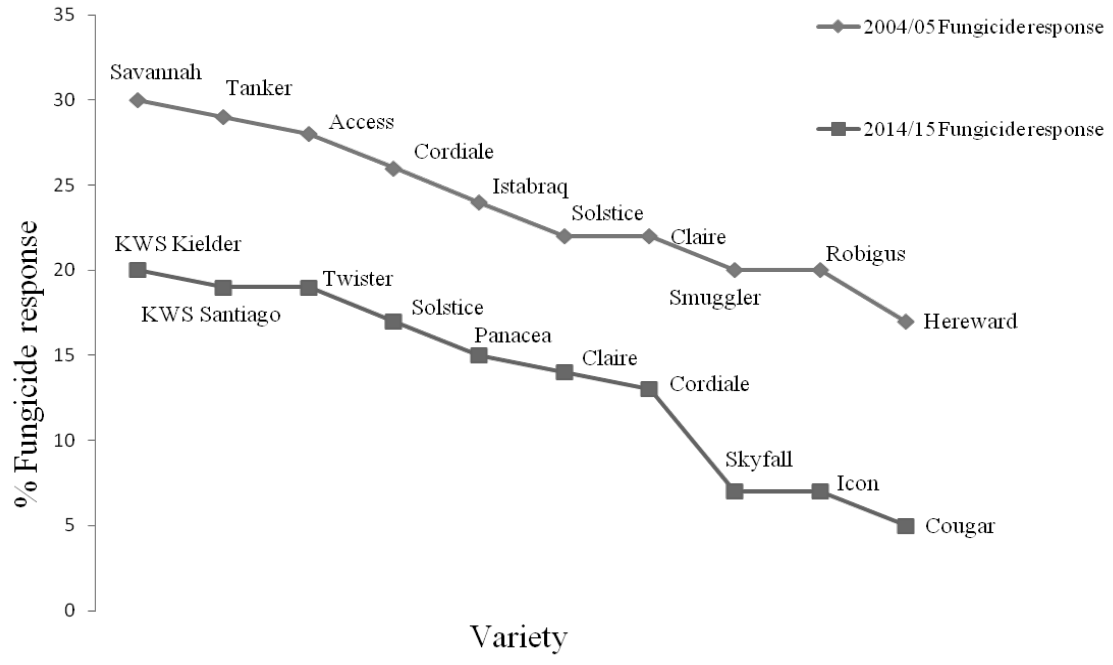

Figure 3 Yield response to fungicide treatment for selected varieties from HGCA RL 2004/05 compared to selected varieties from HGCA RL 2014/15

SELECTION FOR DISEASE RESISTANCE

As already discussed, selection for yield must be maintained and if possible accelerated. This implies that selection for disease resistance must not be at the expense of advancing genetic yield potential. Therefore breeding for disease resistance will rely not just on sourcing and introducing resistance genes but ensuring where possible that the genes have no negative effect on yield through linkage or pleiotrophy. In addition there must be efficient selection methods to reduce any breeding time drag associated with the introduction of the resistance. Finally, if at all possible, more durable sources of resistance should be used together with genes of major effect to reduce the likelihood of catastrophic resistance breakdown, as has been seen recently for yellow rust (*Puccinia striiformis*) resistance (Figure 4). We are fortunate that in the wheat gene pool good resistance exists to some of the important disease, for example Septoria tritici leaf blotch (*Zymoseptoria tritici*), Figure 5. However the genetic basis of resistance is often not yet fully understood, although modern techniques are giving us insights into the genetics much faster than was possible using more traditional methods. For example at RAGT we have used GWAS to identify the chromosomal location of resistance to brown rust (*Puccinia recondita*) in our French winter wheat breeding pool (Figure 6).

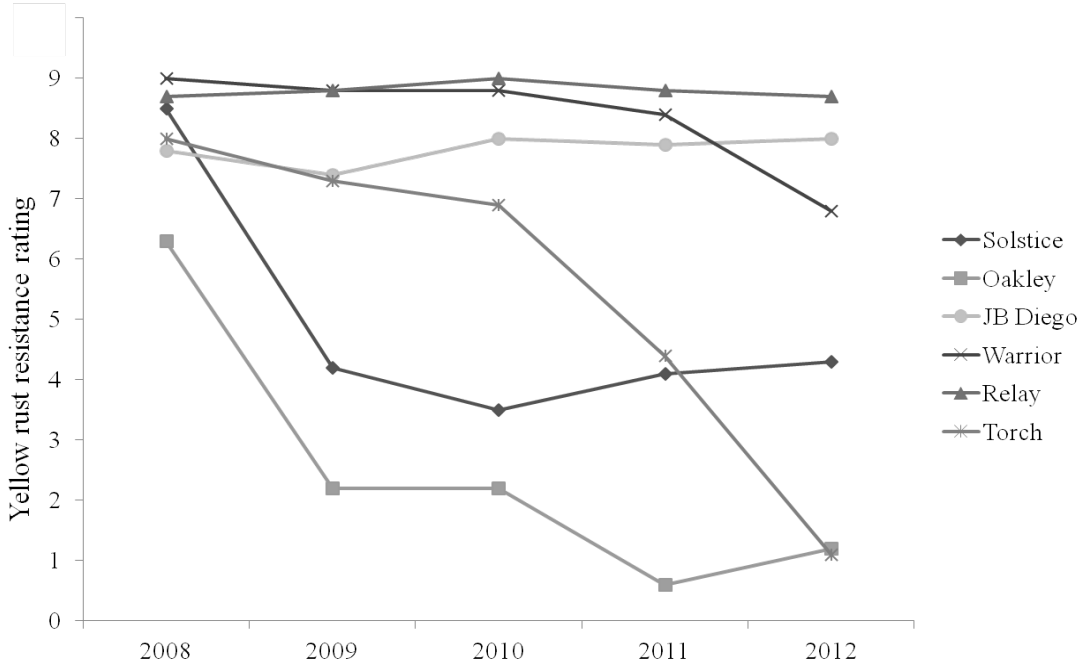

Figure 4 Yellow rust resistance breakdown in the UK 2008 to 2012

A good example of a lack of resistance sources in the cultivated wheat genepool was resistance to the fungal pathogen, eyespot (*Oculimacula spp*). A potent resistance source from *Aegilops ventricosa* was found and transferred into the wheat variety 'VPM' by French workers in the 1980's (Dousinnault et al, 1983). Breeding with the 'VPM' resistance, bestowed by the presence of the gene Pch1 from *Aegilops venticosa*, has had mixed success and it has become clear over the years that although the level of resistance bestowed is high and apparently durable, it is associated with a yield penalty (Figure 7).

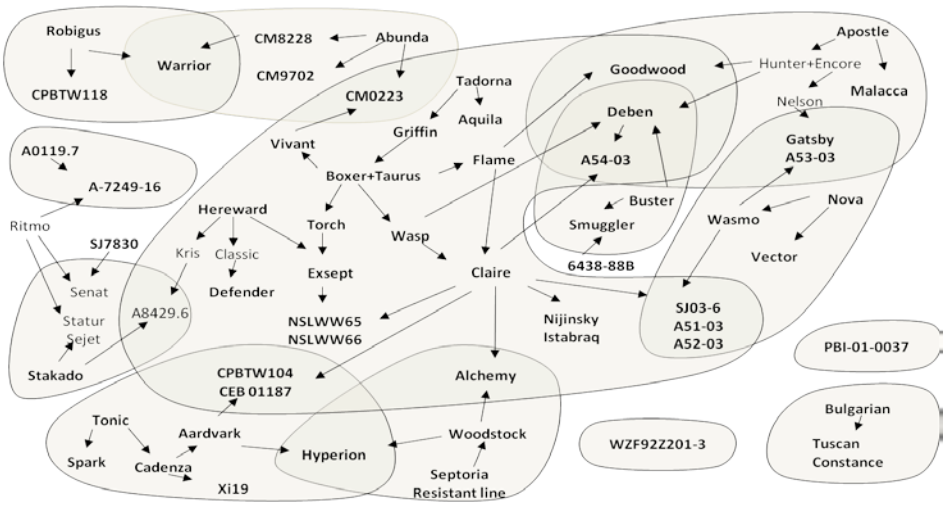

Figure 5 Putative Septoria tritici resistance sources (groups) in the UK winter wheat genepool

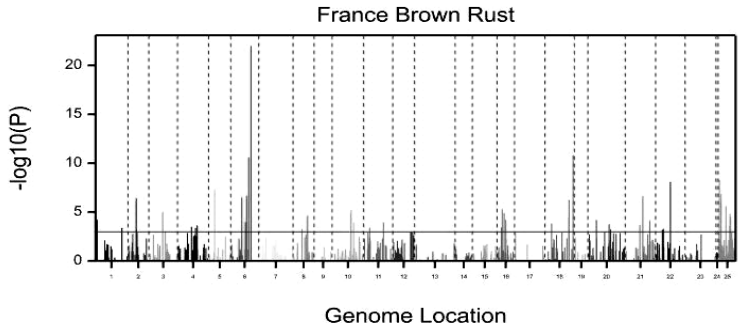

Figure 6 GWAS for brown rust resistance in the French winter wheat genepool

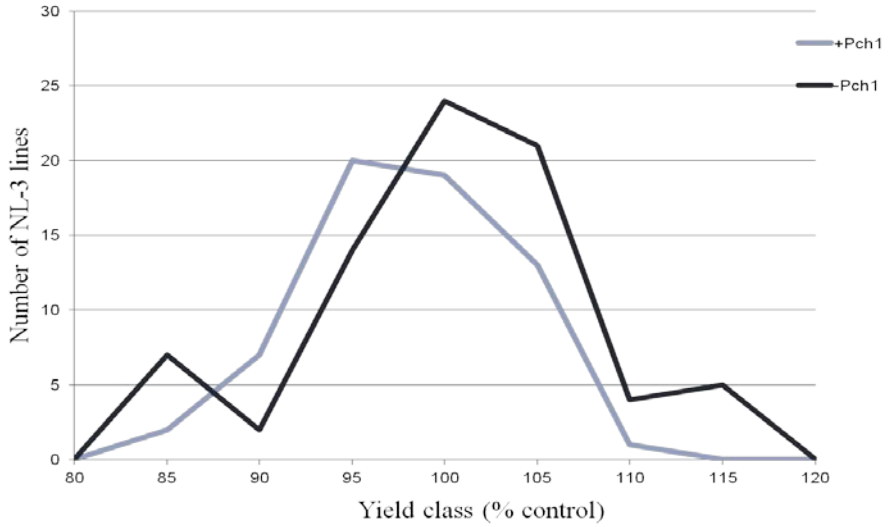

Figure 7 Yield performance of RAGT NL-3 lines (2003) with and without the *Aegilops ventricosa* eyespot resistance gene, Pch1. Lines derived from a common set of crosses. Highest yielding lines did not contain Pch1

Fortunately the yield penalty has been eroded over continual cycles of breeding, although not completely eliminated. RAGT data suggests that the improved yield performance of varieties containing the Pch1 resistance is due in part to the reduction of the *Aegilops venticosa* chromosomal segment containing the resistance gene (Figure 8).

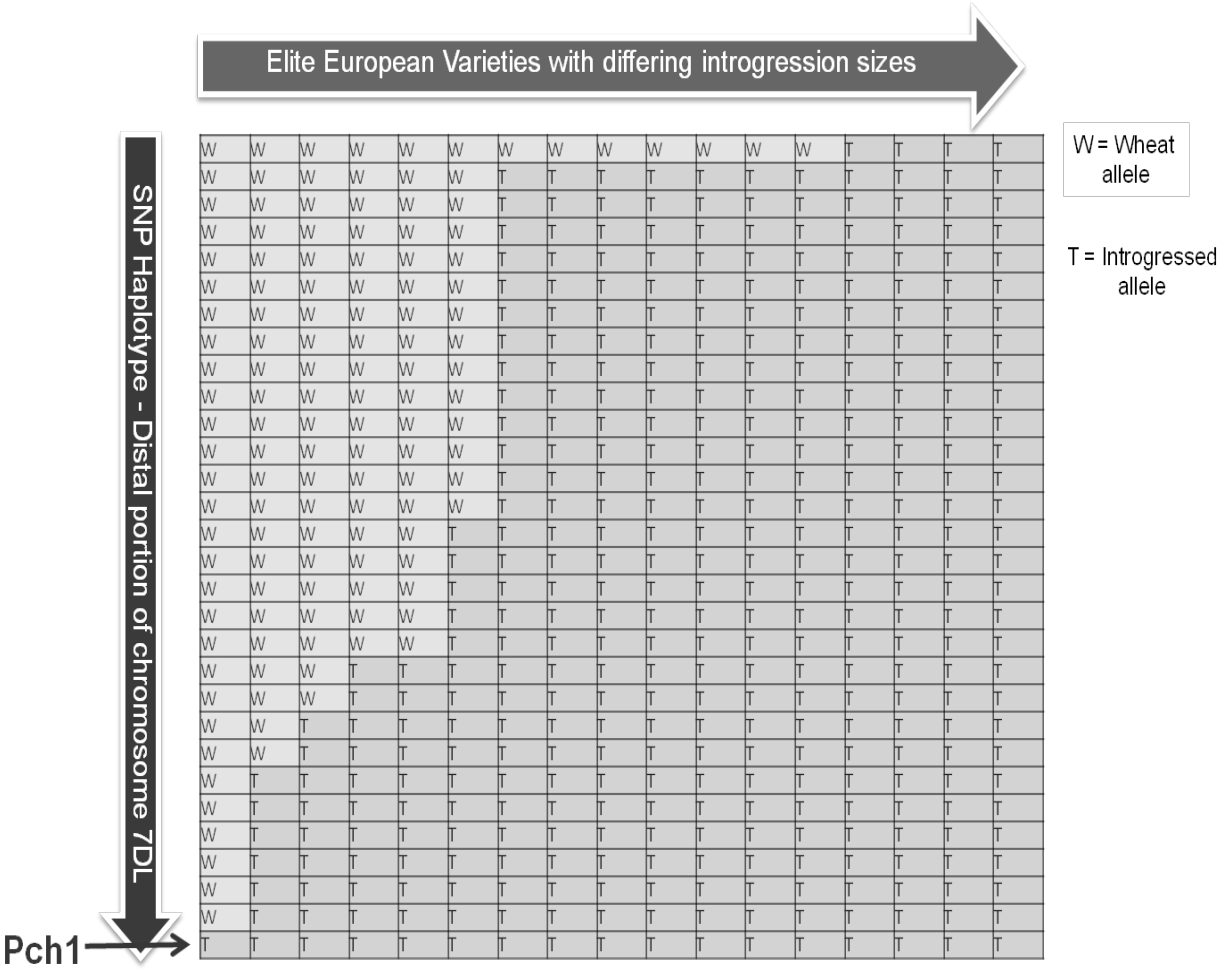

Figure 8 Molecular marker analysis of elite varieties containing resistance gene Pch1

RAGT analysis using single nucleotide polymorphic markers (SNPs) has found that the size of the *Aegilops ventricosa* chromosome introgression has been reduced by recombination in some elite cultivars and there is some evidence that the recombination is related to improved yield performance.

An example of resistance already existing in the elite wheat gene pool is the resistance to the insect pest, Wheat Orange Blossom Midge (OBM), (*Sitodiplosis mosellana*). Resistance to OBM is important both for varietal performance and grain quality, but also to reduce the use of non specific insecticides that need to be applied to control the pest in the absence of resistance. It was possible to select the resistance by phenotype, but selection was very laborious and relied on OBM flights and subsequent crop infestation. Fortunately it has proved possible to develop molecular markers which can tract the resistance in the absence of symptoms (Figures 9 and 10).

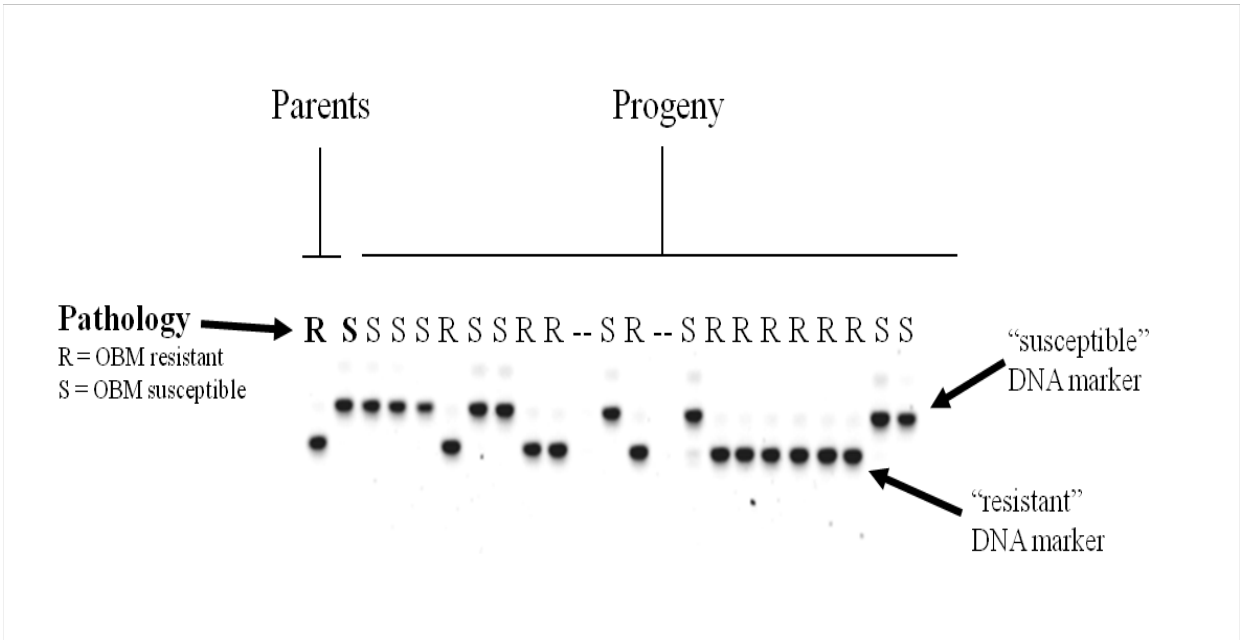

Figure 9: Single sequence repeat (SSR) marker for OBM resistance

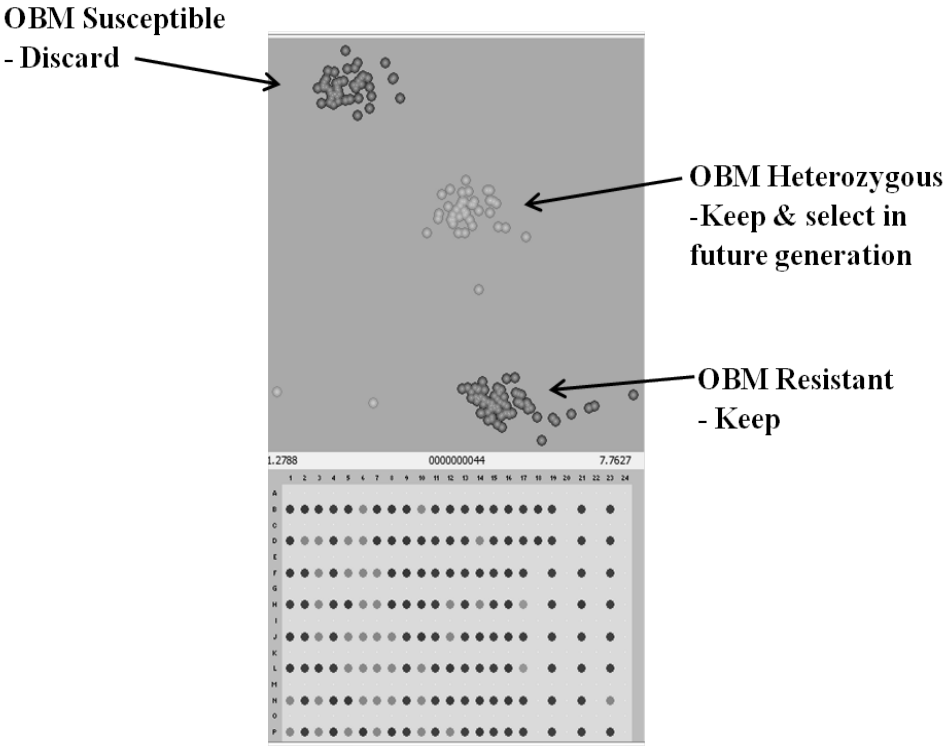

Figure 10 High throughput SNP marker for OBM resistance

Our ability to efficiently select for Pch1 and OBM resistance using molecular marker systems has recently resulted in the development of a new group 1 breadmaking wheat at RAGT, the variety RGT Skyfall, which contains both the eyespot resistance Pch1 and resistance to OBM.

As already mentioned, a current major challenge for UK wheat breeders is the continual break down of useful yellow rust resistance genes as the pathogen evolves new virulence to overcome the genes both singly and in combination. I believe major genes at risk of breakdown should continue to be used because they provide the most potent resistance. However breeders need to attempt to protect these genes, and varieties containing them, from catastrophic resistance breakdown by combining them with incomplete (partial) durable resistance sources in the same variety. This presents a serious selection challenge. In combination with major gene resistance, the partial resistance phenotype often associated with durable resistance is hidden and therefore not amenable to traditional selection methods. Other selection methodology is required and genomic studies and molecular markers are proving essential to achieve this. For example, work at RAGT has identified QTL associated with two putative sources of durable resistance to yellow rust (Figures 11 and 12). We are attempting to stack these resistances together in breeding lines that also contain major genes.

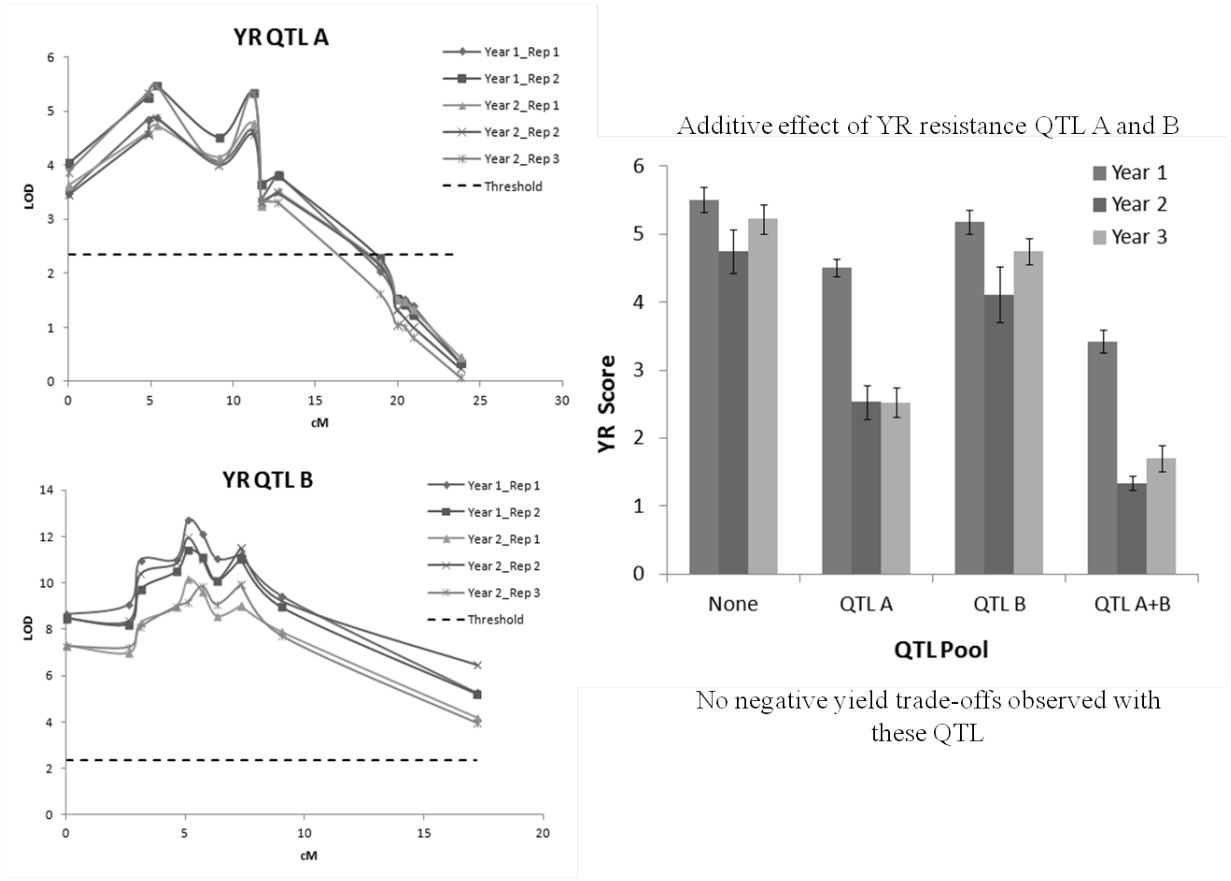

Figure 11 QTL analysis of the first source of durable resistance, 2 QTL, A and B, associated with the resistance have been identified and demonstrate an additive effect

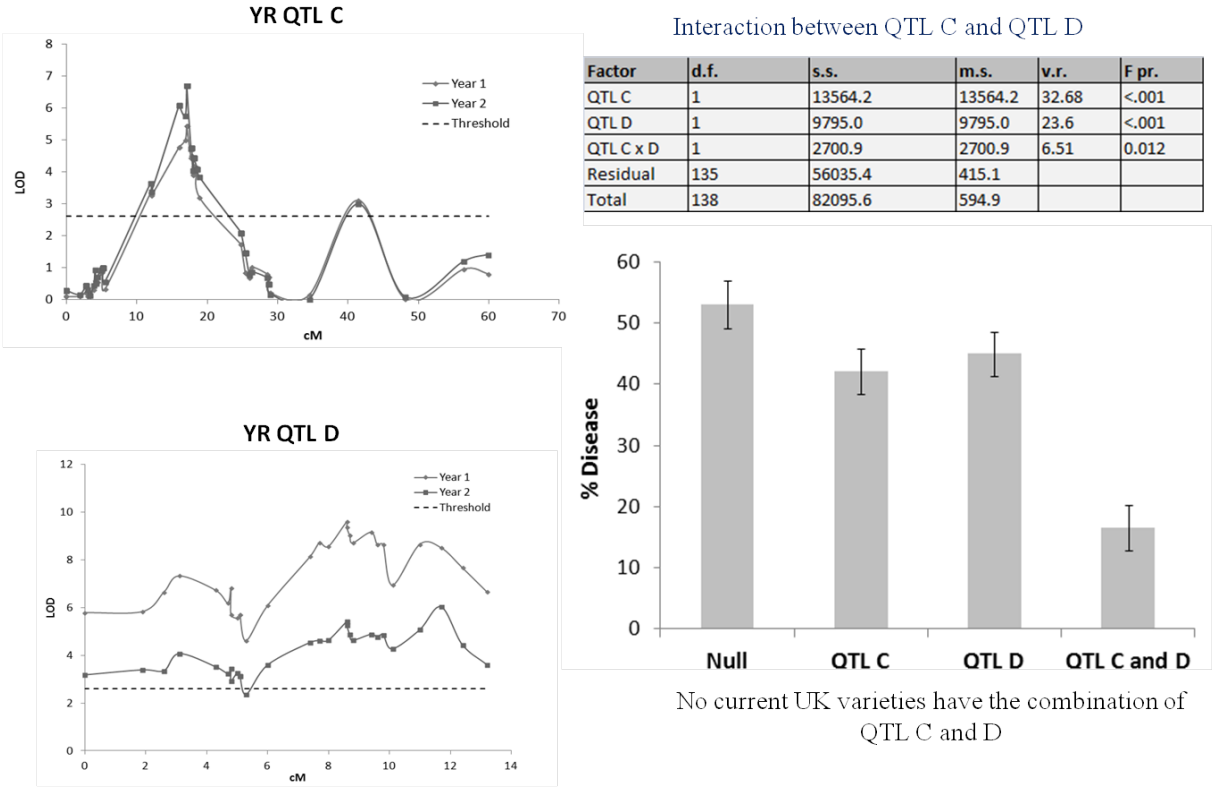

Figure 12 QTL analysis of the second source of durable resistance, 2 QTL, C and D, associated with the resistance have been identified and demonstrate an epistatic effect

With the examples given in this paper it should be clear that although complex, it will be possible to increase levels of genetic resistance in elite commercial varieties. However breeding for better disease resistance must not compromise breeding for higher yield. This will be difficult but new technologies, making use of advances in genomics in particular, will help. With increased genetic understanding and selection tools the introduction of key resistances into the commercial breeding funnel should become more straightforward. When new sources of resistance are required they will need to be sourced from unadapted germplasm and related species and their introduction will require significant pre breeding (Figure 13) before their introduction into commercial breeding pipelines to avoid the problems seen with the introduction of Pch1 eyespot resistance.

As breeding continues to advance, it is possible to imagine the development of very high yielding, possibly hybrid, wheat varieties with high levels of resistance based on major genes protected by additional genes bestowing durable resistance. Due to the high yield and high disease resistance such varieties would also have improved resource use efficiency in terms of nutrient and water use.

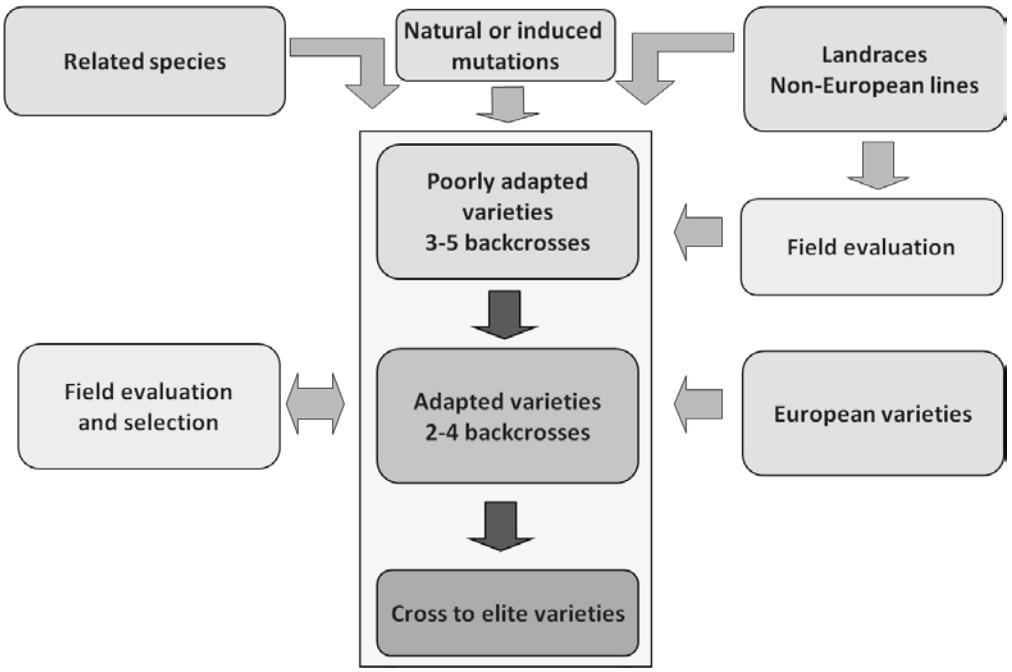

Figure 13 A pre-breeding parental development backcross scheme to introduce novel characters into adapted germplasm

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IDENTIFICATION OF NEW SOURCES OF RESISTANCE TO RHYNCHOSPORIUM IN BARLEY AND THE ROLE OF ASYMPTOMATIC INFECTION

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Summary: A series of qualitative and quantitative assays were used to determine the resistance of a variety of barley lines to the fungal pathogen *Rhynchosporium commune*. To assess asymptomatic infection and the extent of colonisation on different barley accessions, the level of biomass accumulation was coupled with analysis of the growth of the fungus using a strain expressing green fluorescent protein. All three tested strains produced disease symptoms only in highly susceptible cultivar Optic which also had the highest amount of fungal biomass and showed an extensive fungal colonisation pattern. Two Syrian landraces did not develop any symptoms after inoculation with any of the tested *R. commune* strains, contained overall low levels of pathogen biomass and showed a decrease in fungal colonisation at the cellular level.

INTRODUCTION

Similar to other crops, barley is exposed to a vast array of plant pathogens, including *Rhynchosporium commune*, a fungal pathogen which causes leaf scald. Rhynchosporium is by far the most destructive and economically important disease of both spring and winter barley in the UK. The disease is primarily controlled through the application of fungicides. However, the fungus remains in a long asymptomatic phase during infection making timing of chemical treatment difficult (Davis & Fitt., 1990). High fungicide costs, combined with the evolution of fungicide insensitivity, make this type of control an expensive requirement for farmers (Taggart *et al*., 1998). Despite routine fungicide applications, *R. commune* still costs the UK economy £7.2 million per year (HGCA, 2013). Such high losses call for a new, environmentally friendly way to handle *R. commune* infections. Effective cultivar resistance to this damaging disease has long been an important breeding target. However, due to the pathogens high genetic variability, one of the biggest challenges is finding cultivars with longer lasting resistance (Zaffarano *et al*., 2006). The only well characterised resistance interaction *Rrs1* - *AvrRrs1* is associated with a decrease in fungal biomass and a random mycelial pattern formation compared to a compatible interaction. Surprisingly, the fungus is still able to complete its lifecycle on a resistant plant, although its spread is greatly restricted (Thirugnanasambandam *et al*., 2010). Little is known about the intricate mechanisms that underpin a resistant response in this pathosystem. Evaluation of cultivar resistance is generally scored using qualitative and subjective methods based upon visual disease symptoms (Ayliffe *et al*., 2013). To discover potentially resistant barley lines we need to gain an understanding of the type of resistance barley confers against this pathogen. The aim of this work is to identify new sources of resistance to *R. commune* in barley lines using pathogen strains with different race specificities, and to provide further insight into a resistant response through pathogen biomass accumulation and confocal microscopy.

MATERIALS AND METHODS

Fungal culture conditions and spore collection

R. commune strains 13-13, L73a and 214-gfp from the James Hutton Institute culture collection were used in this study. Cultures were grown on CZV8CM agar medium (Newton, 1989) in the dark at 17°C. After 14 days, spores were collected by scraping the mycelial mat with a spatula. Spores were suspended in sterile distilled water (SDW) and filtered through a 60µm mesh. The spore suspension was then centrifuged at 3000 rpm for 3 minutes and re-suspended in SDW. This step was repeated a further two times.

Plant Material

Barley cultivars and Syrian landraces were grown for a period of 10-15 days at 20°C with a 14 hour day photoperiod. Cultivars and landraces used in this study include SLB10-009, SLB19-011, SLB66-024, Atlas, Atlas 46 and Optic.

Detached Leaf Assay

Detached leaf assays were performed as described in Newton et al., (2001). Leaf fragments, 4 cm in length were set out following a randomised design on 0.5% sterile distilled water agar supplemented with 120 mgL^{-1} benzimidazole. Using a fine haired brush, the waxy cuticle was removed in the centre of the leaf and 10 µl of 1 x 10^5 spores/mL were pipetted onto the brushed area. Boxes containing leaf samples were incubated in a controlled environment cabinet at 17°C, light intensity 200 µmol.m-2.s-1. Barley cultivar Optic was used as a control as it is highly susceptible to *R. commune*. Lesion formation was assessed from 10 days post inoculation (dpi). A total of five replicates were used for each assay.

Microscopy

Leaf material was inoculated with *R. commune* strain 214-gfp. Samples were mounted onto a slide with ~20 µl of silica oil and a coverslip and visualised using a Leica SP2 AOBS Spectral Confocal Laser Scanning Microscope. Three replicates were used for the assay.

DNA extraction

DNA was extracted from inoculated plant material (10dpi) frozen in liquid nitrogen and homogenised using a mortar and pestle. DNA was extracted using Qiagen DNeasy Plant Mini Kit following the supplied protocol. DNA was stored at -20 ^oC. DNA extraction was performed on three biological replicates.

Quantitative Polymerase Chain Reaction (qPCR)

Quantitative PCR (qPCR) was used to quantify *R. commune* DNA in infected leaves using primers specific to *R. commune* actin. Barley tubulin gene was used as a reference for plant DNA quantification (Table 1). Primers were optimised with *R. commune* and barley genomic DNA as a template. Each PCR reaction was made of 6µl of SYBR green master mix (Life Technologies), 1µl of forward primer, 1µl of reverse primer, 1µl of template DNA and 3µl of water. PCR was performed on Bio-Rad Chromo4 PCR machine using the following

programme: 95 ^{o}C for 15 minutes followed by 39 cycles 95^{o}C for 15 seconds, 60^{o}C for 30 seconds and 72^{o}C for 30 seconds.

Table 1. Oligonucleotide primers used for quantification of *R. commune* DNA in infected leaves

Primer	Sequence 5'-3'	
Actin Forward	GCGAGGACGACCAACGAT	*R. commune* reference gene
Actin Reverse	AATGTGTAAGGCCGGTTTCG	
Tubulin Forward	AGTGTCCTGTCCACCCACTC	*H. vulgare* reference gene
Tubulin Reverse	AGCATGAAGTGGATCCTTGG	

RESULTS

Barley lines resistance to different *R. commune* strains

Three Syrian landraces that previously showed high levels of resistance to *R. commune* in the field (Looseley et. al, unpublished) were tested for resistance to three *R. commune* strains, 214-gfp, 13-13 and L73A with different race specificities. The highly susceptible barley cultivar Optic was used as a control of fungal pathogenicity. Also included in the test were the near-isogenic cultivars Atlas and Atlas 46. Cultivar Atlas contains resistance gene *Rrs2* (Dyck & Schaller, 1961), whereas cultivar Atlas 46 has, in addition, *Rrs1* (Dyck & Schaller, 1961; Habgood & Hayes, 1971), making it resistant to strain 214-gfp which expresses a functional cognate avirulence gene. Although resistance to *R. commune* appears to follow a gene-for-gene interaction model, it is not mediated by the hypersensitive response. Indeed, *R. commune* may develop symptomless infection in both susceptible and resistant cultivars, with pathogen DNA detectable in resistant cultivars (Fountaine et al., 2007; Atkins et al., 2010). All three strains produced disease symptoms only in highly susceptible cultivar Optic on all 5 replicates. Lesions progressed during the infection with L73A producing the largest lesions which were significantly different from the other two isolates. There was no lesion formation for the duration of the assay on any other lines tested (SLB 10-009, SLB 19-011, SLB 66-024, Atlas and Atlas 46).

Fungal biomass accumulation during infection

The amount of biomass for each cultivar was compared to the level in susceptible control, set to the value of 100%. As expected susceptible barley cultivar Optic had the highest amount of fungal biomass for all *R. commune* strains used in this study. Cultivar Atlas 46, carrying *Rrs1*, inoculated with 214-gfp showed a small amount of fungal biomass compared to cultivar Optic and near isogenic cultivar Atlas (Figure 2). The other 2 strains produced more than triple the amount of biomass on cultivar Atlas 46. Landrace SLB10.009 was unable to restrict the growth of strain 214-gfp and contained almost 70% fungal biomass in comparison to the other two strains 13.13 – 48% and L73A – 32%. Landraces SLB 19-011 and SLB 66-024 contained

higher levels of fungal biomass for strain L73A in comparison to the other strains. However, both of these barley lines had the least amount of overall fungal biomass for all strains with the results indicating similar levels of fungal restriction to cultivar Atlas 46.

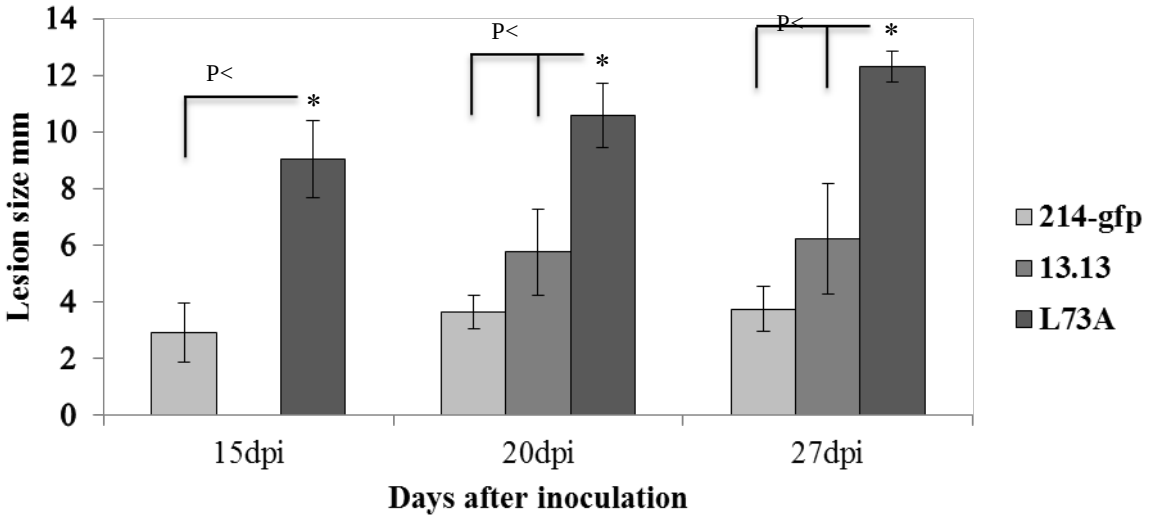

Figure 1. Lesions size observed for *R. commune* strains 214-gfp, 13-13 and L73A in susceptible cultivar Optic at 15, 20 and 27dpi.

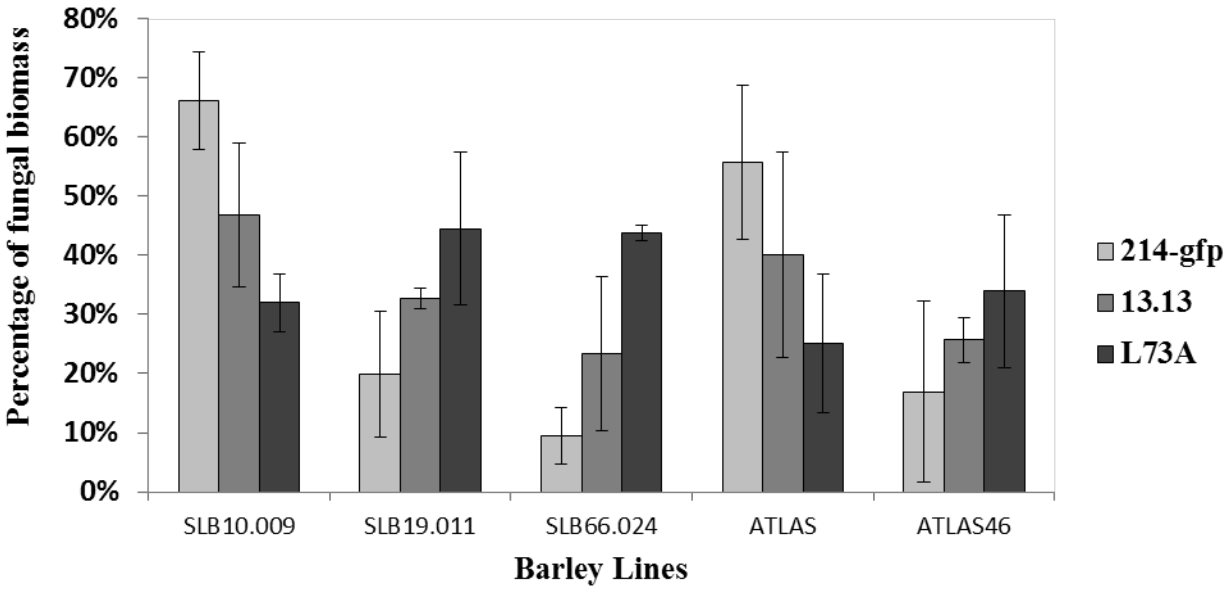

Figure 2. The percentage of fungal DNA on barley inoculated with strains 13-13, L73a and 214-gfp. Susecptible Optic set at 100% biomass

Microscopy

The growth of *R. commune* strain 214-gfp inoculated onto different barley lines was assessed at 8 dpi using confocal microscopy. Epidermal growth pattern was clearly observed on the susceptible control with high levels of colonisation which also occurred outside the inoculation area. The growth of *R. commune* strain 214-gfp on cultivar Atlas was similar but much less than in the control. Resistant cultivar Atlas 46 showed the least amount of growth with a random pattern of colonisation. SLB10-009 showed a moderate level of growth, slightly less than the susceptible cultivar Optic, but fungal growth was not as defined and there were some areas at the infection site that showed random mycelial growth. For barley lines SLB19.011 and SLB 66-024 there was an evident decrease in overall growth with both a mixture of small amounts of epidermal colonisation and some random hyphal growth.

DISCUSSION

Previous research has shown that asymptomatic growth can occur on a resistant cultivar and the growth pattern of the fungus is very different to growth on a susceptible one. (Thirugnanasambandam *et al*., 2010). Therefore, it was of interest to assess the amount of *R. commune* biomass and its pattern of growth on a variety of different barley lines that showed no lesion formation 3 weeks after inoculation with three different *R. commune* strains. The assessment of *R. commune* growth on multiple barley lines using a more comprehensive approach and allows for the assessment of both plant and pathogen responses in different and potentially resistant backgrounds. Syrian landraces were chosen for this research as they are genetically more diverse than cultivated barley which increases the chance of finding novel barley resistance.

The pathogenicity test confirmed the susceptibility of cultivar Optic and highlighted the high level of pathogenicity of strain L73A which was significantly different to the other isolates. Interestingly, despite lack of lesions on the other barley lines tested, there was varying amounts of fungal growth, in some cases up to 70% of fungal biomass accumulation compared to susceptible cultivar Optic. In addition, analysis of asymptomatic infection by *R. commune* GFP-expressing strain confirmed the pathogens ability to grow undetected with no lesion formation. However, in all barley lines tested (except cultivar Optic) there was a correlation between asymptomatic growth and a decrease in fungal biomass in comparison to the susceptible control, indicating the presence of a plant response that restricts fungal growth or interference in the ability of the fungus to colonise properly.

The response to *R. commune* infection by Syrian landraces SLB11.009 and SLB 66.024 shared a high level of similarity to Atlas 46, containing two resistance genes. Further microscopic analysis revealed that decreased fungal growth and random mycelial growth patterns were characteristic of both of these landraces, suggesting that they might contain an allele of *Rrs1*or another *R* gene recognising other avirulence gene(s) present in 214-gfp and 13-13. Further tests are required to investigate this possibility, but the absence of diagnostic markers for *Rrs1* make proving that barley accessions contain *Rrs1* difficult.

This research has highlighted the effectiveness of using qPCR to assess the amount of fungal biomass within barley lines which appear to be asymptomatic. However it is also important to assess the type of fungal growth. The restriction of fungal growth by the plant may not be

sustainable and the fungus may have the ability to overcome this at a later stage during infection. It is therefore necessary to further examine how the pathogen reacts in a variety of potentially resistant backgrounds over a longer period of time. The data obtained from this research further supports the need for more defined approaches to identifying resistance to *R. commune* and predicting resistance durability. In addition, gaining further information on *R. commune* infection may aid in the timing for fungicide application.

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THE EFFECT OF RHYNCHOSPORIUM RESISTANCE RATING ON FUNIGICIDE REQUIREMENT FOR DISEASE CONTROL AND YIELD FORMATION IN SPRING BARLEY

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Summary: A field experiment was conducted to investigate whether the efficacy of disease control and yield response to fungicide application differed between spring barley cultivars of contrasting disease resistance rating for Rhynchosporium leaf blotch. The experiment was sown in Carlow, Ireland during 2014 as a split-plot design with three cultivars in main plots (Sanette, resistance rating 7; Concerto, 5 and KWS Irina, 4) and ten fungicide treatments in sub-plots (untreated, half rate chlorothalonil, and prothioconazole or prothioconazole plus bixafen at 0.25, 0.5, 1.0 and 2.0 times the full rate). Rhynchosporium was the major disease present. Disease development was delayed in cultivars of higher resistance, but final disease severity on the top two leaves of untreated plots at GS77 and the area under the disease progress curve for these leaves did not differ between cultivars ($P>0.05$). Fungicide treatment increased yield by 8 to 14%. There was no cultivar x fungicide interaction for disease severity, yield or yield components indicating that the resistance rating of the cultivar did not influence the efficacy of disease control or yield response to fungicide. There is no evidence from this experiment that fungicide programmes should be adjusted to account for the Rhynchosporium resistance rating of the cultivar which calls into question the value of the narrow range of resistance currently available for developing integrated disease management programmes.

INTRODUCTION

Spring barley yields in Ireland are among the highest in the world. In order to maintain these high yields, disease levels must be managed effectively and this is generally achieved through the application of fungicides. In disease management programmes the requirement for fungicide is typically based on an assessment of the amount of visible disease present in a crop, or the risk of disease developing, and its likely impact on yield. However, recent evidence suggests that other potential effects of the fungicide on the crop should also be taken into consideration. Thus, significant yield responses to a triazole and strobilurin fungicide programme were found in some spring barley crops when there was little or no visible disease present (Bingham *et al.,* 2012). The additional yield has been associated with an increase in grain number formation and radiation use efficiency, most likely resulting from direct physiological effects of the fungicide prior to anthesis (Bingham *et al.,* 2014).

Increasingly, integrated approaches to disease management are being promoted in which non-chemical methods to restrict epidemic development, such as cultivar resistance to pathogens, are combined with the judicious use of fungicides. The potential benefits of this approach include greater economic returns through reduced input costs and improved stewardship of fungicide active ingredients via reduced selection pressure for fungicide-insensitivity in pathogen populations (Dooley *et al.,* 2015). However, the range of host resistance available against some important barley pathogens, such as *Rhynchosporium commune,* is relatively small. Thus, it is unclear to what extent fungicide programmes can be modified to account for the resistance rating of a cultivar and still achieve the required level of disease control and possible physiological effects of fungicides on yield. The objective of the experiment reported here was to determine whether the efficacy of disease control and yield response to fungicide was influenced by the disease resistance rating of the cultivar. In doing so we ask the question, what is the value of the current range of resistance to *R. commune* infection found in spring barley cultivars?

MATERIALS AND METHODS

The experiment was sown in Carlow, Ireland during 2014 as a split-plot design with three cultivars allocated to main plots and ten fungicide treatments allocated to sub-plots; untreated, half rate chlorthalonil (Bravo 500® 1.0 l/ha) and prothioconazole (Proline®) or prothioconazole + bixafen (Siltra Xpro®), at 0.25, 0.5, 1.0 and 2.0 times the full rate (1.6 and 2.0 l/ha, respectively), in four replicate blocks. The cultivars used were chosen for their range of *R. commune* resistance; Sanette (7, Syngenta Switzerland), Concerto (5, Limagrain France) and KWS Irina (4, KWS Germany) based on national recommended lists from the Republic of Ireland and Northern Ireland. Seed was drilled on the 10th April using a Wintersteiger small plot drill into 12m x 2.5m plots at a rate of 325 seeds/m^2. Agronomy and inputs apart from fungicides were in line with local practice. The fungicide active ingredients (a.i.) were chosen as representative examples of the triazole (prothioconazole), triazole plus SDHI (prothioconazole + bixafen) and chlorophenyl (chlorthalonil) groups of fungicides.

All fungicide treatments were applied at GS30 and GS39-45 as part of a two spray programme apart from chlorothalonil which was applied at GS15-25, GS30 and GS45-50. In order to determine the effects of fungicide treatments on contrasting cultivars, visible disease levels caused by *R. commune,* and green leaf area were assessed by leaf and stem layer on 10 randomly selected shoots per plot at GS30, 39, 55 and 77. Pre-harvest sampling was carried out to determine harvest index. Ear counts were conducted in field at GS85. Plots were harvested using a Deutz-Fahr plot combine and an Allegro CX Field PC Data Collector to obtain yields. Moisture and hectolitre weight were determined using DICKEY-JOHN GAC®2100 GI and thousand grain weights using a Contador seed counter. The numbers of grains/m^2 were calculated from measured values of yield and thousand grain weight and the numbers of grains/ear from calculated values of grains/m^2 and measurements of ears/m^2.

To analyse effects of fungicide treatments on contrasting cultivars, data from these plots were analysed as a split-plot design using a two-way Analysis of Variance. All analyses were done using the GenStat 14th Edition statistical software.

RESULTS

Rhynchosporium was observed on untreated plants at the first assessment period (GS39). Disease severity corresponded with the resistance rating of the cultivar ranging from 0.3% (leaf 2; where flag leaf = leaf 1) to 2.6% (leaf 4) for Sanette, the most resistant cultivar, and 1.2 to 10.8% for KWS Irina the least resistant. Disease developed between GS39 and GS77 and spread to the upper two leaves where the final severity ranged from 19.4 to 27.4% for leaf 1 across cultivars and 44.4 to 50.5% for leaf 2. Thus, by GS77 there was little difference between cultivars in disease severity with significant amounts of disease on all cultivars. This resulted in only small differences of low significance (P>0.05) between cultivars in the area under the disease progress curves (AUDPC) for the top two leaves of untreated plants between GS39 and GS77.

Table 1. Disease severity, yield and yield components of spring barley cultivars as affected by differing fungicide treatments. Yield and thousand grain weight (TGW) are expressed at 85% dry matter. Fungicide rate is the proportion of the full recommended rate. [1]Area under the disease progress curve (% disease days). Fungicide treatments are Chlor, chlorothalanil; Pro, prothioconazole; Pro + Bix, prothioconazole plus bixafen For cultivar and fungicide effects, values within a column followed by a different letter are significantly different at P=0.05.

Cultivar	**Fungicide**	**Rate**	**AUDPC[1]**	**Ears/m^2**	**Grains/m^2**	**TGW, g**	**Yield, t/ha**
Sanette			136.7	940^{a}	20511^{a}	44.97	9.21^{a}
Concerto			150.3	785^{b}	17958^{b}	43.98	7.97^{b}
Irina			180.5	963^{a}	20511^{a}	45.85	9.42^{a}
	Untreated	0	339^{a}	887	18854^{d}	42.68^{f}	8.04^{d}
	Chlor	0.5	153^{bc}	907	19897^{ab}	44.59^{e}	8.78^{bc}
	Pro	0.25	141^{bc}	886	19437^{bcd}	44.38^{de}	8.67^{c}
	Pro	0.5	155^{bc}	901	20173^{ab}	44.54^{cde}	9.02^{ab}
	Pro	1	143^{bc}	882	19870^{ab}	44.92^{bcde}	8.93^{abc}
	Pro	2	128^{bc}	882	19695^{abc}	45.39^{abcd}	8.94^{abc}
	Pro + Bix	0.25	169^{b}	810	19116^{cd}	45.84^{abcd}	8.78^{bc}
	Pro + Bix	0.5	109^{c}	939	19947^{ab}	46.26^{a}	9.18^{a}
	Pro + Bix	1	107^{c}	938	19990^{ab}	45.51^{abc}	9.2^{a}
	Pro + Bix	2	115^{bc}	928	19843^{ab}	45.97^{a}	9.14^{abc}
S.E.M.			35.4	59.9	435	0.766	0.197
Significance.							
Cultivar			0.200	0.003	<.001	0.074	<.001
Fungicide			<.001	0.279	<.001	<.001	<.001
Cv x Fung			0.591	0.387	0.889	0.725	0.267

All fungicide treatments provided significant (P<0.001) disease control reducing the AUDPC by 50-69% depending on the treatment (Table 1). There was no significant effect of cultivar (P=0.20) nor a significant cultivar x fungicide interaction (P=0.59) indicating that fungicides were equally effective at reducing symptoms of *R. commune* infection on all cultivars regardless of their resistance rating. Correspondingly, the area under the green leaf area curve (AUGLC; averaged over leaves 1 to 3) was increased by fungicide treatment (P<0.001) and differed between cultivars (P=0.048) (Sanette>Concerto>Irina), but as with AUDPC there no significant cultivar x fungicide interaction for AUGLC (P=0.91).

Fungicide treatment increased yield (P<0.001), an effect that was associated with increases in both the number of grains/m^2 and the thousand grain weight (Table 1). There was no overall effect of fungicide treatment on the individual sub-components of grain number, namely ears/m^2 and grains/ear. Importantly there was no cultivar x fungicide interaction on yield or its components implying that fungicides were equally effective at increasing grain yield irrespective of the Rhynchosporium resistance rating of the cultivar. Averaged across cultivars, the greatest yield responses occurred with prothioconazole and prothioconazole plus bixafen applied at half the manufacturers recommended rate. Chlorothalonil had a marginally smaller effect on yield than either prothioconazole or prothioconazole plus bixafen at half rate (yield increases of 9, 12 and 14% over controls respectively).

DISCUSSION

Development of visible disease was delayed, but not prevented in spring barley cultivars of high Rhynchosporium leaf blotch resistance rating compared to low. This reflects the relatively small range of disease resistance found in commercial spring barley cultivars. The delay in disease development appeared to have minimal effect on yield formation in the current experiment and thus the yield response to fungicide treatment either through disease control alone, or a combination of disease control and physiological effects of the fungicide, was unaffected by the resistance rating of the cultivar. Thus, the results from this one site-season provide no evidence to suggest that the fungicide programme (active ingredients and dose rate) should be altered according to the resistance rating of the cultivar, which calls into question the value of the narrow range of resistance currently available for developing integrated disease management programmes. The experiment is being repeated to determine whether this is a consistent observation across sites and seasons.

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FUSARIUM HEAD BLIGHTS – EPIDEMIOLOGY AND SPECIES DIVERSITY

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Summary: Fusarium Head blight (FHB) is a cereal disease complex which is becoming an increasing problem due to recent warmer and wetter growing seasons. The complex is made up of a number of species that may affect grain quality. Testing of samples from 2011 indicated that many of the species commonly associated with FHB were only present in a small number of samples. Lesser known Fusariums, such as *Fusarium tricinctum* were found to be more widespread than previously thought. The potential implications are discussed.

INTRODUCTION

Cereal production in Scotland in 2015 is estimated at 3.25 million tonnes with barley production contributing about 70% of this total. However, there are increasing concerns about grain quality and in this context one of the major disease complexes that affects grain quality is FHB (Fusarium Head Blight). Many species contribute to the range of symptoms observed. The species that are considered the most significant causal agents contributing to FHB in the UK are *F. avenaceum, F. culmorum, F. graminearum, F. poae and F. langsethiae* (HGCA, 2014). The ears of cereal crops are also infected by *Microdochium nivale*, which has been shown to have a negative effect on seed germination (Cockerell *et al.*, 2009). FHB infection during early flowering in warm and wet conditions can result in the whole or part of the ear becoming bleached. Later infections to the grain can result in yield loss and it is at this stage that natural toxic substances known as mycotoxins are produced. Control is based on cultivar resistance in wheat and effective fungicide applications in all crops to protect the ear. A number of recent summers have contained environmental conditions that encourage fungal attack. Increasing concern about mycotoxin levels in crops both in the field and in store have lead to interest in the incidence of head blight and the relative abundance of the species concerned.

MATERIALS AND METHODS

Samples of harvested wheat and barley have been collected from SRUC trials, the Official Seed Testing Station at SASA and from SAC Consulting advisors and agronomists across Scotland. Samples were tested with a number of techniques to determine the species present and their potential influence on grain quality.

Field Trials

A number of field trails were set up to measure disease incidence, including *Fusarium spp*. control in winter- and spring-barley at Drumalbin Farm (Lanarkshire), Gilchriston (East Lothian) and Bush Estate, Midlothian. The trials were a randomized block design with a standard plot size (2m × 10m). Samples were also obtained from SASA, advisors and agronomists.

Identification of fungi by PCR

The first stage of the DNA extraction process was grain milling. Two hundred randomly-selected grains from the main sample were ground into a fine flour-like powder using a Retsch® ball mill (Retsch, Germany). Grain was placed into the grinding jar along with the grinding ball and then mounted to the ball mill; the machine was set to a frequency of 25 Hertz and run for two minutes. Following the completion of the ball mill programme the ground seed was stored ready for the DNA extraction process. All parts of the milling equipment that were in direct contact with the grain were cleaned with ethanol between samples to prevent cross contamination of DNA.

One gram of ground grain from each sample was weighed into 15ml centrifuge tubes. 4ml of CTAB was added to the tubes as the extraction buffer. The tubes were heated to 70^{o}C for 20 minutes in a water bath then spun for 15 minutes at 14,000g. 2ml micro-centrifuge tubes, each prepared with 900µl of 7.5M ammonium acetate, were filled to the top with the supernatant from the 15ml tubes and mixed on a vortex. The samples were refrigerated at 4^{o}C overnight before being centrifuged for 10 minutes at 10,000g. The supernatant from each sample was transferred to a 2ml tubes prepared with 800µl of ice-cold isopropanol. The samples where kept at 4^{o}C overnight, then centrifuged at 10,000g for 10 minutes after which the supernatant was poured off whilst the presence of a pellet containing the DNA in each sample was observed. The resulting pellet was washed with 400µl of 70% ethanol and then centrifuged at 10,000g for 5 minutes. The supernatant was removed, taking care not to lose the pellet, and the tube was left to dry inverted in a flow cabinet for one hour. After 200µl of sterile distilled water (SDW) was added to each sample they were stored at 4^{o}C overnight.

To re-suspend the DNA, the samples were heated to 50^{o}C for 40 minutes using a heat block and then mixed by vortex to ensure the pellet was fully re-suspended. The DNA concentration of the samples was tested on a NanoDrop® Spectrophotometer (Thermo Scientific, USA), the data from this test was then used to adjust the DNA concentration to 10ng/µl in SDW.

PCR testing of extracted DNA

PCR was carried out using GoTaq® G2 green Master Mix (Promega, USA) and primers from Eurogentec (Hampshire, UK). The volumes of each component in each reaction were as listed (DNA extract 10µl, Forward primer 0.5µl, Reverse primer 0.5µl, Go Taq Green Master mix12.5 µl. The PCR reactions were carried out in a T3000 thermocycler (Biometra, Germany). Primers used were as described in Table 1.

Table 1. Primer sets used in Fusarium testing.

Target Species	Sequence (5' – 3')	Fragment size	Source
F. culmorum	ATGGTGAACTCGTCGTG GC CCCTTCTTACGCCAATC TCG	570bp	Nicholson *et al*., 1998
F. graminearum	ACAGATGACAAGATTC AGGCACA TTCTTTGACATCTGTTC AACCCA	280bp	Nicholson *et al*., 1998
F. sporotrichioides	CGCACAACGCAAACTC ATC TACAAGAAGAGCGTGG CGATAT	310bp	Nicholson *et al*., 2004
F. langsethiae	CAAAGTTCAGGGCGAA AACT TACAAGAAGAGCGTGG CGATAT	332bp	Nicolson *et al*., 2004
F. poae	GCTGCTCATCACTTTGC TCAG TCGTGGTGAAACAATGT AT	400bp	Niessen *et al*., 2004
F. avenaceum	GCTAATTCTTAACTTAC TAGGGGCC CTGTAATAGGTTATTTA CATGGGCG	220bp	Turner *et al*., 1998
F. tricinctum	CGTGTCCCTCTGTACAG CTTTGA GTGGTTACCTCCCGATA CTCTA	215bp	Kulik, 2008

RESULTS

Table 2. Positive Fusarium results in samples (2011-14).

Year	Crop	No of samples	No of positive PCR tests
2011	W Barley	33	19
2011	S Barley	17	17
2012	W Barley	33	23
2012	S Barley	59	35
2012	W Wheat	15	12
2014	W Barley	40	14
2014	S Barley	90	69
2014	W Wheat	31	27

The highest number of positive tests was recorded in 2014 (69). However in 2011, 100% of the spring barley samples tested gave a positive result for *Fusarium spp*. Overall, wheat (39/46) gave a higher proportion of positive results compared with barley (177/272).

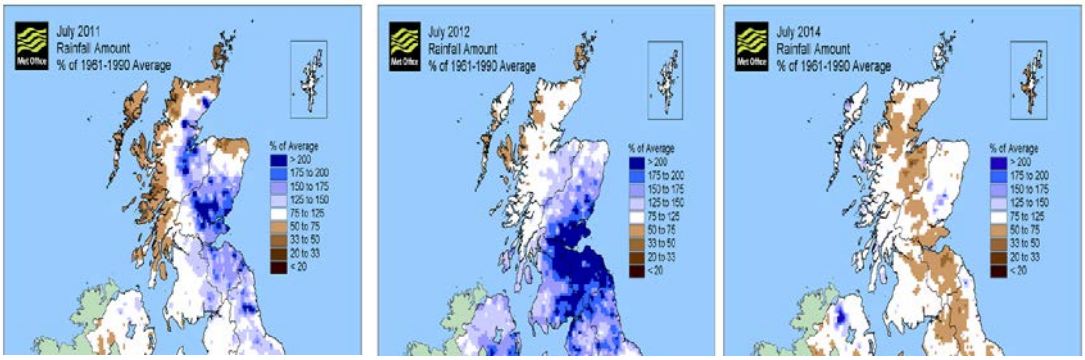
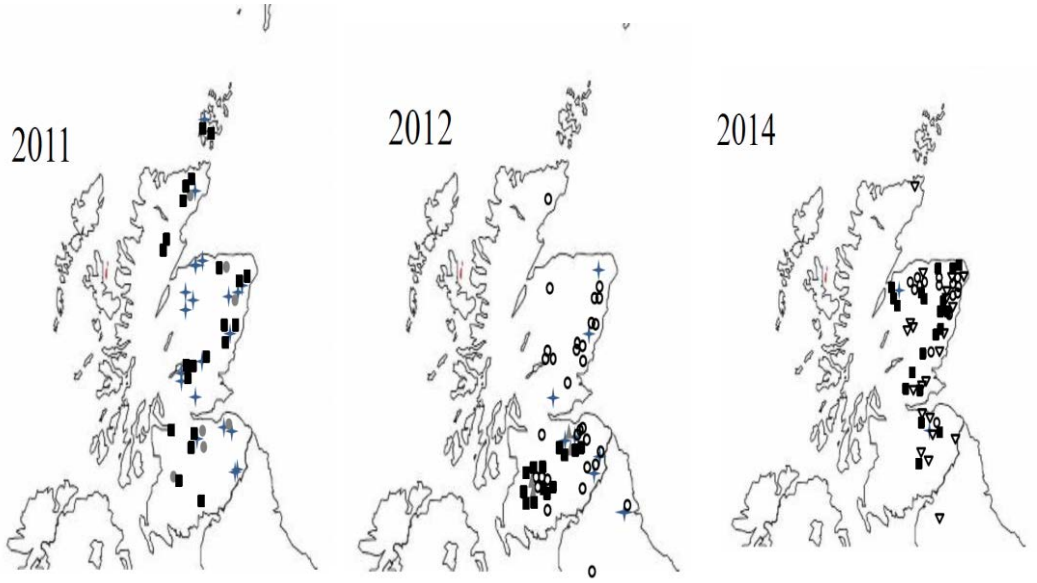


Figure 1. July Rainfall 2011-14 (monthly average compared to 50 year average).

July rainfall was highest in 2012 (Met Office, 2015), when much of the cereal growing area in Scotland experienced more than 200% of the normal average rainfall (dark blue colours). High rainfall levels were more isolated in 2011 but not uncommon in Angus. 2014 was the driest year of the 4 examined.



Symbols ■ *Fusarium avenaceum,* ✦ *F. poae,* ○ *F. tricinctum,* ▲ *F. sporotrichoides,* ● *F. langsethiae,* ▽ *Microdochium nivale*

Figure 2. Fusarium species detected in samples.

In 2011 the most commonly detected Fusarium species were *F. avenceum* and *F. poae.* Other species detected included *F. culmorum* and *F. langsethiae.* By 2012 *F. tricinctum* was the most frequently recorded species. The most abundant species detected in 2014 were *F. tricinctum* and *F. avenaceum.*

DISCUSSION

The 2014 results show that *M. nivale* and several species of *Fusarium, F. avenaceum, F. poae, F. tricinctum, F. langsethiae* and in very limited quantities, *F. culmorum,* were present throughout Scotland. The low incidence of *F. culmorum* would appear to be a contrast to what might be expected in Scotland given that this species has a tendency to exist in cooler climates because the optimum temperature for it to compete strongly is around 15^{o}C (Parry *et al*., 1995). A previous report suggested a decline in *F. culmorum* is underway in Northern Europe, due to increased temperatures during anthesis (Waalwijk *et al*., 2003). Another possible reason for low incidence of *F. culmorum* and the high incidence of others could be competition between species, particularly with *Microdochium*. Simpson *et al*. (2004) observed that *M. majus* was able to co-suppress the colonisation of wheat seedlings by *F. culmorum*, although the presence of *M. majus* has not been confirmed in all samples and this requires further investigation. The low level of *F. graminearum* and high level of *M. nivale* detected in 2014 suggests that conditions were cool since the former requires 25^{o}C conditions to support its colonisation of the plant, and the latter group 15^{o}C. The cooler conditions would support the presence of *M. majus* and also explain why no *F. graminearum* was detected. *F. tricinctum* has been shown to be on the increase in other countries (Castañares *et al*., 2011). This species is known to produce fusarin C, enniatins, and moniliformin toxins and has been implicated in cattle disease (Grove *et al.*, 1970). Since *F. tricinctum* can infect different cereal grains and fescues a large-scale survey of cereals and grasses from Scotland should be considered.

The growing conditions in 2012 gave rise to very high disease levels, and the spray programmes applied gave no significant decrease in disease levels. In stark contrast, the 2013 season was more normal, in terms of rainfall and temperature, and this in turn gave rise to very low levels of disease. 2014 was a warmer and wetter year and disease levels more closely resembled 2012. The increased temperatures in 2014, compared to 2012, could explain the difference in species present. 2015 was a wet cool summer and testing of samples is underway to see if there has been a shift back towards *F. culmorum* and away from *Microdochium spp*. and *F. tricinctum*.

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THE INVISIBLE RISK OF SAPROPHYTES: A *CRYPTOCOCCUS* / *RHYNCHOSPORIUM* CASE STUDY

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Summary: Non-pathogenic microorganisms can have detrimental effects on plants by either promoting disease or impairing host fitness. Here *in vitro* and *in planta* interactions between a yeast isolate, *Cryptococcus victoriae* O6, and *Rhynchosporium commune* (*Rc*), the barley leaf scald causing agent, are presented. The saprophytic yeast could promote *Rc* spore germination, scald lesion appearance and the loss of green leaf area caused by *Rc*. Furthermore, the yeast impaired barley photosynthetic activity in a manner similar to *Rc*. The effect of O6 was then tested in the field, but preliminary results showed no significant effect of the yeast on barley disease levels and yield under the conditions prevailing in this study. This research has demonstrated the potential risk of non-pathogenic saprophytic organisms to enhance disease. However, it remains to be ascertained whether this phenomenon is also found under field conditions other than those examined here.

INTRODUCTION

A multitude of microorganisms live associated with plants. The vast majority of these are benign, but some are pathogenic and they can have significant costs to food production, i.e. yield penalty, loss of crop quality, costs of agro-chemical usage. The microbial interactions *in planta* can range from synergistic to antagonistic. At one end of the spectrum, antagonistic microbes might be exploited as biological control agents (BCA) to control diseases and some have shown applicability in the field. At the other end, synergistic microbes (disease-helpers) should, in theory, be avoided and controlled along with pathogens, but as yet little is known about their interactions with pathogens *in planta* and in the field. The latter research area requires more attention to fully assess the costs associated with potential disease promotion in the field.

Disease-promoters have been documented in various pathosystems. Bacteria, isolated from the wheat phyllosphere, increase *in planta* the pathogenicity of *Phaeosphaeria nodorum*, the causal agent of leaf and glume blotch disease (Dewey *et al.*, 1999). The presence of the bacterial potato pathogen, *Pectobacterium atrosepticum*, correlates with increased pathogenicity of septoria net blotch and powdery mildew on cereals in the field (Newton *et al.*, 2004). Non-pathogenic fungi were also shown to increase the severity of a fungal disease on trees caused by *Drepanopeziza populi* (Busby *et al.*, 2013). Indirectly, saprophytes have also been hypothesised to negatively affect host plant physiology. Glasshouse inoculation of saprophytes including *Alternaria alternata* and *Cladosporium macrocarpum* resulted in accelerated leaf senescence (Bertelsen *et al.*, 2001). This impact of saprophytes on the host is

one possible explanation for the "greening effect" of some fungicides. In situations of low disease pressure, fungicide application can result in a yield increase possibly because detrimental non-pathogenic saprophytes are controlled and leaf lifespan is increased (Bertelsen *et al.*, 2001). However, the mechanisms by which saprophytes impact on crop production, are still a matter of debate.

Barley is a major crop in Scotland. Leaf scald, one of barley's major foliar diseases caused by the fungus *Rhynchosporium commune* (*Rc*), can result in up to 40% yield loss if not controlled appropriately (Zhan *et al.*, 2008). In a previous effort to identify BCA against *Rc*, yeast isolates with disease-promoting properties have been identified *in vitro* (Fountaine *et al.*, 2009). They belonged to the *Cryptococcus* genus. One particular isolate identified as *C. victoriae* O6 showed a strong disease promotion. The aim of this study was to assess the impact of O6 on *Rc* pathogenicity and on barley leaf physiology. Complementary *in vitro* and *in planta* methods were used to measure the interaction of the O6 saprophyte with *Rc*. The effect of the yeast on leaf physiology was assessed by monitoring chlorophyll fluorescence. Finally, the potential for disease promotion by the *Cryptococcus* isolate was tested in a preliminary field experiment.

MATERIALS AND METHODS

Spore suspensions of *Rhynchosporium commune* (Scottish isolate 614.2) were prepared from 3 week-old potato dextrose agar plates and diluted to 10^5 spores/ml. Yeasts were grown in Potato Dextrose Broth (PDB) for 4 days. The cell density was diluted down to 10^6 cells/ml for *in planta* experiments and 10^5 cells/ml for the field experiment.

The indirect effect of O6 on *Rc* was determined by monitoring *Rc* spore germination when placed in contact with yeast culture supernatant. Culture supernatant was obtained after centrifugation (3 min at 1100 *g*) and filter-sterilisation of 4 day old cultures. *Rc* spore inoculum and yeast supernatant were 1:1 mixed in 2X PDB and incubated under constant agitation at 17°C. The proportions of ungerminated and germinated spores were counted under a microscope from 50 spores after 18 h of growth.

Controlled condition experiments were carried out using plants grown for 3 weeks in a climate chamber (Snijders, 16h:8h, 20:16°C, light:dark) in pots containing 10 seedlings of spring barley (cv Cellar). A detached leaf assay was undertaken to quantify yeast effects on symptom appearance and lesion length. Leaf segments were placed on 0.5% agar supplemented with benzimidazole (1 mM, Sigma) and inoculated with a 10 µl droplet containing either O6 or *Rc* spores or both. Plates were incubated at 18°C (12h:12h; light:dark). Lesion appearance was assessed every other day from 13 days post inoculation (dpi) onwards and lesion size was measured using an electronic calliper. The effect of O6 on *Rc* pathogenicity was also assessed on whole plants as follows. Six pots were sprayed until run-off with O6 or SDW. One day later, seedlings were sprayed with the *Rc* spore solution and placed in sealed bags in the dark for 24 h. The loss of green leaf area, expressed as a % of leaf area, was visually estimated for each pot 28 days later. Finally, the effect of O6 and *Rc* on barley photosynthetic activity, i.e. the maximum quantum yield of photosystem II (Fv/Fm) and the electron transfer rate (ETR), was measured on a weekly basis for 3 weeks using a IMAGING-PAM chlorophyll fluorimeter (Walz, Effeltrich, Germany).

A field experiment was conducted in 2015 in Fife. Plots (10 x 2m) of the cv Concerto were grown in 4 replicates according to a split-plot design and all inputs apart from the experimental

treatment were as per local practice. Yeast treatment (O6) was applied at Zadoks growth stages 30, 59 and 75. Severity of *Rhynchosporium* leaf scald and ramularia leaf spot (RLS) were visually assessed at several time points throughout the growing season as % of leaf area infected. Leaf scald is expressed as area under the disease curve progress (AUDPC), whereas RLS is expressed as an infection percentage.

All statistical analyses were carried out using t-test and repeated measurements ANOVA from the Genstat software (VSN International LTD).

RESULTS

The effect of the O6 isolate on *Rc* growth and pathogenicity was assessed using *in vitro* and *in planta* approaches (Fig 1). Firstly, O6 was shown to significantly ($P < 0.001$) promote the germination of *Rc in vitro* (Fig 1a): 20% more *Rc* spores had germinated after 18h of cultivation in the presence of O6 culture supernatant than in controls. Secondly, using a detached leaf assay, co-inoculation of *Rc* with O6 caused scald symptoms to appear significantly earlier ($P < 0.05$) than when leaves were infected with *Rc* alone. No significant effect of the yeast on scald lesion size was observed (Fig 1b). On average, scald symptoms developed more than 1 day earlier after O6 treatment. The yeast alone did not cause any visible symptom development (data not shown). Finally, a whole plant assay indicated that co-inoculation of O6 and *Rc* caused a significant increase ($P < 0.001$) in green leaf area loss (Fig 1c). Three weeks post inoculation, the loss of green leaf area in plants co-inoculated with the yeast and *Rc* was more than twice that of plants inoculated with *Rc* alone. Overall, O6 caused *Rc* spores to germinate faster and co-inoculation of *Rc* and O6 resulted in a faster scald lesion appearance and a greater loss of green leaf area.

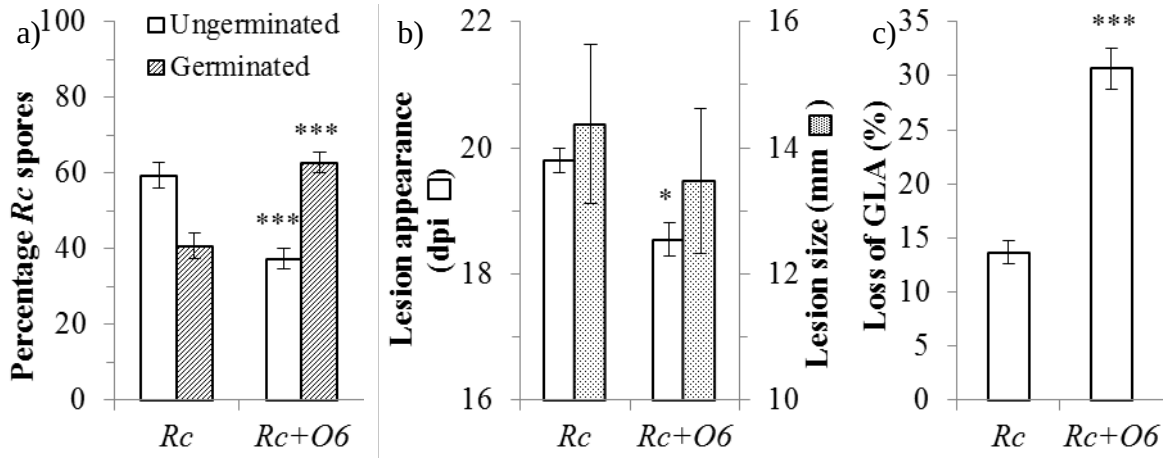

Figure 1: *In vitro* (a) and *in planta* (b, c) interactions between *Cryptococcus victoriae* O6 and *Rhynchosporium commune* (*Rc*). a) After 18h of incubation, *Rc* spores were categorised as ungerminated (white) or germinated (shaded). b) *Rc* lesion appearance (white) and size (shaded) are expressed as days post inoculation (dpi) and mm, respectively. c) Loss of green leaf area, expressed as %, was assessed at 4 weeks post inoculation. (Error bars: standard error; ***: $P < 0.001$; *: $P < 0.05$)

Photosynthetic activity of barley seedling inoculated with O6 and *Rc* alone and in combination was monitored by measuring the chlorophyll fluorescence over 3 weeks. The maximum photosystem II (PSII) efficiency (Fv/Fm) is used to detect stress-induced changes in photosynthesis, because the efficiency declines with photodamage to PSII reaction centres and with the development of slowly relaxing quenching processes (Rolfe and Scholes, 2010). The electron transfer rate (ETR) through PSII of illuminated leaves is related to the rate of CO_2 assimilation (Rolfe and Scholes, 2010). The presence of *Rc* strongly affected Fv/Fm (Fig 2a). Fv/Fm was significantly reduced in *Rc*-infected leaves after 3 weeks, when scald symptoms were visible (Table 1). ETR was affected by the presence of *Rc* and the yeast O6 (Fig 2b; Table 1). Overall, inoculated leaves, either with *Rc* or O6, increased their ETR in the early phases of the infection, but it was dramatically reduced after 3 weeks, when scald symptoms were visible. The effects of *Rc* on ETR were increased by co-inoculating with O6 as shown by the *Rc**O6 interaction (Table 1).

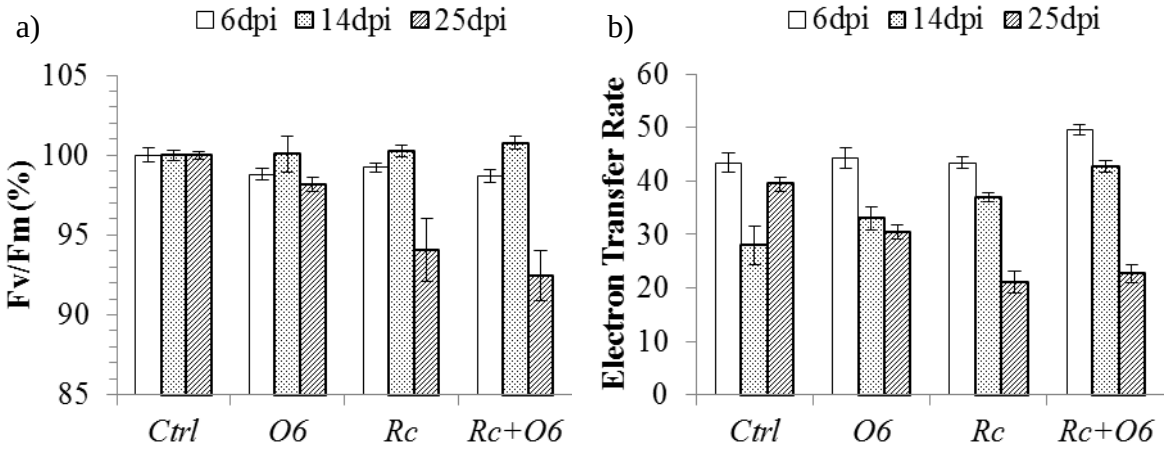

Figure 2: Photosynthetic activity of barley leaves over 3 weeks following inoculation with the *Cryptococcus victoriae* O6 isolate and *Rhynchosporium commune* (*Rc*) either alone or in combination (ctrl: untreated). The maximum photosystem II quantum yield (Fv/Fm) of dark adapted leaves, expressed as relative percentage compared to the control, and the electron transfer rate (ETR) of illuminated leaves were measured. Error bars represent standard error.

Table 1: *P*-values of the repeated measurements ANOVA, comparing the effect of the yeast (O6) and *Rhynchosporium commune* (*Rc*) over time on the host photosynthetic activity: i.e. maximum PSII efficiency (Fv/Fm) and electron transfer rate (ETR).

	P-values						
	Rc	O6	*Rc**O6	Time	Time**Rc*	Time*O6	Time**Rc**O6
Fv/Fm	0.004	0.153	0.735	<0.001	<0.001	0.164	0.895
ETR	0.160	0.557	<0.001	<0.001	<0.001	0.031	0.534

The effect of the saprophytic isolate O6 was tested in the field. Disease scores and yield were compared to untreated plots (Table 2). Two diseases were observed in the field: leaf scald and ramularia leaf spot (RLS). Scald was visible as early as at GS30, whereas RLS was observed only at the last field assessment (GS80). Treating plots with O6 did not result in significant changes in disease susceptibility and yield.

Table 2: Effect of inoculating field grown barley with the *Cryptococcus victoriae* O6 isolate. Severity of foliar diseases, Rhynchosporium leaf scald and ramularia leaf spot (RLS), are expressed as AUDPC and percentage of infected leaf area, respectively. Yield is expressed at 85% dry matter content. *P*-values from t-test comparing control plots to O6-treated plots are indicated.

	Treatments		
	Control	O6	*P*-value
Leaf scald (AUDPC)	303	241	0.521
RLS (%)	6.3	3.5	0.131
Yield (t/ha)	6.00	6.15	0.423

DISCUSSION

This study highlighted that a saprophytic non-pathogenic yeast, identified as *Cryptococcus victoriae* isolate O6, had a synergistic effect on a major barley pathogen, *Rhynchosporium commune* (*Rc*). A direct effect of the yeast on *Rc* pathogenicity can be hypothesised: an unknown compound released in the yeast supernatant accelerated *Rc* spore germination (Fig 1a). This could lead to an earlier colonisation of the host and therefore a shorter time for symptom appearance (Fig 1b), which would result in greater loss of green leaf area (Fig 1c). Similar direct stimulation of pathogenicity has been hypothesised in wheat, where disease-promoting bacteria could produce lipases, thought to increase penetration of the host by pathogens (Dewey *et al.*, 1999).

Other mechanisms may be involved in the disease-promoting effect of O6. Pathogens are known to cause a dramatic reduction in photosynthetic activity as lesions develop (Rolfe and Scholes, 2010). This was observed in *Rc* and *Rc*+O6 treated plants at 25dpi with the maximum photosystem II efficiency (Fv/Fm) and electron transfer rate (ETR; Fig 2). Interestingly, seedlings inoculated with O6 had their ETR and Fv/Fm reduced (relative to controls) in a similar manner to *Rc*-inoculated plants, but to a lesser extend (Fig 2b). It appears that the yeast may stress the host metabolism in a manner conducive for *Rc* pathogenicity. A combination of direct and indirect interactions may therefore explain disease promotion of *Rc* by O6 on barley.

The fact that O6 as well as other disease-promoting bacteria and fungi have been isolated directly from natural environments where diseases occur (Dewey *et al.*, 1999; Fountaine *et al.*, 2009; Busby *et al.*, 2013), raises the question of how extensive disease promotion is in the field. Newton *et al.* (2004) observed directly in the field a correlation between a non-host bacterial pathogen and cereal disease levels. However, results from our study field experiment did not confirm the results from experiments carried out under controlled conditions. Several

factors may have affected the outcome of the experiment: a single Scottish *Rc* isolate was used in this study, whereas *Rc* genetic variability in the field is very large (Zhan *et al.*, 2008); different barley varieties were used for controlled and field experiments: cv Cellar and Concerto respectively; the titre of the yeast inoculum used in the field was not as high as the one used in experiments done under controlled conditions.

Whilst it remains plausible that synergistic interactions between microorgansims could enhance disease development in field crops, their economic impact on food production is yet to be determined. The control of saprophytes by fungicide application could explain the "greening effect" of fungicides, where yields are increased in low disease pressure situations by controlling detrimental non-pathogenic saprophytes and extending the green leaf area period. However, there is conflicting evidence of the costs of saprophytes to the plant (Bertelsen *et al.*, 2001; Bingham *et al.*, 2014). A reliable *in situ* model is required to fully assess the control and cost of disease-promoting microbes on food production.

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USE OF ELICITORS IN BARLEY DISEASE CONTROL PROGRAMMES

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Summary: The potential for resistance elicitors to control crop diseases has been demonstrated previously. A number of experimental compounds alone and in combination have been shown to reduce disease levels both in glasshouse and field trials. However, these products have not been available for commercial growers. Over the past two seasons commercially available products have been evaluated for their ability to reduce disease levels in barley trials at sites across Scotland. Initial results indicate elicitors at the T0 spray timing can allow reductions in later fungicide usage.

INTRODUCTION

The potential for resistance elicitors to confer broad spectrum disease control in a number of crops has been reported previously (Oxley & Walters, 2012) and results on barley presented at the Dundee conference by Walters *et al.* (2010). The authors outlined the potential for a combination of elicitors, which induce both Systemic acquired resistance (SAR) and Induced systemic resistance (ISR) to control foliar pathogens in barely. The authors concluded that resistance elicitors could be used early in the crop protection programme in combination with fungicides to reduce disease and increase yield. Much of this work involved molecules involved in plant pathogen/host signalling. e.g. acibenzolar-S-methyl (ASM; marketed in Europe as Bion®), the non-protein amino acid β-aminobutyric acid (BABA), and the oxylipin, *cis*-jasmone (CJ) (Walters *et al.,* 2007). However, only one of these compounds has official registration as a crop protection product. Therefore it was decided to focus on commercial products in future years. As a result the commercial resistance elicitor/biofungicide Regalia® (Syngenta) was tested in a number of field trials at SRUC websites.

MATERIALS AND METHODS

Field Trials

Winter barley

Winter barley seed (cv. Saffron) was grown in 2014 and 2015 at two SRUC trial sites (Bush Estate, Midlothian and Drumalbin Farm, Lanarakshire) Seed was sown in plots (10 m x 2 m) in a randomised block design. A number of fungicides and elicitors were tested, including prothioconazole (Pro) (Proline 275®), pyraclostrobin (pyr) (Comet®), chlorothalonil (chlor) (Bravo®), extract of *Reynoutria sachalinensis* (reg) (Regalia® biofungicide) and alcohol ethylene (war) (Warrior® adjuvant). Treatments and growth stages are outlined in Table 1.

Table 1. Treatments used in winter barley trials 2013/14 and 2014/15.

Code	GS 24	GS31-32	GS45	GS 55
1	Untreated	Untreated	Untreated	
2		pro 0.18 l/ha+pyr 0.25 l/ha		pro 0.18 l/ha + chlor 0.5 l/ha
3		pro 0.36 l/ha+pyr 0.5 l/ha +		pro 275 0.36 l/ha + chlor 1.0 l/ha
4		pro l/ha +pyr 0.25 l/ha	pro 0.18 l/ha + chlor 0.5 l/ha	chlor 1.0 l/ha
5		pro 0.36 l/ha + pyr 0.5 l/ha +	pro 0.36 l/ha + chlor 1.0 l/ha	chlor 1.0 l/ha
6	reg(2.5 l/ha) + war	reg (2.5 l/ha) + war	reg (2.5 l/ha) + war	
7	reg(2.5 l/ha) + war	reg (2.5 l/ha) + war		
8	reg(2.5 l/ha) + war	pro 0.18 l/ha + pyr 0.25 l/ha	pro 0.18 l/ha + chlor 0.5 l/ha	
9	reg(2.5 l/ha) + war	pro 0.36 l/ha +pyr 0.5 l/ha +	pro 0.36 l/ha + chlor 1.0 l/ha	
10	reg(2.5 l/ha) + war	pro 0.18 l/ha +pyr 0.25 l/ha +reg (2.5 l/ha) + war		pro 275 0.18 l/ha + chlor 0.5 l/ha

Spring barley

Sping barley (cvs. Optic and Concerto) was grown at an SRUC trial site (Drumalbin farm, Lanarkshire) in plots (10 m x 2 m) in a randomised block design. A number of fungicides and elicitors were tested, including prothioconazole (pro) (Proline 275®), pyraclostrobin (pyr) (Comet®), cholothalonil (chlor) (Bravo®), bixafen+prothioconazole (bix+pro) (SiltraXpro®), cyprodinil + isopyrazam (cyp+iso)(Bontima®), epoxyconazole+fluxapyroxad (epo+flu)(Adexar®), (extract of *Reynoutria sachalinensis* (reg) (Regalia® biofungicide) and alcohol ethylene (war) (Warrior® adjuvant).

Table 2. Treatments used in spring barley trial 2015.

Code	GS25-30	GS45	GS 53
1	Untreated	Untreated	Untreated
2	pro 0.36 l/ha + pyr 0.5 l/ha		pro 0.36 l/ha
3	pro 0.36 l/ha + pyr 0.5 l/ha		bix+pro 0.5 l/ha
4	pro 0.36 l/ha + pyr 0.5 l/ha		cyp + iso 1.0 l/ha
5	pro 0.36 l/ha + pyr 0.25 l/ha		epo+flux 0.5 l/ha
6	pro 0.36 l/ha + pyr 0.5 l/ha		pro 0.36 l/ha + chlor 1.0 l/ha
7	reg 2.5 (l/ha) + war	pro 0.18 l/ha + chlor 1.0 l/ha	pro 0.18 l/ha + chlor 1.0 l/ha
8	pro 0.36 l/ha + pyr 0.5 l/ha	pro 0.36 l/ha + chlor 1.0 l/ha	
9	pro 0.18 l/ha + pyr 0.25 l/ha + reg (2.5 l/ha) + war	pro 0.18 l/ha + chlor 1.0 l/ha	
10	reg (2.5 l/ha) + war	reg (2.5 l/ha) + war	pro 0.18 l/ha + chlor 1.0 l/ha

Leaf layers were assessed for the severity of foliar disease 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 Version 11.1 (VSN International Ltd, Hemel Hempstead, UK).

RESULTS

Winter barley

R. commune was the major disease problem in the winter barley trials. Only the three and two Regalia® spray programmes failed to give a significant reduction in leaf scald. The greatest reduction was given by the full fungicide programme (2 sprays of pro and chlor) (60%). Mildew figures followed a similar trend to Rhynchosporium, with no control given by reg alone. Early reg followed by full fungicides gave the greatest reduction in mildew (96%). Brown rust levels were much lower, with no programme giving significant control. Ramularia levels were moderate over the course of the trials but no programme gave significant control. The yield figures reflected disease control, with reg sprays alone not giving a significant increase in yield. The biggest increase in yield was given by the full pro and chlor programme (22%). Specific weight was significantly increased by all except three of the programmes (reg alone and reg + half rate fungicides).

Table 3. Disease levels, green leaf area (AUDPC) and yield figures for winter barley trials 2014-15.

Code	Rhyn	B rust	Ram	Mild	GLA	Yield (t/ha) @15%MC	Specific Weight (KG/HL)
1	1348	24.4	49.7	317.1	4896	7.12	61.89
2	750	16.7	36.4	150.9	5854	8.47	63.83
3	573	3.9	23.7	69.3	6245	8.69	63.8
4	640	19.5	41.9	171.8	6015	8.63	63.57
5	532	17.3	21.1	99.3	6255	8.62	63.23
6	1137	13.4	43.9	279.6	5263	7.54	62.11
7	1237	24.6	40.3	302.6	5120	7.27	61.9
8	645	20.2	49.1	163.1	6071	8.45	63.08
9	561	16.8	41.5	11.32	6213	8.63	63.37
10	685	13.3	33.1	140.8	6012	8.45	63.45
F Ratio	<0.001	0.78	0.08	<0.001	0.239	0.005	0.008
LSD (P=0.05)	265.1	21.57	20.38	80.07	1240.1	1.03	1.25

Spring barley

Rhynchosporium was the major disease in this trial. All of the treatments gave a significant reduction in disease over the trial. The best control in both varieties was given by two spays of pro+pyr followed by pro+chlor (73% and 86%). Most of the treatments controlled the mildew in cv. Optic. There was no control of Ramalaria in the trial. The biggest yield increase in cv. Optic was 2 sprays of reg followed by half rate pro+chlor (23%). In cv. Concerto the biggest increase in yield was produced by pro+pyr followed by pro+chlor mirroring the control of rhynchosporium. All the treatments gave an increase in specific weight but none increased thousand grain weight.

Table 4. Disease levels, green leaf area (AUDPC) and yield figures for spring barley trial 2015.

A. cv Optic

Code	Rhyn	Mild	Ram	GLA	Yield (t/ha) @15%MC	Specific Weight (KG/HL)	TGW
1	1304.2	46.2	1	1443	6.688	59.4	50.88
2	481	9.3	7.5	2072	8.274	64	51.41
3	444	9.3	5.5	2174	8.146	64.7	48.03
4	434.8	0	7	2294	8.045	64.65	50.56
5	601.2	0	4	2007	7.89	64.05	51.91
6	351.5	0	4	2350	8.28	65.2	51.83
7	370	0	2	2303	8.105	65.1	50.5
8	453.2	9.3	7.5	2164	8.056	64.45	51.83
9	490.2	0	4.5	2164	8.19	64.15	50.93
10	397.8	0	3	2386	8.275	65.25	48.04
P value	0.063	0.53	0.69	0.03	0.74	0.03	0.14
LSD (P=0.05)	115.9	31.3	4.89	261	0.68	1.02	3.66

B. cv Concerto

Code	Rhyn	Mild	Ram	GLA	Yield (t/ha) @15%MC	Specific Weight (KG/HL)	TGW
1	1193.2	0	6	1415	7.12	60.65	48.22
2	351.5	0	8	2128	7.966	62.8	49.23
3	259	0	5.5	2248	8.162	64.05	49.27
4	212.8	0	7.5	1989	7.996	64.05	50.86
5	249.8	0	5.5	2322	7.595	63.65	48.89
6	157.2	0	8	2322	8.181	64	47.29
7	185	0	4	2544	8.078	63.85	49.76
8	203.5	0	11	2202	8.093	64.2	46.86
9	370	0	8	1998	7.864	63.25	47.38
10	342.2	0	8.5	2072	7.771	63.65	49.65
P value	0.063	0.53	0.69	0.03	0.74	0.03	0.14
LSD (P=0.05)	115.9	31.3	4.89	261	0.68	1.02	3.66

DISCUSSION

Previous work at SRUC has highlighted the importance of controlling *R. commune* at stem extension (Havis *et al.,* 2014). In winter barley, the treatments with an effective fungicide at GS30 (Zadocks *et al.,* 1974) reduced *R.commune* and this reduction in disease contributed to an increase in yield over the trials analysed. The treatments which combined reg + half rate pro + chlor gave a significant increase in yield, but did not give a significant increase in specific weight. However, the combination of reg + pro + chlor at the normal rate gave significant increase in both measurements. The addition of reg to a two spray half rate programme reduced disease levels and increased green leaf area levels but this benefit was not carried over into yield figures. In spring barley the pro+pyr followed by pro + chlor gave the biggest reduction in rhynhcosporium disease. All the treatments with an effective fungicide reduced rhynchosporium. The treatment with reg at GS 24 followed by half rate pro+chlor gave significant reduction in rhynchosporium and a significant increase in yield and specific weight but not thousand grain weight.

The results from these trials indicate that resistance elicitors do have a potential role in controlling disease in barley, as proposed by Walters et al. (2010). An early application followed by half rate fungicides can give significant disease control and yield increases. This work will be extended to look at other disease/crop interactions and also incorporate other commercially available elicitors.

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EVALUATING TESTING METHODS FOR FUSARIUM HEAD BLIGHTS

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Summary: Fusarium Head Blight is a serious problem for cereal growers. A number of methods exist to estimate infection in harvested grain. This paper will describe a project comparing visual methods to incubated plate assays and and end point PCR assays. The advantages and disadvantages of each method will be highlighted.

INTRODUCTION

Fusarium (teleomorph *Gibberella*) and *Microdochium* species make up a group of fungal pathogens commonly known as Fusarium Head Blight (FHB) complex. These cause a number of diseases which have significant damaging effects on most small grain cereals, including Foot Rot, Seedling Blight and Head Blight (Kelly *et al.*, 2010). All varietiesof barley and wheat are vulnerable (Maiorano *et al.*, 2012).In the UK, the list of potential hosts includes oats, rye, triticale, maize and grasses (HGCA, 2014a; Kelly et al. 2010).Infection can result in reduced yield and grain quality. *Fusarium* species also contaminate grain with mycotoxins which are poisonous to both humans and animals (EMANa, b; HGCA, 2014b).

MATERIALS AND METHODS

Samples of harvested wheat grown in 2014 from across Scotland were collected from the Official Seed Testing Station at SASA and SRUC trials in Aberdeen. Visual assessments were compared to incubation of seeds on agar plates and PCR analysis.

Tombstone Counts

The method used was similar to that used by the University of Arkansas plant pathology department. One hundred wheat seeds from each of the 31, samples harvested in 2014, were counted out into ten batches of ten. . This sample division was used to save time and improve accuracy and ease the observations. The process was repeated three times. Each batch was inspected for *Fusarium* levels by counting and recording the number of seeds showing signs of infection

Agar Plate Tests

At SRUC, two hundred seeds were incubated on potato dextrose agar (PDA) plates at 20°C for 7 days and mycelial colonies grew out from the seed. The plates were then scored for infection levels and the species of *Fusarium* or *Microdochium* present. The method followed was based on a process advised by SASA. Agar plates were made up using Potato dextrose Agarose (PDA) (Potato extract 4.0 g/l; Glucose 20 g/l; Agar 15g/l, Oxoid Ltd) with streptomycin as an antibacterial agent to reduce non-target bacterial growth on the agar. Seed surface inoculum was reduced by surface sterilising the seeds by washing in 8% sodium hypochlorite for 8

minutes followed by two rinses in sterilised distilled water (SDW) for 5 minutes. The rinsing water was drained off and 25 seeds from each sample were plated onto PDA, (5 seeds per plate). The plates were sealed with parafilm and kept at 20 °C for 7 days to allow and fungi present to grow onto the agar. *Fusarium* and *Microdochium* species infection levels were assessed by visual comparisons of fungal growth and colour with an image library of colony growth on PDA. Number of infected seed and species present was recorded for each plate. At SASA 200 seeds from each sample were evaluated, using validated International Seed Testing Association method 7-022 for detection of *Microdochium* species. 200 seeds were incubated on PDA plates at 20^{o}C for 7 days. Plates were then assessed by an experienced analyst for the presence of *Fusarium* and *Microdochium* species. Any identified *Fusarium* colonies were followed up with further analysis of inoculation and incubation on Carnation Leaf Agar, with final microscopic morphological examination of the spores produced by an experienced analyst, to identify individual Fusarium colonies to species level.

Identification of fungi by PCR

The DNA extraction techniques have been described in detail in Havis *et al*, 2016.

PCR testing of extracted DNA

PCR was carried out using GoTaq® G2 green Master Mix (Promega, USA) and primers from Eurogentec (Hampshire, UK). The volumes of each component in each reaction were DNA extract 10µl, Forward primer 0.5µl, Reverse primer 0.5µl, Go Taq Green Master mix12.5 µl. The PCR reactions were carried out in a T3000 thermocycler (Biometra, Germany). Primers used were as described in Havis *et al.*, 2016. The results from the PCR were visualised by agarose gel electrophoresis. The qualitative PCR used in this study cannot quantify fungal DNA but merely confirms the presence or absence of the target pathogen. The results were analysed by generalised linear models using Genstat Version11.1, (VSN International Ltd, Hemel Hempstead, UK).

RESULTS

The General Linier Regression (GLR) tests on the tombstone counts showed that the effect of region and variety on the level of infected grains was significant with an F pr. value of 0.042 and less than 0.001 respectively. The mean infection levels for each region were; North: 5.5% (± 5.2), central: 3.4 (± 2.3) and south: 5.8 (± 4.0). The highest counts appeared to be in the north and south region. However, based on this analysis of the results, there does not appear to be a significant difference in counts between the regions. This does cast some doubt on the implications of the GLR results. Comparison of the tombstone counts with the plate assays showed an underestimation of Fusarium and Microdochium levels in the former. This was especially true when compared to the results obtained from SASA. The plate assay yielded more meaningful results when it was carried out by an experienced technician. The highest recorded levels of fungal species were in samples from the South and mean levels were higher than those in the Central region.

Statistical tests did not suggest that region or variety were influencing factors on the species of *Fusarium* or *Microdochium* present. The results for the GLR are listed in Table 5 below. The only significant result is that of *F. avenaceum*, suggesting that region has an influence. It is likely that the results above have been caused mostly by the lack of replications in the dataset and the uneven representation of the variables, particularly varieties. As discussed the location data was converted into regions. There is a skew towards the central region since it has twelve

samples and away from the north region which has eight. The total number of positive results was 79, table 4 below shows the distribution of these across the regions.

Table 1 – Results from 2014 wheat samples

Region	Variety	*Fusarium Species*			*Microdochium*			Tombstone counts
		PCR[a]	Plate test		PCR[a]	Plate test		% discoloured
			1	2		1	2	
Central	Alchemy	2	7.5	1.6	0	1	0	10.7
South	Relay	0	4.5	0.4	1	1	3.6	14.3
Central	Viscount	2	2	2.6	0	1	1	0.7
South	Horatio	3	8.5	1.4	1	1	2	4.3
South	Tuxedo	2	6	2.2	1	1	2	3.0
South	Leeds	0	1	0	1	1	3.8	13.7
South	Consort	2	31.5	0.4	0	1	3.4	11.0
Central	Viscount	0	2	0.6	1	1	1.8	1.3
Central	Alchemy	2	6.5	2	0	1	0.6	8.7
South	Leeds	1	0.5	1.6	1	1	2.6	5.3
South	Consort	1	7.5	0.2	1	1	2.2	3.0
Central	Alchemy	3	16.5	1.2	0	1	1.8	3.3
Central	Alchemy	3	17	1.4	0	1	2	2.7
Central	Horatio	3	5.5	1.6	1	1	1.6	1.3
South	Leeds	0	0.5	0.4	1	1	4.2	4.0
South	Denman	2	6.5	2.2	1	1	0	1.0
Central	Unknown	2	5.5	1.6	1	1	2.2	1.3
Central	Istabraq	1	2.5	1.2	1	1	1.4	3.7
Central	Viscount	3	2.5	0.6	1	1	2.2	4.0
Central	Istabraq	1	0.5	0.6	1	1	2.2	2.0
South	Mayriad	2	22.5	1.6	1	1	2.4	4.0
South	Viscount	3	8.5	2	1	1	0.4	0.0
Central	Leeds	1	0	3.4	1	1	0	0.7
North	Leeds	2	0	3.4	1	1	0.2	0.7
North	Viscount	2	1.5	1.4	0	1	0	1.0
North	Horatio	3	*		1	*		3.3
North	Panacea	2	*		1	*		10.3
North	Leeds	1	*		1	*		4.7
North	Alchemy	2	*		1	*		5.7
North	Evolution	3	*		1	*		12.0
North	Zulu	1	*		1	*		6.0

[a]= no of Fusarium species detected by PCR, 1 detected? 0 not detected?, 1= results from SASA plate tests % infection, 2 = results from SRUC plate tests. * = no data. Plate results are % seeds infected, tombstone counts are % seeds observed with discolouration.

Table 2 fungal species detected by SASA plate assay and SRUC PCR assay

Species	SASA	SRUC
F. avenaceum	3	8
F. culmorum	2	2
F. graminearum	7	0
F. langsethiae	12	8
F. poae	21	11
Other	15	13
M. nivale	25	18

Table 3 Generalised linear regression on SRUC PCR results

Species	Variety	Region
F. avenaceum	0.563	0.045
F. culmorum	0.966	0.545
F. graminearum	*	*
F. langsethiae	0.312	0.25
F. poae	0.521	0.193
F. sporotrichioides	*	*
F. tricinctum	0.249	0.747
M. nivale	0.175	0.136

Table 4 PCR Results by region

	North	Central	South
No of samples tested	8	12	11
No. of positive results	23	30	26
Percentage of positive results	35.9	31.25	29.5

Based on this PCR data alone, the presence of *Fusarium* and *Microdochium* species generally appears to be almost evenly spread across the regions. Statistical tests did not suggest that there was a significant difference in the level of infection generally, when the species of pathogen was ignored, between the regions or varieties. When the positive results are displayed as a percentage of the total number of tests conducted in each region, the suggestion is that the north has the highest level of infection. There is still little difference between the regions however, so the only conclusion that can be drawn with regard to region is that *Fusarium* and *Microdochium* species are distributed throughout the regions tested.

Table 5 No of positive PCR tests

Species	No of positive samples	Species	No of positive samples
F. avenaceum	13	*F. poae*	13
F. culmorum	2	*F. sporotrichioides*	0
F. graminearum	0	*F. tricinctum*	17
F. langsethiae	10	*M. nivale*	24

There are noticeable differences between the species detected, Table 5 above shows the number of positive PCR results for each. There are a number of interesting findings that have

some significance. *Microdochium sp.* are noticeably higher than any other species. The absence of *F. graminearum* and *F. sporotrichioides* and the very low presence of *F. culmorum* is also noteable. There appears to be little evidence of any variety of wheat being particularly resistant or susceptible to infection. The differences in infection between varieties could be attributed to their relative abundance in the sample set.

DISCUSSION

The results show that *M. nivale* and several species of *Fusarium, avenaceum, poae, tricinctum, langsethiae* and in very limited quantities *culmorum,* were present throughout wheat crops in Scotland in 2014. The low incidence of *F. culmorum* would appear to be a contrast to what might be expected in Scotland given that this species has a tendency to exist in cooler climates because the optimum temperature for it to compete strongly is around 15^{o}C (Parry *et al*., 1995a). This result is in line with the report, discussed earlier, from Northern Europe that suggested a move away from *F. culmorum,* in the FHB disease complex, due to increased temperatures during anthesis (Waalwijk *et al*., 2003). Another possible reason for low incidence of *F. culmorum* and the high incidence of others could be competition between species, particularly with *Microdochium*. Simpson *et al.*, (2004) observed that *Microdchium sp* compete with *F. culmorum* in wheat seedlings.. The low level of *F. graminearum* and high level of *M. nivale, F. avenaceum and F. poae* detected in 2014 suggests that conditions were cool, since the former requires 25^{o}C conditions to support its colonisation of the plant, and the latter group 15^{o}C. The cooler conditions would support the presence of *Microdchium sp*and also explain why no *F. graminearum* was detected.

As has been discussed by Madgwick *et al*. (2011) climate change has the potential to provide these conditions on a more permanent basis. The complex of species detected in 2014 could be said to be less pathogenic because neither *F. culmorum* nor *F. graminearum* are dominant, however, there are other species which can create similar problems. *F. langsethiae* appears in one third of the 2014 samples, this particular species is known to be a prolific producer of highly toxic HT-2 and T-2 mycotoxins (Imathiu *et al*. 2012). This suggests that mycotoxins produced could also be highly changeable between seasons. However, even though mycotoxin producing pathogens have been detected this does not necessarily mean that mycotoxins are present. The differences between the two tests seen in Table 3could be explained by the sampling method for DNA extraction (3 x 1g sub samples for the milled seed). In the SASA test each seed was examined individually for *Fusarium* infection

The analysis of the data was unable to show differences in resistance between varieties. Differences in the variety of wheat that was infected by each species of the pathogen could be observed in the data but none of the varieties appeared to show significant signs of resistance to the disease complex generally. Further work would have to be carried out to investigate differences in varietal resistance. Recent research suggests that host resistance is limited to certain species of *Fusarium* (Nicholson *et al*. 2003). Future environmental conditions in Scotland, as a result of projected climate change, are likely to increase the risk of *Fusarium* infection and therefore mycotoxin contamination in Scottish wheat crops. Although increased temperatures are forecast to be only slight, in warm wet years with an early anthesis date, this will still likely be enough to increase FHB risk. It is difficult to say exactly what can be expected given the limited accuracy of many climate change models. An increase of the more pathogenic *F. graminearum* is likely but, at least in the near future, may be an intermittent problem rather than a feature of every harvest. The possible increase of other species particularly, *F. langsethiae* with is abilities to produce high levels of HT-2 and T-2 toxins could be a significant emerging issueThere were clearly differences in the level of accuracy

between the assays. The most clear and specific was the detailed SASA plate assay followed by the PCR assay. For increased accuracy using PCR, quantitative PCR assays using an increased number of sub samples of milled grains would need to be developed. PCR assays can give falsely elevated pathogen levels due to the variability of mycelial loading on individual seeds. These assays also detect non-viable as well as viable mycelium, and surface-borne as well as embedded infective mycelium.

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SENSITIVITY OF IRISH *ZYMOSEPTORIA TRITICI* POPULATIONS TO THE MOST COMMONLY APPLIED FUNGICIDES

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Summary: Septoria tritici blotch (STB) caused by the fungal pathogen *Zymoseptoria tritici* is the most economically destructive disease of winter wheat crops in North-Western Europe. Due to the inability of wheat varieties to adequately resist *Z. tritici* infection and subsequent STB development, control is heavily reliant on the application of fungicides. Currently the range of fungicides available to achieve this control is limited to specific azole, SDHI and multisite fungicides. Due to the specific nature of inhibition of both the azole and SDHI fungicides, and the ability of *Z. tritici* to adapt to stressful environments, the potential for resistance development is regarded as medium to high. As part of the deployment of fungicide anti-resistance strategies it is essential to monitor *Z. tritici* populations and alter programmes to reflect changes in sensitivity. Since 2005 Teagasc has undertaken a *Z. tritici* field population fungicide sensitivity monitoring programme. During this period decreases in sensitivity to the main azole fungicides have been detected in the Irish *Z. tritici* population. In addition a small number of isolates collected in 2015 exhibited reduced levels of sensitivity to the main SDHIs used for control.

INTRODUCTION

The combination of high yielding varieties and a maritime climate afford Irish winter wheat crops the capacity to produce some of the highest yielding crops globally. Unfortunately this same combination provides the ideal conditions for the development and spread of septoria tritici blotch (STB). Caused by the fungal pathogen *Zymoseptoria tritici*, STB has the potential if left untreated to reduce yields by up to 50%. To prevent these losses and to maximise the potential of the crop control programmes incorporating varietal resistance, agronomic practices and fungicide applications are recommended. In current cropping environments the success of both varietal resistance and certain agronomic practices are limited due to their negative impacts on yield. Consequently control of STB in Irish winter wheat crops is heavily reliant upon the application of fungicides. These include specific azole fungicides and the more recently introduced SDHIs. Whilst both groups of fungicides have different modes of action, their specific nature of activity combined with the biology of *Z. tritici* and its ability to adapt to stressful environments, such as the presence of a fungicide, place them at a medium-high risk of loss of activity due to resistance development.

To prolong the activity of both these groups of fungicides it is essential that they are applied in a manner which reduces the potential for the development and spread of fungicide resistance. As a means of optimising such strategies, routine sensitivity monitoring of Irish *Z. tritici* field populations are conducted. Field populations have been sampled post fungicide application on

an annual basis since 2005. Sensitivity is subsequently determined using a microtitre assay and molecular characterisation performed on isolates exhibiting changes in sensitivity (Dooley et al. 2016).

CURRENT SENSITIVITY STATUS AND RECOMMENDATIONS

Azole Sensitivity

Since 2008 a decrease in sensitivity to the main azole fungicides have been detected. These changes have been associated with changes in the *CYP51* gene including the emergence of the mutations D134G and S524T and increasingly complex combinations of previously detected mutations in individual strains. Furthermore since 2010 isolates exhibiting *CYP51* overexpression, resulting from inserts in the promoter regions, most notably a 120bp insert, have been detected in the Irish population. The possible presence of strains exhibiting a putative alternative resistance mechanism, namely overexpression of efflux pumps, remains to be determined.

SDHI Sensitivity

Following the commercialisation of the newer SDHIs including bixafen, fluxapyroxad, isopyrazam, and penthiopyrad since 2010 their usage on winter wheat has steadily increased. Most Irish winter wheat crops now receive at least a single application of an SDHI based fungicide. To establish a baseline range of sensitivity to the SDHIs a collection of isolates sampled during the 2005-2010 seasons was determined. Since 2011 routine sensitivity of field populations have been determined to isopyrazam. Prior to 2015 no changes in sensitivity to the SDHIs were detected. In 2015 a small number of isolates exhibiting reduced sensitivity to all commercial SDHIs and with lower sensitivity values than those previously recorded in the baseline collection were detected in two commercial crops. Further analysis of these isolates is on-going to determine their potential impact on field performance.

Current Teagasc Recommendations

All fungicides must be applied in a manner that achieves both the desired disease control whilst minimising the potential for resistance development and/or spread. Key to this is ensuring fungicides are only applied when necessary, as part of mixtures with different modes of action and at the minimal rates required. For STB control the SDHIs should not be applied more than twice (and only if necessary) and always in mixture with an azole and a multisite fungicide.

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CONTROLLING SEPTORIA USING MANAGEMENT PRACTICES

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Summary: Seeding rate and variety were included in winter wheat field trials to test the effect of crop density on the progress of *Zymoseptoria tritici*, known as septoria tritici blotch (SBT), and on final yield. Disease progress was measured as area under disease progress curve (AUDPC), yield adjusted to 15% moisture and data was analysed using ANOVA. Disease progress increased as seeding rates increased ($P = 0.015$). While yields also increased as seeding rate increased, there was no difference in yield between the medium to high seeding rates. Disease progress varied between varieties ($P > 0.001$); disease progress was greatest in the most sensitive variety Cordiale and least in the most resistant variety, Stigg. Results presented are based on preliminary analysis from the first year of a 3 year experiment.

INTRODUCTION

The worldwide average yield of wheat is approx. 3 t/ha, but Irish winter wheat crops averaged over 8 t/ha between 2007-2010 (J. Spink, personal communication). Septoria tritici blotch (SBT) caused by *Zymoseptoria tritici* is the main disease of winter wheat in Ireland and many other wheat growing regions throughout the world (Cools & Fraaije, 2013); Burke and Dunne (2008) recorded yield reductions of up to 50% resulting from STB under Irish growing conditions. Given the importance of wheat locally and globally, and considering the potential losses due to STB, adequate control of *Z. tritici* is important for the continuation of profitable wheat cultivation in Ireland and elsewhere.

The application of fungicides has been shown to contribute substantially to the yield of wheat (Blake et al., 2011, Dunne et al., 2008) and intensive cereal production has come to rely on chemical fungicides to secure yields in high disease pressure situations (Anon, 2014). However, the development of insensitivity, and in some cases resistance, to fungicides has limited the number of active ingredients available for control. Additionally, recent changes in European regulations mean some of the fungicides which are available may be removed from the market in the near future, specifically the azoles (Blake et al., 2011, Jess et al., 2014).

Whilst STB control is reliant on fungicides, other tools for managing STB are available. Integrated pest management utilises all available crop protection resources and crop management techniques, and STB control should utilise all available means to reduce disease pressure. Taking into consideration local conditions and expected disease pressure, this includes agronomic practices and STB host resistance. Although currently imperfect, host

resistance can reduce the associated risk of yield loss and therefore the reliance currently placed upon fungicides.

In addition as STB spreads mainly via rain-splashed spores, facilitating transfer up the crop canopy it has been suggested that management of the crop canopy in order to reduce horizontal or vertical transfer (disease escape) of disease would help to reduce the incidence of STB (Arraiano et al., 2009). This can be achieved through manipulation of seeding rates, and variety choice based on physiological traits, amongst other factors. The current work aims to quantify the effect of crop density on disease progress and eventual yields under high disease pressure in the high yield environment often experienced in Ireland. Preliminary results from the first year of a three year project are reported here.

MATERIALS AND METHODS

Field trials studying the effects of seeding rate and variety on the progress of STB were conducted in Oak Park, Carlow, Ireland, over the 2014-15 growing season using a complete randomised block design with 4 replicates. Four varieties of winter wheat were used, Cordiale, J.B. Diego, Lion and Stigg (not all are now included in current recommended lists but these were rated 4, 6, 7 and 8 respectively in official varietal testing results in Ireland in 2012 (Anon, 2012)) in order from the most to the least susceptible) and five seeding rates of each variety (100, 200, 300, 400 and 500 seeds/m^2) were sown. Plots of 12 x 2.5m in size were sown on 2nd Oct 2014. Plots received the same nutrient and weed management as the surrounding commercial crop, and STB was allowed to develop naturally, without management by fungicides. Visual disease assessments were carried out at three time points, GS 37, 55-61 and 73-77. Flag leaf and leaf 2 were assessed by leaf layer for % surface area infected on ten randomly selected main stems per plot, and the average disease per plot per leaf layer at each time point was used to measure the progress of disease between GS 37-77, as area under disease progress curve (AUDPC). Plots were harvested using a small plot combine harvester, and yield was calculated to 15% moisture content.

Differences in disease progress and differences in yield were analysed using General Analysis of Variance, with seeding rate and variety as main factors. An association between disease progress and yield was measured using Pearson's correlation. All data analysis were carried out using the GenStat (14th edition) program.

RESULTS

Flag leaves of Cordiale were dead at the final assessment time; hence flag leaf AUDPC was not calculated for any plot. Instead, AUDPC for leaf 2 was calculated and reported in Table 1 and presented in Figure 1. Seeding rate and variety had a significant effect on disease progress in this trial (P = 0.015 and P < 0.001 respectively, Table 1), but there was no seeding rate by variety interaction. The lowest seeding rate (100 seeds per m^2) had the least disease over all the seeding rates, and disease progressed at a similar rate for the remaining seeding rates. The most susceptible variety, Cordiale, had the most STB, and the least susceptible variety, Stigg, had the lowest amount of STB (Table 1). Disease progress corresponded with yield; lower seeding rates had lower yields, although the difference between yields in plots with seeding rates of 200, 300, 400 and 500 seeds/m^2 was not significant based on LSD of 0.299. There was

a significant negative correlation between disease progress and yield (r = -0.66, $P < 0.001$), with the exception that while Lion had significantly less disease than J.B. Diego; it also had a significantly lower yield (Table 1 and 2).

Table 1. AUDPC (disease progress of STB between GS 37 and 77) for leaf 2 from plots with four varieties of winter wheat, all sown at five different seeding rates. Effect of these factors on disease progress were analysed using ANOVA.

	AUDPC				
Seeding rate (seeds/m^2)	Cordiale	J.B. Diego	Lion	Stigg	**Mean**
100	519	279	171	65	**259**
200	728	330	239	118	**354**
300	727	335	289	58	**352**
400	659	451	259	59	**357**
500	671	439	329	64	**376**
Mean	**661**	**367**	**257**	**73**	

	d.f.	*P* value	LSD
Seeding rate	4	0.015	71.2
Variety	3	< 0.001	63.7
Seeding rate x Variety	12	0.365	142.4

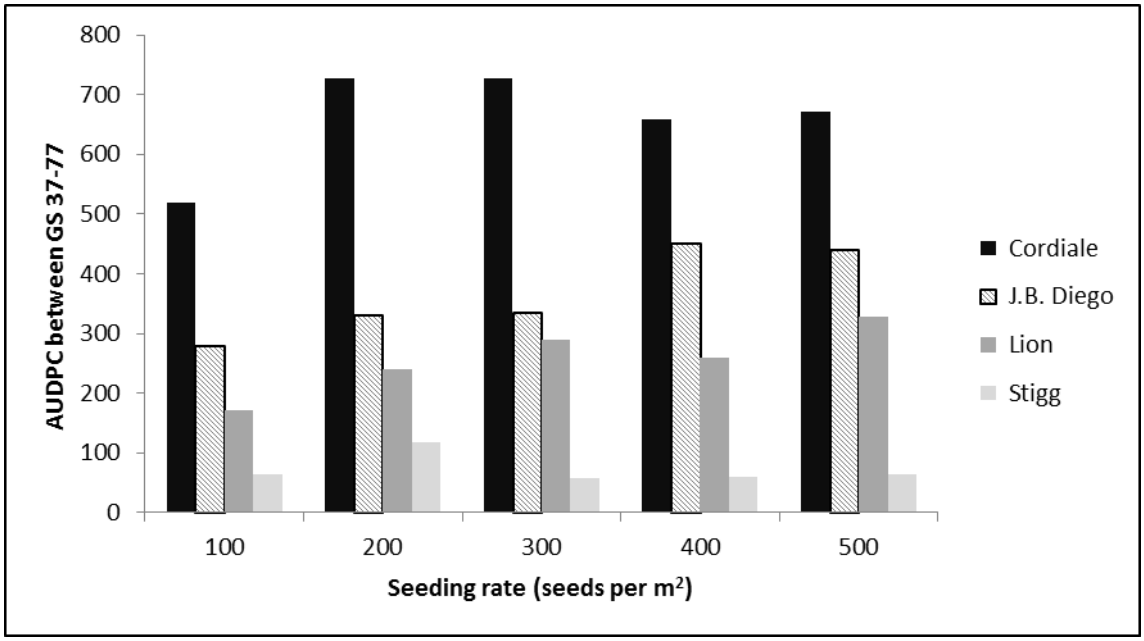

Figure 1. STB progress between growth stages 37-77, represented by the AUDPC for leaf 2, in plots of four winter wheat varieties. Each variety was sown 5 different seeding rates.

Table 2. Yield (t/ha at 15% moisture) after treatments (mean of four reps).

Seeding rate (seeds/m^2)	Yield (t/ha)				
	Cordiale	J.B. Diego	Lion	Stigg	Mean
100	8.48	10.32	9.53	11.19	**9.88**
200	8.69	10.85	9.85	11.95	**10.33**
300	8.91	10.84	10.00	11.92	**10.42**
400	9.06	10.83	9.94	11.94	**10.44**
500	8.86	10.99	10.17	12.02	**10.51**
Mean	**8.80**	**10.77**	**9.90**	**11.80**	

	d.f.	*P* value	LSD
Seeding rate	4	< 0.001	0.299
Variety	3	< 0.001	0.268
Seeding rate x Variety	12	0.99	0.599

DISCUSSION

Crop architecture and density have been identified as factors in disease development; Arraiano et al. (2009) studied the effect of plant architecture and host resistance on disease escape, Baccar et al. (2011) modelled disease progress in different crop densities, and Tivoli et al. (2013) provided a recent review of the work in this area. Disease levels in each variety in this trial were related to their susceptibility rating; plots of Cordiale had almost double the amount of STB than J.B. Diego and Lion, and nine times more STB than Stigg. Host resistance has been linked to a reduction in yield and, as yield is the main consideration when choosing a variety, resistant varieties are sometimes seen to be less commercially appealing (Brown, 2002). However, results from this one site/year contradict the yield penalty theory somewhat and, if found to be consistent at the end of the project, they will support the case for more resilient varieties reaching the recommended lists. Crop density can influence disease development by altering the microclimate in the crop and/or facilitating disease escape, and while we show that crop density had the effect of increasing disease progress, results from previous similar studies are inconsistent (Broscious et al., 1985, Baccar et al., 2011). Yield is also positively associated with crop density, but as there was no statistical difference in yield between plots sown with 200 and 500 seeds/m^2, using the lower rate would not only reduce the incidence of STB, but also reduce production costs. The current results are based on one site/year only, and further research is required to confirm these findings

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DETECTION OF THE CYCTOCHROME B MUTATION G143A IN THE IRISH *RHYNCHOSPORIUM COMMUNE* POPULATION USING TARGETED 454 SEQUENCING

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Summary: *Rhynchosporium commune*, cause of barley scald, is the most destructive pathogen of both winter and spring barley crops in Ireland. Control is achieved using a combination of varietal resistance, agronomic practises and foliar fungicide applications. Currently a range of fungicide actives with *R. commune* activity are available, however *R. commune* has the potential to develop resistance to these fungicides. Amongst these currently available fungicides are a number of the Quinone outside inhibitors (QoI). The interaction of *R. commune* and QoI fungicides is regarded as being at a medium resistance risk, with the potential for *R. commune* to develop the QoI resistance mutations F129L, G137R and G143A in the cytochrome *b* target site. Monitoring of Irish *R. commune* populations detected the G143A mutation in a single sample in 2013 and in four samples in 2014.

INTRODUCTION

Barley is the most widely grown cereal in Ireland, accounting for over 70% of all cereals grown. Whilst spring barley accounts for most of this, in recent years improvements in winter barley yields coupled with difficulties associated with winter wheat production have resulted in an increased area of winter barley. Favourable climatic growing conditions together with long day lengths during the grain filling period provide the ideal conditions to maximise yields in both crops. Similar to all other cereals, these climatic conditions are also ideal for the development and spread of wet weather diseases. Barley scald, caused by the ascomycete pathogen *Rhynchosporium commune*, is amongst the most destructive of these diseases (Avrova & Knoge, 2012). Depending on levels of infection prior to stem extension *R. commune* can reduce the number of tillers available to generate yield and subsequently, post stem extension, can reduce the green leaf area available during grain filling. To prevent such losses disease control programmes integrating varietal resistance, agronomic practises and foliar fungicide applications are utilised in crops. Whilst varietal resistance is improving positive yield responses continue to be attained from fungicide applications and Irish winter barley crops annually receive between 2-3 foliar fungicide applications, whilst spring barley crops annually receive 1-2 foliar fungicide applications.

Currently a range of fungicide groups including the azoles, the Quinone outside Inhibitors (QoIs), the succinate dehydrogenase inhibitors (SDHIs) and other individual actives (e.g. cyprodinil and chlorothalonil) are available to growers for the control of *R. commune*. The availability of these different actives allows for the implementation of effective anti-resistance

strategies which can include limiting the number of applications of individual fungicide groups and / or the mixing of fungicides with different modes of actions but with similar efficacy profiles. Ensuring the availability and effective lifespan of all currently available actives is essential. Unfortunately due to the adaptability of *R. commune* and the properties of the QoI, azole and SDHI actives the potential for resistance development to these groups is regarded as medium – high risk (Grimmer et al., 2014). Complementary to the above anti-resistance strategies is the need to monitor *R. commune* populations for changes in sensitivity. This can include both phenotypic assays, and where resistance mechanisms are known molecular assays. As the mechanisms for QoI resistance in *R. commune* has been confirmed molecular assays have been developed, including a pyrosequencing assay (Fountaine, 2011) which can allow for the rapid screening of high numbers of isolates and hence a better chance of detecting changes early.

DETECTION OF G143A

The presence of the cytochrome *b* mutation G143A known to confirm high levels of resistance to the QoI fungicides (Fountaine, 2011) was detected in the Irish *R. commune* population both in 2013 and 2014 using a 454 amplicon sequencing assay (*data unpublished*). In 2013 the mutation was detected in a single sample obtained from a spring barley crop, while in 2014 the mutation was detected in four samples obtained from three different sites.

RECOMMENDATIONS

Given the availability of a range of different fungicide groups with good or excellent activity against *R. commune* it is imperative that fungicide mixtures with different modes of actions are applied. The efficacy of each of the individual active included in these mixtures must be assessed to ensure equal activity of the fungicide partners. Furthermore, to reduce the exposure of the pathogen and given the range of fungicide groups available repeated applications any one fungicide can be avoided.

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CHANGES IN LEAF MICROBIOME LINKED TO DISEASE SUSCEPTIBILITY

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Summary: Plant pathogens cause significant losses to crop production, but they represent only a small fraction of the microbiome populating crops. Little is know about the effects of the majority of non-pathogenic microbes on the crop fitness. Using molecular tools, it was shown that the leaf-associated bacterial populations differed between barley accessions (commercialised cultivars and landraces). Leaves were collected from tussocks grown in the field, which were visually scored for the following diseases: leaf scald, powdery mildew and ramularia leaf spot (RLS). Plants with high amount of leaf scald had low bacterial diversity, whereas the opposite trend was observed in plants with high amount of mildew and RLS. Landraces also showed a larger variability in bacterial diversity than cultivars. The genetic variability of landraces could be mined for crop breeding to achieve more sustainable disease management through phyllosphere manipulation.

INTRODUCTION

The phyllosphere, i.e. the leaf habitat, is a large and rich environment, where bacteria are the most abundant leaf coloniser (Lindow and Brandl, 2003). Host genotype is a major factor affecting the phyllosphere composition and significant differences in bacterial composition have been observed between various plant species as well as between cultivars (De Costa *et al.*, 2006; Sapkota *et al.*, 2015). Other factors have also been shown to have an effect and these include weather conditions (O'Brien and Lindow, 1989; Copeland *et al.*, 2015), location (Cordier *et al.*, 2012; Sapkota *et al.*, 2015) and agricultural practices (Behrendt *et al.*, 2004).

Although these factors have been identified and studied under controlled and natural conditions, little is known about the effects of the microbial populations as a whole on plants (Schlaeppi and Bulgarelli, 2015). Behrendt *et al.* (2004) found a correlation between forage quality and microbial density. The phyllosphere was also shown to be modified in the presence of pathogens. Bacterial diversity and richness increased on cucumber and Japanese spindle leaves naturally infected by powdery mildew (Suda *et al.*, 2009), whereas maize varieties resistant to southern leaf blight (SLB) hosted a greater bacterial diversity compared to the susceptible ones (Balint-Kurti *et al.*, 2010). Here, we hypothesise that the barley phyllosphere is also affected by diseases and its composition may be differentially affected by different types of pathogens.

Studies of the barley phyllosphere are few (Fountaine *et al.*, 2009; Sapkota *et al.*, 2015) compared to other cereals and grasses (De Costa *et al.*, 2006; Balint-Kurti *et al.*, 2010; Sapkota *et al.*, 2015). Barley is an economically important European crop whose major diseases in Scotland are leaf scald (*Rhynchosporium commune*, *Rc*), ramularia leaf spot (*Ramularia collo-*

cygni, *Rcc*) and powdery mildew (*Blumeria graminis* f.sp. *hordei*, *Bgh*). Using a molecular DNA fingerprinting method, namely Ribosomal Intergenic Spacer Analysis (RISA), a selection of field-grown barley varieties, including both commercial cultivars and ancient landraces, have had their phyllospheric endobacterial communities characterised at the end of the growing season over 2 years. The objectives of this study were to investigate the following issues: i) the potential difference in bacterial communities between cultivars and landraces; and ii) the effect of naturally-occurring disease on bacterial populations.

MATERIALS AND METHODS

Twenty six barley accessions, including 6 cultivars and 20 landraces (Table 1), were grown in tussocks of 20 seeds at two Scottish locations: Gilchriston, East Lothian (2012) and Boghall, Midlothian (2014), using conventional agricultural practices. We obtained seed material for landrace accessions from European (IPK Gatersleben, Nordic Gene Bank, GRC-INRA, the Centre for Genetic Resources) and North American (NSGC) gene banks and commercially available cultivars from UK breeding companies. At growth stage 72 (GS72) (Zadoks *et al.*, 1974), 10 flag (F) and F-1 were harvested and stored at -20°C until required. At GS72, tussocks were also visually scored for foliar symptoms. In 2012, leaf scald (*Rhynchosporium commune*, *Rc*) and ramularia leaf spot (*Ramularia collo-cygni*, *Rcc*) were observed, whereas in 2014, only powdery mildew (*Blumeria graminis* f.sp. *hordei*, *Bgh*) was visible.

Table 1: List of barley accessions grown in the field.

	Name / Accession	Origin	Gene bank
Cultivars	Forensic		
	Propino		
	Quench		
	Shuffle		
	Waggon		
	Garner		
Landraces	5.1	Germany	INRA
	5.6	Great Britain	INRA
	5.8	France	INRA
	5.10	France	INRA
	5.19	Finland	NGB
	5.23	Germany	NSGC
	5.25	Ireland	NSGC
Landraces	5.33	Poland	IPK
	6.49	France	INRA
	6.81	West EU	CGN
	7.92	East EU	CGN
	7.115	East EU	CGN
	7.129	Georgia	IPK
	8.140	Denmark	IPK
	8.142	Norway	IPK
	8.147	Denmark	IPK
	8.149	DDR	IPK
	8.179	Sweden	IPK
	9.189	CCII Harlan	
	9.201	CCII Harlan	

Five leaves were thawed and sonicated for 7 min in 10 mL potassium phosphate buffer (0.01M, pH7). Washing solutions were centrifuged and stored at -20°C, along with the washed leaves. The former fractions represented the epiphytic bacterial populations and the washed leaves were used to characterise the endophytic bacterial communities. Only the latter communities were characterised in the present study. Leaves were ground into a fine powder using liquid nitrogen, a mortar and pestle and DNA was extracted from 100 mg of ground leaves using a phenanthroline-based buffer, as described in Fountaine *et al.* (2009). Similarly, the RISA PCR conditions were carried out as described by Fountaine *et al.* (2009) using the following primer

pair: 1406F (5'-TGYACACACCGCCCGT-3') and 23SR (5'-GGGTTBCCCCATTCRG-3'). The PCR products were run on the 2100 Bioanalyzer (Agilent, USA) using the 7,500 bp chip, according to the manufacturers instructions. Each sample was defined by a list of operon taxonomic units (OTUs, i.e. bacterial ribotypes) associated with an area under the peak (i.e. PCR band intensity). Results were organised in a matrix and expressed as relative abundance for each OTUs. Using this matrix of bacterial profiles, a Principal Component Analysis (PCA) was performed to group similar populations, using Genstat (VSN International Ltd, version 15). The structure of the bacterial communities was estimated using the Shannon diversity index. Differences of PCA scores and Shannon indices between phyllospheric populations of cultivars and landraces were compared using t-tests.

RESULTS

The PCA plots for 2012 and 2014 (Figure 1a and 1b) showed that the phyllosphere of the barley cultivars were more similar, i.e. data points not spread, than that of landraces with data points more spread. The bacterial communities of landraces were significantly segregating from the cultivars according to PC4 (7.8%, *P*=0.046) and PC2 (15.5%, *P*=0.029) (Figure 1a and 1b respectively). The Shannon diversity index, which represents the community structure (i.e. OTUs homogeneously or heterogeneously represented), was visualised using box plots (Figure 1c). It confirmed that cultivars had a more conserved bacterial diversity, whereas it was more variable for landraces. The diversity observed in cultivars phyllosphere was similar between 2012 (1.77) and 2014 (1.87) (*P*=0.15, lsd=0.34). However, phyllo-bacterial diversity of the landraces appeared to exhibit a greater variability, but was not significantly different (*P*=0.15, lsd=0.19). While the average diversity observed in landraces phyllosphere appeared greater than the one of cultivars in 2012 (1.91), it was lower in 2014 (1.73), but in neither cases significant differences were observed (*P*=0.15, lsd=0.28).

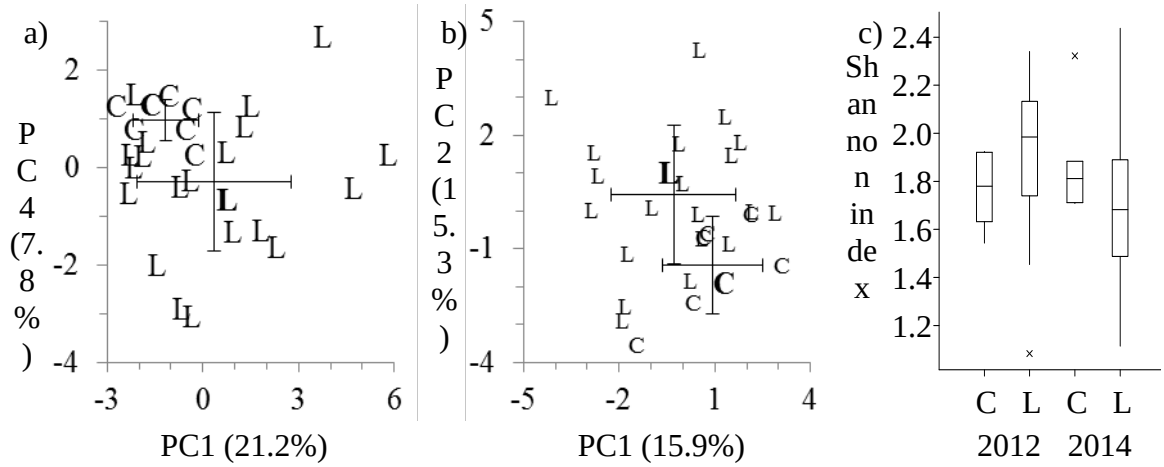

Figure 1: Estimations of endophytic phyllo-bacterial communities in barley cultivars (C) and landraces (L) in 2012 (a, c) and 2014 (b, c) using PCA plots (a, b) and box plots of Shannon diversity index (c, n.s.).

Disease symptoms in the field were visually estimated concurrently with leaf sampling in both years. The correlations between disease infection rates and the bacterial diversity of the various barley landraces (14 out of 20) were analysed using linear regression (Figure 2). Three diseases

were observed in the field: leaf scald (*Rc*) and ramularia leaf spot (*Rcc*) in 2012 and powdery mildew (*Bgh*) in 2014. A significant correlation between the percentage of leaf area affected and the phyllosphere diversity was observed for *Rc* (*P*=0.01), but not for *Rcc* (*P*=0.07) and *Bgh* (*P*=0.12). The more *Rc* symptoms were visible, the lower was the bacterial phyllospheric diversity. Although not significant, opposite trends were observed with *Rcc* and *Bgh*: more visual symptoms correlated with greater bacterial diversity.

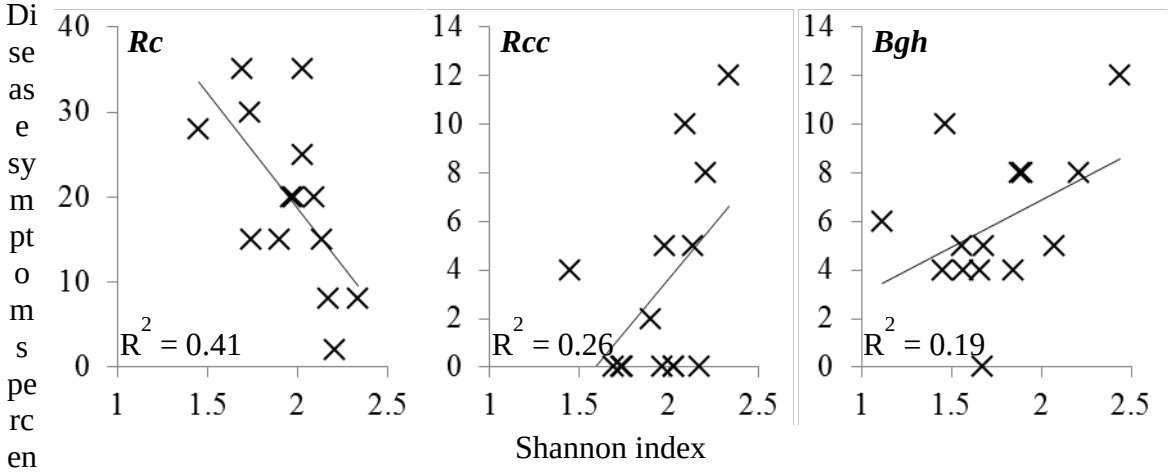

Figure 2: Linear correlation plots between the Shannon diversity index and visual disease symptoms (%). Three diseases were recorded: leaf scald (*Rc*) and ramularia leaf spot (*Rcc*) in 2012 and powdery mildew (*Bgh*) in 2014. Regression analyses were significant with *Rc* (*P*=0.01), but not with *Rcc* (*P*=0.07) and *Bgh* (*P*=0.12).

DISCUSSION

This study had 2 major findings: i) ancient landraces exhibited more variable bacterial communities than modern cultivars; ii) the structure of bacterial communities differs depending on plant pathogens infecting the host. Here, the hemi-biotrophic pathogen *Rhynchosporium commune* caused more infections on plant with a low bacterial diversity (*i.e.* dominated by certain bacterial ribotypes), whereas the biotrophic pathogen, *Blumeria graminis* f.sp. *hordei* tended to have greater infections on plants with a high bacterial diversity (*i.e.* with a more homogenous representation of the bacterial ribotypes). The infection pattern of the hemi-biotrophic pathogen *Ramularia collo-cygni* had a similar trend that that of the *Bgh*. This could be explained by the fact that *Rcc* goes through an extensive biotrophic phase during its life cycle (Havis *et al.*, 2015). Furthermore, at GS72 *Rcc* is about to enter its symptom causing phase. Molecular quantification of *Rcc* may provide better correlation with bacterial diversity, especially as 7 out of 14 landraces had no visible RLS symptoms.

Hence, the life style of fungal pathogens correlated with the bacterial microbiome structure. Correlations between bacterial diversity and susceptibility to fungal diseases were previously observed in other crops and with other pathogens. Diversity increased on powdery mildew-infected cucumber and Japanese spindle leaves (Suda *et al.*, 2009), whereas it decreased on leaves of southern leaf blight (*Bipolaris maydis*) susceptible maize varieties (Balint-Kurti *et al.*, 2010). This again seems to confirm that biotrophs and hemi-biotrophs with a long biotrophic phase are more infectious on plants with a high bacterial diversity, whereas

necrotrophs and hemi-biotrophs with a short biotrophic stage are more infectious on plants with a low bacterial diversity.

Biotrophs and necrotrophs are controlled by differently induced plant-defence mechanisms. It is generally accepted that biotrophic pathogens are susceptible to SA-dependent defences, whereas necrotrophic pathogens are susceptible to JA-induced defences (Glazebrook, 2005). Both sets of defences have shown to influence the abundance and diversity of leaf-associated culturable bacterial communities on *Arabidopsis thaliana* (Kniskern *et al.*, 2007). Plants with constitutively SA-induced defences had a reduced endophytic population, whereas mutants deficient in JA-dependent defences had a greater epiphytic diversity. Our results corroborate previous observations concerning the biotrophs-endobacteria interactions. Plants infected by biotrophic pathogens trigger their SA-induced defences. As a result, plants able to defend themselves exhibit a reduced endophytic bacterial population (Kniskern *et al.*, 2007); whereas successful infection by biotrophic pathogens results in increased endophytic bacterial diversity (Figure 2; Suda *et al.*, 2009).

Even though this study highlights a correlation between bacterial diversity and disease resistance, it does not give any information on whether a change of bacterial diversity is a pre-requisite or a consequence of disease development. Furthermore, other factors may have influenced the phyllosphere between 2012 and 2014. Weather conditions greatly differed between the summers of 2012 (rain, low temperature and low amount of sunshine) and 2014 (warm and sunny) and may have also affected the overall bacterial diversity. It is known that bacterial density increases under wet and low light conditions (O'Brien and Lindow, 1989). Shifts in bacterial relative abundances have also been observed after heavy precipitations (Copeland *et al.*, 2015). Finally as the experimental fields differed between 2012 (Gilchriston) and 2014 (Boghall), the location may have had an effect on the phyllosphere composition (Cordier *et al.*, 2012; Sapkota *et al.*, 2015).

This study highlights that landraces possess more genetic variability, which may affect the phyllosphere in an unknown way, as they produced more variable bacterial diversity than cultivars (Figure 1). This bacterial variability correlated with a greater genetic variability of landraces compared to the commercial cultivars used in this study. A subset of the landraces (accessions: 5.1, 5.6, 5.8, 5.10, 5.19, 5.23, 5.25) and all cultivars have been genotyped using 384 single nucleotide polymorphisms (Hoebe *et al.*, in prep) to estimate genetic diversity differences between the two groups. The barley landraces showed an average of 1.5 alleles per locus compared to 1.3 alleles per locus for the cultivars. Locally adapted barley landrace materials are genetically and phenotypically more diverse than pedigree bred cultivar material (Hoebe *et al.*, in prep; Russell *et al.*, 2000). Therefore, landraces represent a great resource for genetic crop improvement (Newton *et al.*, 2010a), including potentially manipulation of the phyllosphere (Newton *et al.*, 2010b).

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RECENT ADVANCES IN BIOTECHNOLOGY AND INFORMATION TECHNOLOGY IN THE POTATO INDUSTRY

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Summary
Recent advances in potato production particularly concern those in biotechnology and information technology. This paper discusses two examples of these advances from the Netherlands. Developments in biotechnology are demonstrated using the methodology and results of the DuRPh project. In this 10 year project, a proof of principle was developed to make potato resistant to late blight (*Phytophthora infestans*) by the introduction of resistance genes from wild Latin American potato species without the use of selectable markers: cisgenesis. Advances in information technology are exemplified by the national Netherlands effort of Smart Potato Farming in which a potato ontology is developed, decision support systems are upgraded with geographical data details and a national data exchange platform (Akkerweb) is successfully exploited.

INTRODUCTION

Since 1995 most resistance genes against diseases and pest have been located in the potato genome and cloned. Through genetic modification many of these resistance genes have already been deployed in new potato varieties resulting in resistance against viruses which drastically reduces the degeneration rate of seed, reduced losses and sprays related to late blight and control of cyst nematodes allowing potato more frequently in the crop rotation. A gene from a bacterium made one variety resistant against Colorado beetle and another against tuber moth potentially reducing losses and use of insecticides. Quality characteristics are also being altered through these techniques, for example, reduction in bruising damage and browning when frying. Precision farming translates as giving potato crops and tuber lots the right treatment at the right time at smallest scale possible. Introduction of GPS systems, sensors and e-Science allows treatment of plants at grid and eventually individual plant level, and offers new opportunities for chain management with real-time data. Decision support systems are being developed for many parts of the production and processing chain. The expectation is that innovative genetic, sensor and big data techniques will benefit potato more than other crops due to the many solvable issues from which this clonally multiplied crop currently suffers. .

Biotech

The first genetically modified potato products were plants with resistance to Colorado beetle (*Leptinotarsa decemlineata*), *potato leafroll virus* (PLRV) and *Potato virus Y* (PVY). These were registered in the United States of America and allowed to grow there. Monsanto brought

NewLeaf to the market, the variety Russet Burbank incorporated with a Bt-gene from *Baccillus thuringiensis* making it resistant to Colorado beetle. Risks of non-acceptance coupled with the high costs of regulatory compliance resulted in the withdrawal of these products from the market at the turn of the century.

BASF created varieties Amflora, Modena and Amadea by modifying the GBSS gene with RNAi resulting in solely amylopectin production in the tubers. BASF also modified the variety Fontane into Fortuna with two late blight resistance genes from *Solanum bulbocastanum* with the aid of *Agrobacterium tumefaciens*. Meanwhile the potato company Simplot in the USA developed a GM line of so called 'innate' potatoes through RNAi, RNA interference disabling poly phenol oxidase reducing black spot bruising and reducing asparagine formation thereby reducing brown discoloration when frying. Future "innate" potatoes are being supplied with the Rpi-vnt1.1 gene to make the crop resistant to late blight.

Information technology

More and more data in agriculture are being generated and captured in databases for further use. The developments in information technology, speed of data transfer, data processing and storage capacity are such that they started to assist growers in managing their crops and customers to make use of data generated in the process of production. To structure data, an ontology of the domain needs to be established first. Subsequently, the database needs to be populated with information regarding the genotype, environment and management. Lastly, use should be made of the database in subsequent strategic, tactical and operational decisions, both of growers and customers. In this contribution, a recent advance in information technology in potato production will be discussed as an example: Smart Potato Farming consisting of a consortium of related projects aimed at collecting, organizing and exploiting data in potato production.

DuRPh PROJECT

The DuRPh (Durable Resistance against Phytophtora through cisgenesis) project at Wageningen UR was carried out from 2006 through 2015.The project aimed at proving the principle that sustainable resistance can be achieved through cisgenesis. This involves the use of multiple resistance genes from crossable (with *Solanum tuberosum*) wild potato species from North and South America through genetic modification (cloning genes with *E. coli* and transform them with *A. tumefaciens*). The use of existing varieties is essential as these have proven to be of market value whereas the introduction of new varieties is costly and the success rate usually is low. Moreover the introgression of a single resistance gene takes decennia. In 1959, *S. bulbocastanum* (2x) that cannot be crossed directly with *S. tuberosum* (4x) was crossed with *S. acaule* to create the AB-material (3x) that after polyploidisation (6x) six years later was crossed with *S. phureja* (2x) yielding ABP material (4x) that was crossable with *S. tuberosum* (4x) leading to the widely used breeding material ABPT to introduce the *S. bulbocastanum* into modern varieties. The varieties Bionica and Toluca only contain one R-gene which in the past, when only single genes of *S. demissum* were used proved risky, and indeed currently the resistance of these new varieties has not kept up. The nomenclature of the Resistance genes is explained with the aid of the following example: Rpi-vnt1, meaning R= Resistance gene, pi = *Phytophthora infestans*, vnt = *S. venturi*, 1 = the first Rpi gene found in this wild species. Globally costs of control in developed countries but mainly losses due to late

blight in developing countries are estimated to be close to € 9 billion (Haverkort et al., 2015). The DuRPh project concluded in 2015 and consisted of five subprojects that are detailed below.

Cloning

The main interest regarding cloning new R genes is to find and clone genes with a broad spectrum of recognition, meaning that the gene allows the potato plant to recognize and offer resistance to many late blight pathotypes, each pathotype differing in a typical protein (an effector) they contain. Late blight resistance is based on the host potato plant being able to detect that it is being attacked by *P. infestans* and effect a hypersensitivity reaction, killing a great number of cells around the invaded spot on the leaf (apoptosis) and resulting in the death of the pathogen. To be able to clone R-genes, first they have to be detected by crossing a wild species with a susceptible variety. If Mendelian segregation in a 50% to 50 % ratio the presence of an R-gene is assumed. If it is more complicated in case of the presence of a stack of R-genes the result may be backcrossed with the susceptible genotype, so-called de-stacking. Through successive mapping, fine-mapping and landing in a BAC-library (bacterial artificial chromosome), some 20 R genes that segregated in 50-50 had been cloned by late 2015.

Transformation

The DuRPh project used three potato varieties to transform into late blight resistant ones, the early table variety Premiére, the mid late variety Désirée and the late maturing starch variety Aveka. Single R genes or stacks up to three were cloned into a binary *A. tumefaciens* vector. At the start of the project, a selectable marker gene, kanamycin resistance, was used to quickly explore which R genes were effective but as this is not a Solanum gene this could not be classed as a cisgenic plant. The following approach was taken to overcome this issue. First, most of the R genes were transferred with *A. tumefaciens* to the variety Désirée to create a set of R genes in an iso-genic background but with varying spectrum of resistance. Broad spectrum resistance are resistant to over 80 % of all isolates occurring in an area, narrow spectrum to less than 10 %. This set was then used in the subproject 'resistance management'. Selected R genes with known resistance spectrum were transferred to study the influence of the genetic background on transformation efficiency and resistance expression, both showing considerable variation. Next combinations of two R genes were made, not all of these were stable in the vector and they varied in transformation efficiency associated with slow growth of the vector. Also it was discovered that if two genes were transferred in one stack, they were not always both effective. Next, marker-free events were created by mass transformation and each transformed plantlet was screened for the presence of the R gene using PCR (de Vetten *et al*., 2003). Transformed plantlets were also checked for freedom of vector backbone. Within the time frame of the project it was not possible to obtain a cisgenic event containing 3 R-genes so an event with 2 R genes was re-transformed with another single different R gene to obtain an event with 3 R genes.

Selection

After a successful transgenic or cisgenic event, with the vector *A. tumefaciens* transferring one or more R-genes to a cell of recipient variety and the production of plantlets through a callus stage, a number of tests are carried out to finally select genotypes. Genotypes that carry the target genes with as few copies as possible, without vector backbone (parts of the plasmid of *A.*

tumefaciens) of which the R gene stack is a part are selected. The resulting plant and subsequent tubers are resistant to late blight and true to type. The number of transfer DNA, T-DNA insertions or the number times the same R-gene is transferred to the genome could not be ascertained with the Southern blot technique in marker free events so it was done with PCR and R-gene specific primers. Similarly, backbone screening took place to remove undesired events. Subsequently, late blight resistance was assessed with a detached leaf assay in the lab and with field trials. Finally, it is important that the new genotype only distinguishes itself from the original variety, the wild type through its resistance to late blight but that all other characteristics remain the same so growers and processors do not have to take special measures regarding the new genotype. In 2014, 22 events mainly transformed Désirée but also Première and Aveka were tested in the field.

Resistance management

In the past, in conventional breeding programs, a single R gene from *S. demissum* was introgressed that conveyed resistance to all hitherto present late blight pathotypes. As soon as a new pathotype is introducedthrough the atmosphere, through mutation, or because of variation following sexual replication of the two mating types A1 and A2, that is not recognized by the resistant variety, its resistance is overcome. Resistance is overcome more rapidly if it is based on a narrower spectrum of *Phytophthora* isolates and subjected to high spore densities caused by incomplete control and large areas of this variety. Supplying a variety with two, three or four R genes considerably reduces the chance of the variety becoming susceptible to new pathotypes.. Monitoring the single R-gene events by planting them as a differential set in three different areas in the country allows the recording of broken R genes. When two R genes in a stack are left unbroken, seed potatoes with ~ a new stack should be bulked to have a new stack ready for growers within a few years. In the unlikely event that only a single R-gene is present, growers are advised to spray a few times during the season with a low dose fungicide. Monitoring plots with Désirée events in 2013 showed that the weak, narrow R3a gene from *S. demissum* delayed infection by three days. The results showed that there was a clear effect of specificity and of stacking. In the two final years of the DuRPh project a field trial was conducted near Wageningen to demonstrate the effectivity of resistance conveyed by specificity and stacks combined with low doses (25 % of conventional dose) of fungicide sprays. The synergistic effect of stacking was similar to that of adding low dose to a narrow spectre resistance gene.

Communication

The DuRPh project dedicated 10 % of its budget to communication. The objective of this was to inform society, all stakeholders such as consumers, consumer organizations, growers, non-governmental organizations, policymakers, journalists and peer scientists of the motifs, procedures and outcomes of the project. This was in order to allow all involved or interested to judge the advantages and disadvantages of the cisgenic breeding approach. It was not an objective to convince or to achieve societal acceptance of this approach nor to stress its advantage compared to transgenesis. Over the years hundreds of interviews and presentations were given for laymen and scientific peers and many articles in newspapers appeared and films shown on television channels in the Netherlands and abroad (example http://youtu.be/veX6VXAfUoU). The website (www.durph.nl) was updated when related news appeared and contributions to webinars (example http://nas-sites.org/ge-crops/2014/09/22/webinar-november-6/ were recorded.

SMART POTATO FARMING

The national aspiration in the Netherlands to arrive at Smart Potato Farming consists of various research and development efforts. First is the description of the domain or creation of an ontology; second, the collection of crop data, mainly from recordings and decision support systems (DSS); third, data collection from sensing associated with precision farming; and fourth, the development of a platform (Akkerweb) to facilitate user interaction. In this section, each of these aspects are shown under the national Netherlands umbrella activity of **S**mart **P**otato **F**arming (SPF),

Ontology

In structuring data, it is most helpful to create an ontology so those working with the data have a common understanding and to allow for numeric techniques to identify correlations. An ontology is a description of a domain, say 'seed potato production' in terms of instances in classes, super-classes and sub-classes that are described with features and attributes. The ontology completed with instances is a knowledge base.

One approach described by Haverkort *et al*. (2006) and Haverkort and Top (2011) is to organize the ontology in five groups: 1) **S**ociety with e.g. consumer classes and classes of products such as organic and labelled that determine the required performance, 2) **P**erformance of the crop resulting from yield and recovery as determined by seed, environment and management, 3) **G**enotype or propagation material as determined by variety, seed size, health and physiology, 4) **E**nvironment consisting of climate and soil and 5) **M**anagement of the crop. In a squence: :

S » P » G »E » M.

The ontology makes use of concepts or classes. For example, biocides are a subclass of agrochemicals and have their own subclasses i.e. fungicides, herbicides, nematicides and insecticides (which are therefore sub-subclasses of agrochemicals). General properties of agrochemicals are their timing of application and dose rate giving them "slots" or "restrictions" such as not allowed to apply 3 weeks before harvest. An instance elsewhere in the database may be e.g. variety Pentland Dell planted on April 15 in field New Acre, seed size 40-55 mm, class E and purchased from SeedCo. A widely used standard language is the Ontology Web Language (OWL) called Protégé that was developed by Stanford University in the USA.

Crop management (DSS)

Well-developed potato farming systems increasingly make use of decision support systems (DSS) for strategic (at the farm level, future oriented), tactical (before planting a particular field) and operational decisions. DSS all have in common that measurements are made, that the resulting data is inserted into a quantitative tool (a table or a model) which produces a quantitative output which the grower or the advisor uses to make a decision. An example of a decision support based strategic decision would be whether to grow potatoes in a given field if a heavy infestation with potato cyst nematodes is present, or to grow starch potato only as there are sufficient starch varieties available with resistance (reducing the population) and tolerance (yields not overly depressed by the current nematode population). The measurement here consists of sampling the soil, determining species and pathotype and the number of cysts and

viable eggs. The outcome is benchmarked against response curves of varieties with different tolerance and resistance against the various potato cyst nematode races and the optimal combination is chosen. If needed the DSS may also suggest type and dose of a nematicide.

Tactical decisions are the choice of variety and the use of the variety: a table variety requires a different nitrogen application regime from a crop destined for chipping. Based on the pre-planting residual amount of nitrogen in the soil determined from soil samples and depending on the variety and use, a pre-planting nitrogen rate is advised. After planting, a new supplemental rate of nitrogen is advised based on the amount of nitrogen present in the soil and/or foliage, or on the amount of nitrate in the leaf petioles, or based on crop reflection in infrared (shows biomass) and in green (shows percentage nitrogen in the biomass). The findings in sampling and sensing are compared to previously established calibration lines and appropriate advice is provided.

A wide application is operational decision support for late blight control. This DSS consists of a grower completing a form online detailing which variety is grown, which fungicide was applied previous;y (when and at which dose). The DSS collects past late blight development related weather events such as amount and duration of rain, dew, leaf wetness, and temperature; it collects spore density and presence of foci in the area and from information on variety maturity it assumes how many new unprotected leaves have appeared since the last treatment. Based on the weather forecast the grower is given spray advice including if, when, which fungicide and at what dosage. An irrigation planner also uses past irrigation amount, past and forecast weather, ground cover, and soil water holding capacity to advise on the time and rate of irrigation. A crop growth model such as LINTUL-POTATO-DSS (Haverkort *et al*., 2015b) may be used in strategic decision to calculate expected average yields and deviations due to erratic temperature (frosts and heat waves) and precipitation (floods and drought) events. It may, however, also be used for the operational decision to harvest a particular field based on the predicted yield at any given moment. This model requires information on planting depth time daily maximum and minimum temperatures, solar radiation, precipitation and evapotranspiration; these weather related data are readily available from a weather station. Soil water holding capacity following rooting depth and percentage clay and silt of the soil is also required. There are more DSS, such as planting rate, in MAPP, the management advisory package of potato developed at the James Hutton Institute at Dundee. There are the DSS based on insect trapping such as aphid counts to advise on haulm killing dates for certain seed potato classes in the Netherlands, Colorado beetle trapping for insecticide sprays in America and pheromone trapping of tuber moth for control in stores in North Africa. A most comprehensive DSS is NemaDecide developed in the Netherlands to reduce populations of potato cyst nematodes and free living nematodes based on rotation frequency of potato, type of nematode, choice of variety and support of a granular nematicide. This very successful and effective program is widely applied by seed potato growers to answer questions such as how do I detect the presence of PCN at an early stage? Which measures should I take to avoid infection and thereby losing my licence to grow seed potatoes? And what is the effect of potential control measures? For growers of starch and ware potatoes NemaDecide answers questions like ‘how do I control PCN infection and should I apply a granular nematicide at what rate of return?’ NemaDecide was extended in 2015 with a geographic interface and the sampling report is now accompanied by geographic coordinates so a grower can apply location specific control or management measures.

Precision farming

Precision farming is aimed at not treating the whole field in a similar manner but rather location specific, just in time, and with as little input as possible. This means that measurements and sensing data are needed to detect variation; consequently, variable rates of applications (VRA) inputs such as seed potatoes, topdressing of nitrogen (Van Evert *et al*., 2012), herbicides (Kempenaar *et al*., 2014, Van Evert *et al*., 2012) and fungicides following DSS are applied which requires specific adaptations to machinery. There are a few more challenges: 1) acquiring spatial information from soil and crop on time and at the right level of resolution i.e. as a multiple of the width of the planter, spreader or sprayer; 2) adequate communication between sensor, farm computer and machine - this is not always feasible and despite the wealth of DSS that work on the whole field level with non-machine mounted sensors there is a need to adapt real time application on a tractor and applying machine. More than 65 % of the Dutch arable farmers nowadays use global positioning systems, mainly for navigation. Gradually, more and more growers are purchasing sprayers that allow spatial variation in application. To mimic the real farmer and to assist growers in taking spatial and temporal strategic and operational decisions, a so called "Smart Farm" was created in 2012 at the research and demonstration farm of the Agricultural college CAH Vilentum at Dronten. Gamma radiation based soil scans yielding information on soil organic matter were carried out by the Altic and Dacom companies and crops were scanned with remote and proximal reflection sensors to calculate NDVI values at various resolutions. Electric conductivity (EC) and electromagnetic (EM) soil sensing technologies were used to map spatial variation in soil properties. Combined with yield maps the objective was to generate VRA maps of the fields that machines use as input. WDVI and NDVI indices from remote and nearby sensors can be used to make application maps. An example being the use of VRA Reglone dosing for potato haulm desiccation based on crop NDVI values. .

A user exchange platform

In the Netherlands an information exchange platform exists that standardizes information to facilitate exchange between growers, advisors, contractors and clients, and avoids redundancy of information supply. The umbrella organization is "Agroconnect" for exchange of any agricultural data such as poultry, flowers etc. "Editeelt" was established under this umbrella specifically for field crops, and mainly for registration purposes. . From the examples above in the sections "Crop management" and "Precision farming" it is obvious that the amount of data generated in potato production with the use of DSS increasingly assisted by geo-information and sensing rapidly increases. To facilitate exchange for all users in 2012 an information exchange platform Akkerweb (www.akkerweb.nl) was introduced. It originated from the development of the decision support system for the control of plant parasitic nematodes NemaDecide (GeoNema). Akkerweb offers GIS functionality and a number of generic applications, such as web services, to download satellite data or provide the ability to generate an application map. Third parties can use the Akkerweb platform to rapidly develop new applications. The Akkerweb environment serves as a connection between geographical field data and relevant additional information. Akkerweb is deployed by the largest farm cooperative in the Netherlands, and open for any other user. Wageningen UR uses the Akkerweb and FiSpace platforms/environments to develop new advisory concepts and specific Apps for farmers such as a recent yield predictor based on LINTUL-POTATO-DSS.

Akkerweb offers accounts for users, different background maps, basic apps for crop rotation and field boundary information, soil and soil moisture information, and an increasing number of management Apps. Users (farmers and farm advisors) can share data with otherselected users. Akkerweb has links to farm management information systems and can be used to order soil and crop analyses from agricultural laboratories.

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UPDATE ON PLANT HEALTH

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Summary: This paper summarises recent developments in plant health legislation and updates on harmful organisms of relevance to potato production in Northern Britain. New European Union (EU) plant health law, which it is hoped will strengthen arrangements, is being finalised. Amendments to domestic legislation have been introduced and the UK Plant Health Risk Register has been created and is used to prioritise actions by official services. The risk register is also a resource that can be used by stakeholders for planning their actions. Pests continue to be of concern including *Epitrix* spp. and '*Candidatus* Liberibacter solancearum'.

LEGISLATION

New plant health regime

The main EU plant health requirements for potatoes are contained in the Plant Health Directive (PHD, 2000/29/EC). Associated with that are four EU Control Directives (for *Clavibacter michiganensis* subsp. *sepedonicus* (ring rot), *Globodera rostochiensis* and *G. pallida* (potato cyst nematodes), *Ralstonia solanacearum* (brown rot) and *Synchytrium endobioticum* (wart disease)) and a number of specific emergency measures.

As the EU plant health regime had been in place since 1993, it was agreed that there was a need to review and, where appropriate, strengthen the regime. A review was therefore initiated in 2009 and negotiations on a Commission text started in May 2013.

The new plant health law will come within the scope of an amended Regulation on official controls (currently Regulation (EC) No 882/2004), which will bring together requirements for food safety, animal feed, animal health and plant health. Together these are known as the 'Smarter Rules for Safer Food' package. The aim is to simplify and streamline the existing legal framework on official controls and to establish a unique set of rules applicable to all sectors. This should improve the efficiency of official controls performed by member states along the food chain while minimising the burden for operators.

The aims of the new plant health regime are also to strengthen arrangements, particularly for plant imports and plant passporting. EU quarantine pests will continue to be listed and a proportion will be termed 'priority pests' for which specific actions will need to be taken in order to reduce the likelihood of their introduction and spread. For seed potato growers in Scotland, current arrangements are likely to remain largely unchanged as most plant health requirements are dealt with in conjunction with seed potato certification requirements. More surveillance for specific pests and contingency planning will be required, as well as enhanced

requirements for 'professional operators'. A large number of subsidiary regulations will need to be adopted in addition to the main official controls and plant health regulations. The anticipated time scale for completion of negotiations is 2016 with implementation of the measures by 2019.

EU emergency measures

Until the introduction of the new plant health regime, existing requirements continue to apply. Over the last two years, several emergency measures have been amended. Emergency measures for *Potato spindle tuber viroid* (PSTVd) in *Brugmansia* sp. and *Solanum jasminoides* plants were adopted in 2007 (2007/410/EU). Surveillance for PSTVd in these species in trade over several years demonstrated that the organism was present in these plants within the EU, although there were no symptoms. The European Food Safety Authority (EFSA) advised that the impact of PSTVd in these ornamental plants was likely to be minimal. As there was no evidence of spread of PSTVd from them to other host plants, the emergency measures were repealed in 2015 (2015/749/EU).

Following findings of *Epitrix* spp. in Portugal and parts of Spain, emergency measures for *Epitrix cucumeris, E. similaris, E. subcrinata* and *E. tuberis* were adopted (2012/270/EU). These included a requirement for all member states to survey for the pests and, if found, to designate an area around the infested plots and take measures to eradicate or at least contain the pest and prevent its spread. In 2014, the emergency measures were strengthened to include requirements for movement of potato tubers from a designated infested area to packing stations outside the area (2014/679/EU).

In the UK, statutory notification schemes are in place for seed potatoes and also for ware potatoes introduced from Poland, Romania, Portugal and Spain (The Plant Health (England) (Amendment) (No.2) Order 2014, The Plant Health (Scotland) Amendment Order 2015). Traders must notify the official plant health services of any movements of seed or ware potatoes so that a risk based assessment can be undertaken to consider whether the tubers should be inspected. For Portugal and Spain, the notification relates to risks posed by *Epitrix* spp. and an update on *Epitrix* taxonomy and findings is provided below.

Review of Annex II organisms

The EFSA plant health panel was asked by the European Commission to give a scientific opinion on the pest categorisation or risk to plant health of organisms listed in Annex II of the PHD, including several pests of potato ('*Ca.* Phytoplasma solani', *Tomato spotted wilt virus* (TSWV) and *Ditylenchus destructor*).

For '*Ca.* Phytoplasma solani', the causal agent of potato stolbur, the assessment related to solanaceous plants. Although the organism is present in a number of EU countries together with vectors, there are a number of Northern European countries where the pest and vector are absent. There are uncertainties associated with the analysis but as the host species are widespread throughout the EU, there is potential for the pest to spread to unaffected parts of the EU based on extension of distribution range of vector species (http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3924.pdf).

For TSWV, the EFSA panel considered the risk to plant health from the virus and evaluated risk reduction options. The panel identified that the current legislation was limited in that only a small number of the potential hosts are regulated and visual inspection is used to certify freedom from TSWV (http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3029.pdf).

For *D. destructor*, the EFSA opinion regarding pest categorization indicated that the pest was present sporadically in the majority of member states. It has the potential to cause severe damage if introduced on plants for planting, although recently only minor damage has been reported apart from in Eastern member states (http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3834.pdf).

The regulatory status of these pests is being determined by EU member states based on the EFSA opinions. Only *D. destructor* had been considered by September 2015 and it is recommended that a full PRA is developed to determine whether it should be a quarantine pest or regulated non quarantine pest.

UK PLANT HEALTH RISK REGISTER

As a result of the UK Tree Health and Plant Biosecurity Expert Task Force report (https://www.gov.uk/government/publications/tree-health-and-plant-biosecurity-expert-taskforce-final-report), Defra, Scottish Government and the rest of the UK Plant Health Service and stakeholders, developed the UK Plant Health Risk Register (https://secure.fera.defra.gov.uk/phiw/riskRegister/). The UK Plant Health Risk Group (PHRG), composed of all parts of the UK Plant Health Service, owns the Risk Register and meets monthly to review information on pests and to agree priorities for action. The register is updated after the meetings. Actions include recommendation for regulation, pest risk analysis, development of contingency plans, actions by industry and publicity campaigns.

More than 790 pests had been listed by August 2015, of which 94 could infect or infest potatoes. The Risk Register allows not only government, but also industry to consider the risks posed by new and existing pests and to determine any actions that should be taken to prevent the introduction or spread of the pests or to manage the risks.

UPDATES ON HARMFUL ORGANISMS

Epitrix spp. (Potato flea beetles)

A number of *Epitrix* spp. (*E. tuberis, E. cucumeris, E. similaris* and *E. subcrinita*) feed on potatoes. As indicated above, findings were made of *E. similaris* in Portugal and parts of Spain and *E. cucumeris* in Portugal (Boavida, 2009; Boavida & Germain, 2009) and emergency EU legislation was adopted. *E. cucumeris* is recorded in North, Central and parts of South America as well as Portugal and *E. tuberis* and *E. subcrinata* are recorded largely from North America and Ecuador (EPPO, 2015).

The taxonomy of *Epitrix* species and our understanding of their behaviour continue to evolve. For example, examination of specimens from Portugal has recently shown that they do not

correspond to *E. similaris*, which is a rare species that has previously been recorded only from California (Orlova-Bienkowskaja, 2015). In addition, *E. similaris* does not damage potato in its native range. It has therefore been proposed that the Portuguese specimens should be considered a new species, *E. papa*, whose native range is not known (Orlova-Bienkowskaja, 2015). In addition, *E. pubescens*, a European species, has been found to infest potatoes (Highet & Pearson, 2015).

Following findings of surface damage and dead larvae in Spanish ware potatoes from areas outside the demarcated infected zones in 2015, the UK Plant Health Service agreed the measures that would be taken if further findings were made and on further consignments from Spain (http://www.nfuonline.com/assets/48123).

'*Candidatus* Liberibacter solanacearum' (Zebra chip)

Risks of '*Ca*. L. solanacearum' to Northern Britain were described previously (Pearson *et al.*, 2014). Two haplotypes infecting potatoes have been found in North America, Central America and New Zealand and are spread by the potato psyllid *Bactericera cockerelli* (EPPO, 2015). Infected *B. cockerelli* from America is believed to be responsible for the outbreak in New Zealand. Three different haplotypes have been found in carrot (*Daucus carota*) and celery (*Apium graveolens*) crops in Europe and Morocco (EPPO, 2015; Tahzima *et al.*, 2014) and are spread by European psyllid species. The recent PHYLIB EUPHRESCO project (Pearson *et al*., 2014, http://www.sasa.gov.uk/content/phylib-end-project-meeting) included psyllid surveys in both England and Scotland which failed to find any vector reported to transmit the bacterium.

In addition to carrot crops, carrot seed has been found to be infected with the organism (Bertolini *et al*., 2015). In some cases, liberibacter-infected potatoes have been found in Northern Europe associated with infected carrot crops (A Nissinen 2014, personal communication), however, it is not confirmed whether the European haplotypes can systematically infect potatoes.

Following an EPPO pest risk analysis (PRA), which recommended regulation of the vector and potato haplotypes, the UK has also undertaken a rapid PRA and undertaken a public consultation (https://secure.fera.defra.gov.uk/phiw/riskRegister/plant-health/pest-risk-analysis-consultations.cfm?type=previous#pra_C). The PHRG has recommended regulation of this pest and *B. cockerelli* and the UK Plant Health Service is pressing the European Commission for early consideration of EU regulation.

To evaluate the threat to Scottish potato production the Scottish Government has commissioned work on 'Detection and Monitoring of Psyllid Vectors of *Candidatus* Liberibacter Solanacearum in Scotland'. This project is led by Rothamsted Research in collaboration with Science and Advice for Scottish Agriculture (SASA) and the Natural History Museum. The aims of the project are to understand the diversity of psyllid species in the UK and map the abundance of possible *Ca*. L. solanacearum vectors at a landscape scale through the development of sentinel monitoring methods in conjunction with rapid molecular identification assays (see pp 279-282 in this volume).

***Synchytrium endobioticum* (Potato wart disease)**

A localised finding of wart disease was reported in Denmark in 2014 (EPPO, 2014). Potatoes in four fields of the cultivar Kuras grown for starch production were found to be infected. It is possible that the pathogen had been present in one of the fields for a considerable time. This is the first finding in Denmark for more than 30 years and is a reminder of the need to remain vigilant against this pathogen.

'*Ca.* Phytoplasma solani' and '*Ca.* Phytoplasma asteris' (Potato phytoplasmas)

In May 2014, the first finding of the organism causing potato stolbur ('*Ca.* Phytoplasma solani') was found in the UK in strawberry plants imported from Spain (Hodgetts *et al*., 2015). The infected plants were destroyed and statutory action will be undertaken on any future findings of the pathogen or the vector, *Hyalesthes obsoletus*, a polyphagous plant hopper, which is currently absent from the UK.

As part of the PHYLIB project, Scottish carrot growers were asked to report symptoms of '*Ca*. L. solanacearum'. Submitted carrot samples with symptoms of leaf curling, leaf yellowing and reddening were instead found to be infected with '*Ca*. Phytoplasma asteris' (Nisbet *et al*., 2014). This pathogen has been found in Europe in a number of hosts and it can infect potatoes, but it has never been found in European potato production. A Rapid Assessment was considered by the PHRG in relation to the Risk Register entry (https://secure.fera.defra.gov.uk/phiw/riskRegister/viewPestRisks.cfm?cslref=27047&riskId=27336) and it was agreed that based on current evidence no statutory action will be taken in the UK on carrots infected with '*Ca*. Phytoplasma asteris' This will be kept under regular review as new information becomes available.

***Potato spindle tuber viroid* (PSTVd) and other pospiviroids**

The Dutch national plant protection organization (NPPO) reported in March 2014 that PSTVd was found in *in vitro* propagation material of one potato accession line at a potato breeding company (https://www.nvwa.nl/txmpub/files/?p_file_id=2206234). The NPPO initiated an extensive testing programme on all breeding material at the company and material linked to the finding. There were no links found to commercial potato production. The possible origin of the infection may go back several years and may be linked to other breeding material (https://www.nvwa.nl/txmpub/files/?p_file_id=2206624). The NPPO investigation is still on-going and further information will be provided when available.

There are continued risks, especially to minituber producers, from pospiviroids in ornamental plants that have the potential to infect potatoes. In order to minimise risks from infection or cross contamination between plants being handled at production facilities, there is a requirement in ISPM 33 (FAO, 2010) to ensure separation between pathogen-free plants and plants with a different testing history. In recognition of the potential risk, the Scottish Government produced a leaflet on threats associated with viroids to raise awareness with mini-tuber producers.

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IDENTIFICATION OF FACTORS WHICH MAY BE INFLUENCING THE INCIDENCE OF BLACKLEG IN SCOTTISH SEED POTATOES

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Summary: Blackleg, caused by *Pectobacterium atrosepticum*, remains the biggest cause of down-grading and rejection in the Scottish Seed Potato Classification Scheme. For many years its worst excesses were managed by initiating production from disease-free planting material, limiting the number of seed generations, enforcing disease tolerances and industry good practice in terms of agronomy, variety and field selection, and the use of modern storage facilities. Up until 2006 this approach was widely judged successful; however, more recently blackleg incidence has risen steadily, peaking in 2011 to levels not seen for more than 20 years. The purpose of this study was therefore to investigate what influence social changes/industry practices may have on blackleg incidence with a view to identifying contributing factors, should they exist, and ultimately to use this information to inform more effective control strategies.

INTRODUCTION

Up until relatively recently blackleg in Scottish seed potatoes, which is primarily caused by *Pectobacterium atrosepticum*, has been effectively controlled through a range of complementary approaches which comprise initiating production from disease-free planting material, limiting the number of seed generations, enforcing disease tolerances in field-grown crops and, in equal measure, industry good practice. Under the Scottish system, disease-tested micro-plants are multiplied through mini-tuber production under controlled conditions and field grown potatoes are multiplied initially as pre-basic (PB) seed. Blackleg is not permitted in PB seed crops primarily as a means of limiting disease build-up/impact on subsequent seed and ware production.

A steady increase in the seed area downgraded or rejected in Scotland as a result of blackleg was observed from 2006, peaking in 2011/12 at levels not seen for 20 years. Research findings, reported in these Proceedings (Toth *et al*., 2016), have identified that this recent rise cannot be solely or collectively attributed to the introduction of new blackleg-causing species, the emergence of new pathogenic strains of *P. atrosepticum*, nor the withdrawal of sulphuric acid as a means of haulm destruction. Yet it is clear from this research that infection can occur in the first year of field production (~PB1). Additionally, data from the Scottish Seed Potato Classification Scheme (SPCS) demonstrates that once symptoms are observed in early field generations this generally leads to a worsening disease incidence picture with each subsequent seed multiplication (McCreath, unpublished data). Further, related studies exploring the link between prevailing environmental conditions and blackleg incidence cannot fully explain this rise as similar weather conditions were experienced in previous decades without a resultant

increase in blackleg (Saddler *et al.*, unpublished data). The possibility therefore exists that economic, social and cultural drivers have altered practices within the Scottish seed potato production chain which may be contributing to the recent rise in blackleg.

An initial attempt was made in 2011 to identify industry practises which could explain the rise in blackleg incidence. By focusing on growers responsible for early field generations it was hoped that any issues identified could lead to improvements which would have a positive impact throughout the production chain. Therefore, a postal survey of 22 Scottish PB growers was conducted (A'Hara, unpublished data), in which it was possible to categorise respondents into three broad groups, depending on their experience with blackleg:-

A. Growers that did not observe any blackleg in any of their crops in the previous 3 years
B. Growers that observed blackleg in less than 20% of their crops in the previous 3 years
C. Growers that observed blackleg in more than 20% of their crops in the previous 3 years

From this study, although it was difficult to generalise, growers in Group A tended to be smaller, producing on smaller production areas, carry out less spray passes and were more rigorous in washing and disinfecting equipment between fields. Group A growers also tended to harvest in order of health and cleaned their harvester and grader when moving between crops/stocks.

However there were many exceptions to this categorisation and as this earlier survey focused solely on current practice, a more intensive survey, covering all aspects of production and how it had changed over a 5, 10 and 20 year period based on face-to-face interviews was performed.

MATERIALS AND METHODS

The sample comprised all 28 Scottish Pre-Basic seed growers and the largest PB grower from both Northern Ireland and England and Wales. The distribution of mainland growers is shown in Figure 1.

One-to-one, semi-structured interviews were conducted between February and September 2015 and were based on a series of questions around social, financial and economic change and related to:-

1. Business organisation and size
2. Business complement and technical competency
3. Crop/store management
4. Use of outside contractors
5. The inspection regime
6. Sources of advice on crop management
7. Burn down regime

Data was collected by taped interview, transcribed and analysed using QDA MINER 4 (Provalis Research, Montreal, Canada).

RESULTS AND DISCUSSION

The sample comprised a highly skilled and knowledgeable group of growers with more than half those interviewed (n = 16) having more than 20 years' experience working in the industry. Two thirds of the growers interviewed produced up to 60 crops each year with 2 producing more than 100 annually. Generally, the number of varieties for all growers had increased in recent years. More than half produced on less than 20 ha with in contrast, one grower cultivating more than 100 ha of PB seed each year. The vast majority cited an increase in production costs as the greatest current burden on their business with land rent and the cost of chemicals and fertiliser increasing markedly in recent years. A number of interviewees linked these economic pressures to the expansion (either in their own business or the businesses of others), but in general this was viewed negatively, either in terms of the effort required and the financial reward gained. In addition, the majority (n = 16) cited 'just-in-time' delivery as a particular problem, with the gradual change to later delivery adding pressure late in the season. However, there was a general acceptance that that was the way things were and it was unlikely to change in future. In addition, half those interviewed (n = 15) stated that conditions at planting or harvest were important factors in disease development, with less common reasons given including poor desiccation, poor hygiene, extended storage in jumbos sacks and destoning, all contributing to increased disease incidence and severity.

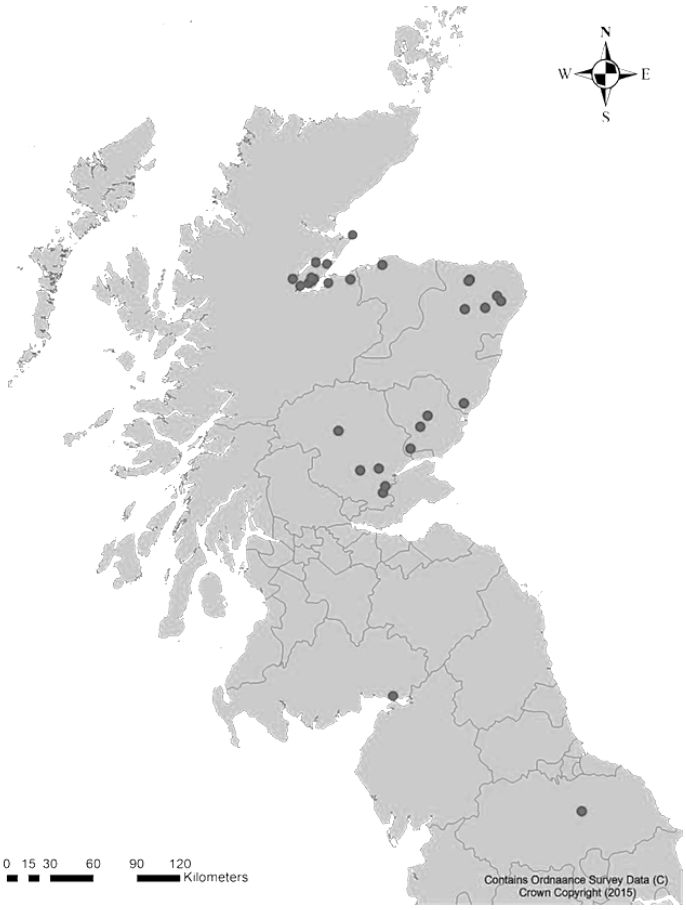

Figure 1. Distribution of Pre-Basic Growers interviewed as part of the blackleg survey from England & Wales and Scotland.

All 30 growers were asked to name what they thought were the main factors driving disease development in crops, with the vast majority citing variety choice as the most commonly identified contributing factor (n = 24), this was followed by environmental conditions during the growing season and 24 PB growers gave more than one answer indicating that disease development, in their opinion, is multifactorial (Figure 2). A third of those interviewed mentioned poor seed health as being a contributing factor to blackleg development, though there were some differing opinions with one grower suggesting that seed may be 'too clean' and therefore more susceptible to infection.

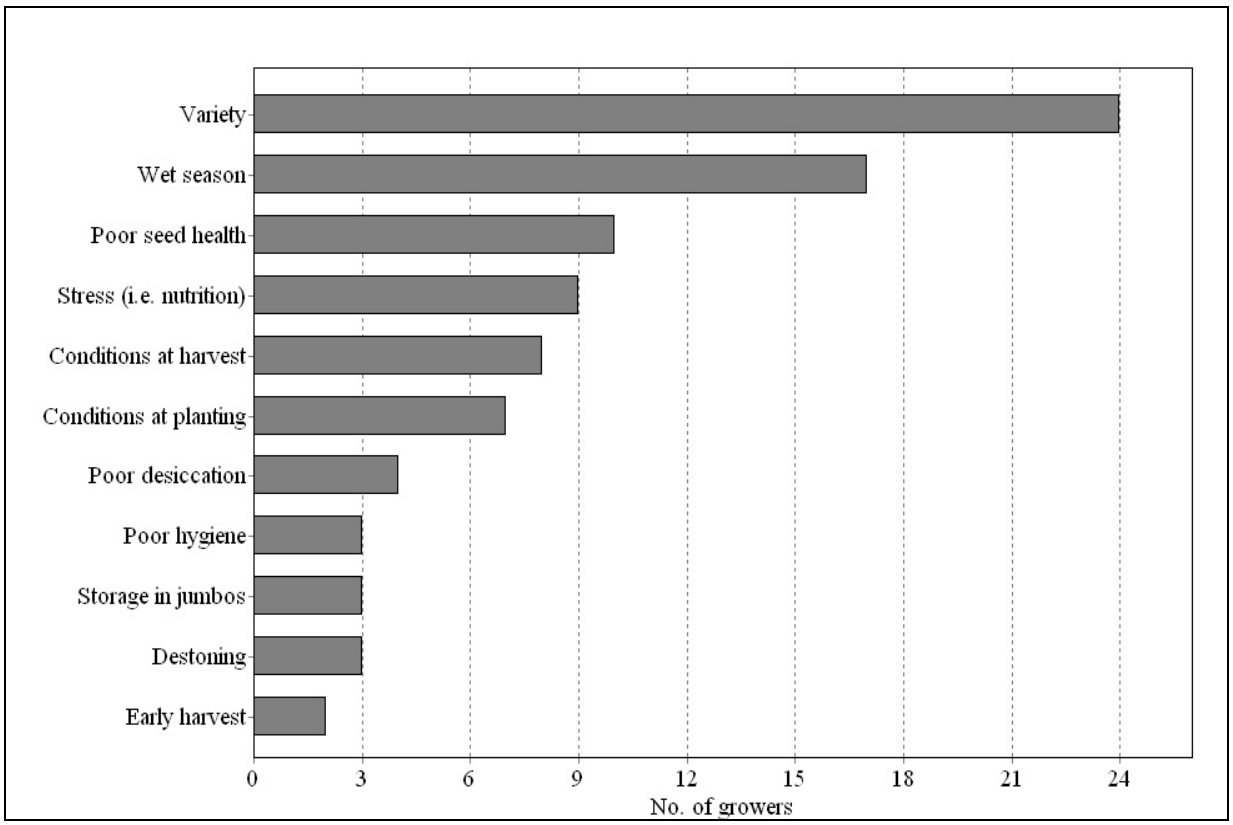

Figure 2. Causal factors identified by Pre-Basic growers driving the increase in blackleg development.

When asked what they thought were the main factors that could be used to control or prevent blackleg from entering a crop; growing or choosing less susceptible varieties was mentioned by 10 growers (Figure 3). It is interesting to note that this is significantly less than the 24 growers who mentioned variety as being a main contributing factor to blackleg incidence. Less than a third of those interviewed (n = 7) mentioned the importance of drying/curing a crop after harvest and appropriate field selection. There was no consensus amongst this group that the removal of sulphuric acid for haulm desiccation had led to the increase in blackleg incidence.

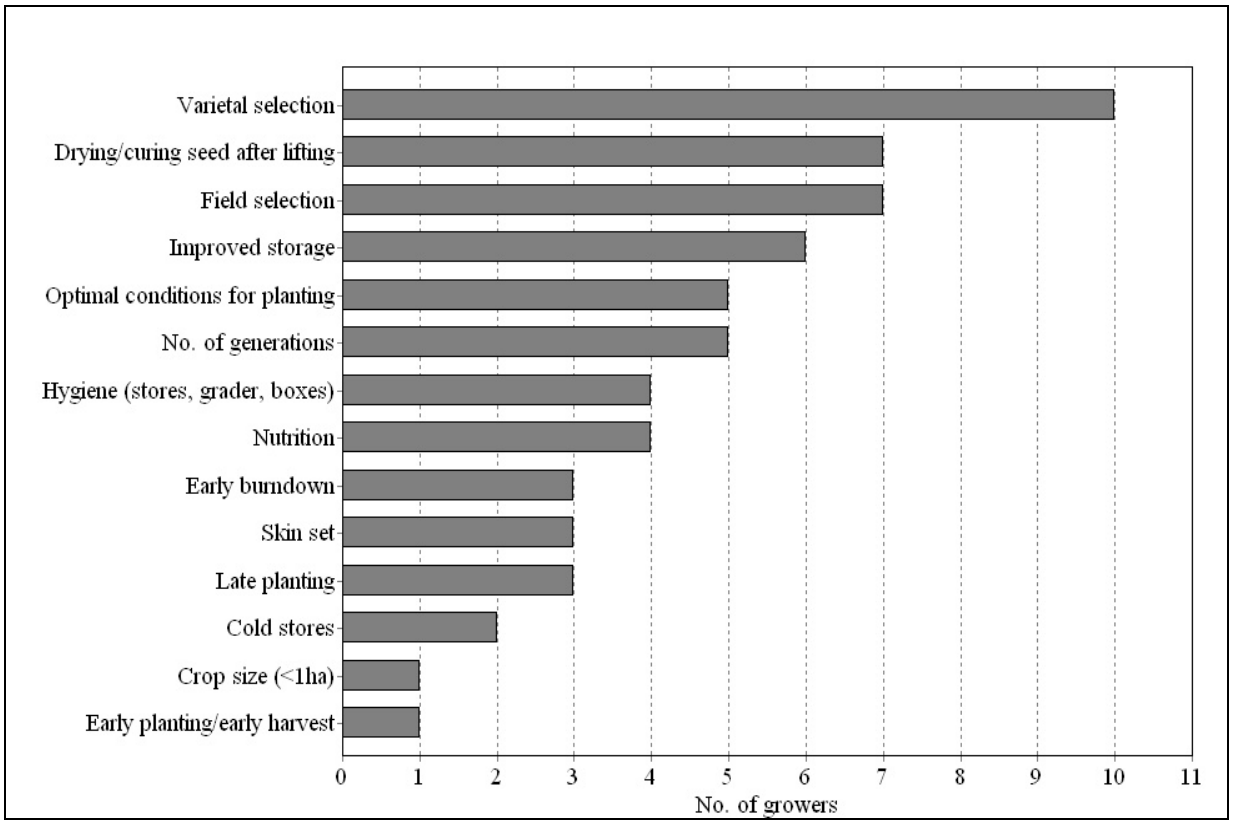

Figure 3. Factors identified by Pre-Basic growers that have the potential for generating greater control of blackleg.

It was clear from the survey that increasing economic pressures are being felt by most PB growers with the need to increase business size and complexity an almost inevitable consequence. There was however tacit recognition that large complex businesses could in themselves be contributing to the increasing incidence of blackleg as attention to detail was acknowledged to be an inevitable casualty under such circumstances. There was substantial support amongst those interviewed that the current portfolio of varieties in cultivation has contributed towards the worsening blackleg situation. However this view is not borne out by related and unpublished analysis of the top 30 varieties with the highest blackleg incidence in Scotland during recent years (Saddler, unpublished data). This analysis shows that that there is a mix of new and old varieties, with no clear link between blackleg incidence and origin identified as both 'continental' and 'domestic' varieties featured heavily in the top 30. Finally, it was evident that little consensus exists between those interviewed as to the best way forward in terms of improving management strategies. As a consequence, further analysis of the data described here is planned as is a wider survey of commercial seed growers across the UK to fully understand key factors which may contribute to our understanding of the worsening blackleg situation in recent years.

ACKNOWLEDGEMENTS

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AN UPDATE ON THE POTATO COUNCIL / SCOTTISH GOVERNMENT-FUNDED BLACKLEG PROJECT - YEAR 2

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Summary: Blackleg continues to be a major problem to potato seed production world-wide and particularly throughout Europe. In Scotland it is the main cause of downgrading and failed seed. In England and Wales it is also commonly detected in seed stocks entered for classification.

INTRODUCTION

Blackleg disease is caused by the bacterial pathogens *Pectobacterium* and *Dickeya*, and a current AHDB Potatoes / RESAS jointly-funded project is investigating initial sources of contamination of high grade potato seed stocks (Field Generation [FG] 1-3) and the subsequent spread of the pathogen within and between the field and generations; whether these pathogens have become more virulent in recently years; whether loss of sulphuric acid has reduced pathogen numbers at haulm destruction; and how data from crop inspections can help to better understand disease occurrence and spread.

RESULTS AND DISCUSSION

After two years of this three year project information is emerging on a number of factors that have provided new insights into these pathogens. For example, we now know that irrespective of the source, bacterial contamination commonly occurs in FG1 and may build in subsequent generations, and that this contamination appears to be from bacteria already in the local environment, as well as from near-by infected plants. Contamination increases as the season progresses, becoming detectable only late in the season. Blackleg disease first becomes evident in FG2 or later (although it may occur in FG1 on occasions) and often continues to develop after field inspections and before haulm destruction, allowing us to offer practical advice to growers, e.g. that early lifting is likely to reduce both contamination and subsequent disease.

DNA sequence-based methods to differentiate species and subspecies of *Pectobacterium* have been developed that have allowed us to identify strains of both *P. wasabiae* and *P. carotovorum* as being responsible for a low number of blackleg incidences (10-20%) across the UK. However, *P. atrosepticum* remains the dominant species (80-90%). Low levels of *Dickeya solani* and *D. dianthicola* (<1%) have also been recorded from blackleg plants in seed stocks grown in England and Wales but originating from outside GB. Therefore another

practical message is that sourcing seed from within GB will limit/avoid contamination and disease by *Dickeya* species. The new sequence-based methods (MLST and VNTR) have shown that increased blackleg in recent years does not appear to be due to the introduction of new strains of the pathogens, and have allowed us to trace different populations of *P. atrosepticum* from environmental sources, machinery, and in blackleg plants and tubers through the production system.

Modelling of inspection data for blackleg disease, using the Scottish Government's SPUDS database, has shown that the occurrence of blackleg occurs in clusters, which are found in different production areas in different years. This knowledge may offer us an opportunity to compare a number of different factors, including local weather patterns or seed distribution, in order to better understand this clustering.

Finally, there is some evidence to suggest that the use of sulphuric acid at haulm destruction does reduce bacterial contamination substantially in tubers (although due to variability significant reductions were not found) and may therefore have a direct benefit to reducing the incidence of blackleg disease. However, as its use is unlikely to be reintroduced into Europe any time soon, another practical message would be that new approaches are needed to ensure minimum contamination by *Pectobacterium* during haulm destruction and, indeed, research by commercial organisations has already provided some interesting new possibilities.

ACKNOWLEDGEMENTS

This project is funded by AHDB Potatoes and the Scottish Government's Rural and Environmental Sciences and analytical Services Division (RESAS) under project reference number 114R475. We gratefully acknowledge the support of our industry collaborators including those that have allowed us access to their growing crops, machinery, provided samples etc.; to the inspectors working for the Rural Payments and Inspections Division of the Scottish Government; and to Stephen Fotheringham and Wilma Sloan, Potato Section, SASA for extracting and quality checking the inspection data from the SPUDS database.

THE CONTRIBUTION OF HOST RESISTANCE ELICITORS TO THE CONTROL OF POTATO FOLIAR BLIGHT IN SCOTLAND

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Summary: The finding by others that the elicitor BABA reduced blight lesion size on potato leaflets prompted the development of a control system in which elicitors should enhance the efficacy of curative fungicides. The objective was to investigate if suppression of leaf colonisation rate by BABA and acibenzolar-S-methyl, in field plots, was sufficiently large to significantly boost the efficacy of well-timed applications of cymoxanil. Blight lesions were significantly smaller for integrated elicitor and cymoxanil treatments, compared with cymoxanil alone; lesions were between 10% and 31% smaller. The results for blight severity (whole plot) for elicitors alone differed substantially from those obtained for integrated elicitor and cymoxanil treatment. With integrated use, each elicitor decreased severity significantly on the more susceptible King Edward; the level of control was similar for both, i.e. 21% and 23%. Without cymoxanil the two elicitors did not significantly improve control compared with the untreated.

INTRODUCTION

The efficacy of elicitors against late blight of potato is limited compared with established blight fungicides therefore some previous studies investigated the integrated use of BABA and contact fungicides (Cohen, 2002; Liljeroth *et al.,* 2010). It is significant that BABA can reduce the size of blight lesions. Liljeroth *et al.,* (2010) demonstrated that lesion sizes were reduced by 40 to 50%, compared with the untreated control, provided treated leaves on glasshouse-grown plants were challenged within 4 to 5 days of treatment. Clearly BABA can reduce the rate of pathogen development within the leaf. The aim of the present study was to investigate if the suppression by BABA of leaf colonisation rate was sufficiently large to significantly boost the efficacy of well-timed applications of a curative fungicide. Experiments focussed on elicitor application during the stable canopy phase of crop development. This was because in a bioassay in 2012, using field-grown King Edward plants in which upper leaves were challenged 3, 7 and 10 days after the application of BABA @ 1.0 g/L + Warrior @ 0.5% at the start of rapid haulm growth, the elicitor had no effect on either the incidence of blight or on lesion size (Bain *et al.,* unpublished).

Many studies that examined the efficacy of host resistance elicitors on potato frequently used glasshouse-grown plants (Andreu *et al.,* 2006; Altamiranda *et al.,* 2008). The work reported in this paper was field-based because this is the environment in which elicitors will ultimately be used. In recent years an early challenge from *Phytophthora infestans* is normal and therefore elicitors are likely to be applied to crops with some symptoms of blight. Walters *et al.,* (2011) demonstrated for the barley/*Rhynchosporium commune* system that prior resistance induction (in this case by infection of lower leaves with *R. commune*) can diminish the response to the elicitors applied to leaves one and two.

MATERIALS AND METHODS

The field experiment described in this paper was established on Auchincruive Estate, Ayr, Scotland in 2013. Table 1 details the elicitors and reference fungicides used. Two cultivars with contrasting foliar blight resistance ratings were tested: King Edward (foliar resistance rating 3) and Ambo (6). Both cultivars have maincrop maturity. The experimental design was a split-plot layout with four replicate blocks. Spray treatments were the whole-plot level and cultivars the subplot level. Cultivar sub-plots were 3.4 m (four rows) wide and 3.0 m long, separated longitudinally by 2.1 m of unplanted row length. Seed spacing within rows was 30 cm. A single row of King Edward was planted longitudinally between plots to act as an untreated infector row.

Table 1. Details of elicitors and fungicides

Treatment	Active ingredient	g/kg or L product	Product (kg or L/ha)
Ranman Top SC	cyazofamid	160/L	0.5 (L)
Revus SC	mandipropamid	250/L	0.6 (L)
Intracrop Warrior	primary alcohol ethylene (adjuvant)	192/L	0.5% v/v
BABA	β –aminobutyric acid		0.2
Bion WG	acibenzolar-S-methyl	500	0.035
Option WG	cymoxanil	600	0.15
Morph SC	dimethomorph	500	0.3 (L)
Electis 75 WG	zoxamide + mancozeb	83 + 667	1.8

All plots, including the untreated, were protected during rapid canopy development with a common fungicide programme: alternating applications of cyazofamid (8 & 22 July) and mandipropamid (15 & 30 July and 6 August). Spray intervals were mostly 7 days with one at 8 days. The test treatments (Table 2) were applied from the start of the stable canopy phase until desiccation. There was some foliar blight in the plots, ranging from 0.05 to 0.36%, when the test treatments were first applied. The trial had not been inoculated because infection spread naturally from adjacent trials. These other trials had been inoculated on 10, 18, 24, 26 & 31 July and 1 August with isolate 7654A (13_A2). Test treatments were applied in 200 litres of water per hectare, using a tractor-mounted modified AZO compressed air sprayer. The nozzles were Lurmark F03-110 flat fans and the spray pressure was 350 kPa. The intervals for the 7-day treatments were 7, 7, 8, 6 and 8 and those targeted at 3 or 4 days were 3, 4, 3, 4, 3, 5, 2, 4, 3 and 5. The early or late sprays occurred if weather conditions were unsuitable on the due date and there was a risk of inaccurate spraying. In the absence of an alternative validated blight weather risk system for the UK, the timing of cymoxanil applications was dictated by the days on which the Smith criteria were met. Data from the Met Office station on the Auchincruive Estate were used. There were few Smith Periods in 2013. The four cymoxanil applications were on: 16 August (the second day of a Smith Period), 22 August (the first day of a Smith Period), 3 September (on the day Smith criteria were almost met) and 13 September (1 day after a Smith day).

Table 2. Elicitor and fungicide treatments applied to King Edward and Ambo during the stable canopy phase of crop development in 2013

	With cymoxanil	Without cymoxanil
untreated	✓	✓
acibenzolar-S-methyl @ 0.175 g/L (7 days)	✓	
acibenzolar-S-methyl @ 0.175 g/L (3 or 4 days)	✓	✓
BABA @ 1.0 g/L + adjuvant @ 0.5% (7 days)	✓	
BABA @ 1.0 g/L + adjuvant @ 0.5% (3 or 4 days)	✓	✓
adjuvant @ 0.5% (3 or 4 days)		✓
adjuvant @ 0.5% (7 days)	✓	
dimethomorph @ 0.3 L/ha (7 days)	✓	
zoxamide + mancozeb @ 1.8 kg/ha (7 days)	✓	

Foliar blight in each subplot was assessed regularly as a percentage of leaf area destroyed by blight. For King Edward only, the impact of treatment on lesion area was assessed for the two elicitors @ 3 or 4 days, zoxamide + mancozeb @ 7 days and the untreated. All of these treatments included cymoxanil applied curatively. Twenty-five randomly selected lesions per subplot were assessed for area. Lesions were placed in one of nineteen categories, ranging from 0.5 to 5.0 cm^2 (in increments of 0.25cm^2), depending on which area matched most closely. Replicate subplots 1 & 2 were assessed on 23 August and the remaining two subplots 1 week later. The foliar blight severity scores were used to generate AUDPC (Area under Disease Progress Curve) values for each subplot and analyses of variance carried out. The lesion area data were analysed using Mann Whitney U tests because the data were categorical. All analyses used GenStat for Windows, 15th edition.

RESULTS

Blight lesions were significantly smaller in plots treated with the two elicitors or the reference fungicide zoxamide + mancozeb compared with those treated with cymoxanil alone (Table 3). However, there was inconsistency between the two pairs of replicates for BABA + adjuvant. The application of acibenzolar-S-methyl or BABA + adjuvant produced lesions that were on average 31% or 10% smaller respectively.

The AUDPC results obtained when elicitors were used alone differed from those obtained when elicitor use was combined with curative applications of cymoxanil (Table 4). Where the curative fungicide wasn't used the AUDPCs for the two elicitors on both cultivars were not significantly smaller than those for the untreated controls. However, the adjuvant alone significantly reduced blight severity compared with the untreated. On King Edward the adjuvant gave 17% control whereas the BABA + adjuvant and acibenzolar-S-methyl gave 12 and 6% respectively. The corresponding levels of control on the more resistant cultivar Ambo were 27% for the adjuvant and 17% for both elicitors. When used together with the curative

Table 3. Blight lesion area (cm^2) on King Edward in relation to disease control programme applied during stable canopy in 2013

Base treatment	23 August[1] Reps 1 & 2	30 August[2] Reps 3 & 4
untreated	2.04	1.99
acibenzolar-S-methyl @ 0.175 g/L (3 or 4 days)	1.42***	1.35***
BABA @ 1.0 g/L + adjuvant @ 0.5% (3 or 4 days)	1.67***	1.95
zoxamide + mancozeb @ 1.8 kg/ha (7 days)	n.a.	1.27***

Lesion size after: [1] three and two applications of elicitor and fungicide base treatments respectively, and two of cymoxanil [2] five and three applications of elicitor and fungicide base treatments respectively, and two of cymoxanil
*** (P<0.001)
n.a. indicates plots were not assessed

Table 4. Foliar blight severities (AUDPC) in relation to resistance elicitors applied during stable canopy either a) alone or b) as a base programme with cymoxanil applied curatively

	King Edward	Ambo
a) Elicitors alone		
untreated	1446	1002
adjuvant @ 0.5% (3 or 4 days)	1195*	732*
BABA @ 1.0 g/L + adjuvant @ 0.5% (3 or 4 days)	1278	835
acibenzolar-S-methyl @ 0.175 g/L (3 or 4 days)	1365	827
b) Elicitors used in conjunction with cymoxanil		
untreated	1268	805
acibenzolar-S-methyl @ 0.175 g/L (7 days)	1007*	648
acibenzolar-S-methyl @ 0.175 g/L (3 or 4 days)	936*	675
BABA @ 1.0 g/L + adjuvant @ 0.5% (7 days)	986*	636
BABA @ 1.0 g/L + adjuvant @ 0.5% (3 or 4 days)	1022*	729
adjuvant @ 0.5% (7 days)	1232	726
dimethomorph @ 0.3 L/ha (7 days)	373*	188*
zoxamide + mancozeb @ 1.8 kg/ha (7 days)	56*	36*
F pr.(cultivar x treatment)	<0.001	
LSD (P=0.05)	208.3	

*AUDPC significantly lower than respective untreated (P=0.05)

fungicide cymoxanil, the two elicitors reduced blight severity significantly, compared with the appropriate untreated control, on the more susceptible cultivar King Edward; the level of control was similar for BABA and acibenzolar-S-methyl and also for the two spray intervals. The mean percentage control for acibenzolar-S-methyl was 23% and for BABA + adjuvant was 21%. The levels of control for the reference fungicides dimethomorph and zoxamide + mancozeb were 71% and 96% respectively. On the more resistant cultivar Ambo the acibenzolar-S-methyl and BABA + adjuvant treatments achieved average percentage control levels of 18% and 15% respectively but these levels were not significantly less than the appropriate untreated control. The levels of control with the two reference fungicides were similar to those for King Edward: 77% and 96% for dimethomorph and zoxamide + mancozeb respectively.

DISCUSSION

The principle of using host resistance elicitors to enhance the performance of curative blight fungicides has been demonstrated. However, the efficacy of the system needs to be improved, chiefly in four areas. 1. More effective elicitors, or combinations of them (Oxley & Walters, 2012), need to be identified, or the efficacy of existing ones boosted, e.g. through the use of adjuvants. 2. Better information on the optimum timing of elicitor applications is required because results for their efficacy during the rapid canopy and stable canopy phases of potato growth are not consistent. In the 2013 experiment elicitors were applied during the stable canopy phase because an experiment the year before demonstrated that under field conditions the application of BABA + adjuvant to King Edward did not improve protection of new growth compared with the untreated (Bain *et al*., unpublished). In contrast, Andreu *et al.,* (2006) demonstrated greater efficacy for BABA if it was applied 15 or 35 days after the emergence of glasshouse-grown plants compared with 55 or 75 days. However, the influence of plant age/growth stage was greatest for the two cultivars of intermediate resistance but more limited for the most resistant cultivar and the very susceptible cultivar. Also, elicitor timings in field experiments have tended to mimic standard blight fungicide timings, which may not be appropriate. 3. Curative fungicides that are more potent than the one in the 2013 experiment should be used. Some are available. 4. Clearly, elicitors are more likely to be accepted and used by the UK potato industry if their use gives a substantial response in a high proportion of the cultivars grown. There is insufficient information on which cultivars have their resistance significantly boosted by compounds such as BABA. In a 2011 field trial, BABA + adjuvant was applied during the stable canopy phase to six representative UK cultivars (rated 3 to 5 for foliar blight resistance): King Edward, Maris Piper, Premiere, Marfona, Sante and Vales Sovereign. Elicitor treatment reduced foliar blight severity compared with the untreated to a similar extent for all six cultivars but the reduction, although always statistically significant, was modest (Bain *et al.,* unpublished). In contrast, Andreu *et al.,* (2006) found that foliar protection against late blight of potatoes by BABA was greatest for cultivars of intermediate resistance. Protection when the elicitor was applied 15 days after emergence was 85 to 95% for these cultivars but only 48% for the most resistant one and 31% for the most susceptible. Also, in the experiment described in this paper the efficacy of the elicitors, compared with the untreated control, was greater for the cultivar rated 3 on the 1 to 9 scale compared with the one rated 6. It appears therefore that the responses of different cultivars to elicitor treatment are not directly related to their foliar resistance ratings (Andrivon, personal communication). For the elicitor/curative fungicide system being developed, the effect of cultivar on the early rate of leaf tissue colonisation is more important than the cultivar's 1 to 9 rating. The former needs to

be assessed because it can't be assumed that the two variates are strongly correlated across cultivars.

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POTATO LATE BLIGHT RESEARCH UPDATE; POPULATIONS, PRIMARY INOCULUM AND PREDICTIONS

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Summary: Potato late blight caused by *Phytophthora infestans* continues to threaten crop yield and quality and intensive fungicide use has environmental and economic consequences. Our multidisciplinary late blight research programme aims to improve late blight management. The programme encompasses resistance breeding, effector biology, disease modelling and applied epidemiological work on the pathogen population change and improved decision support. In this paper we will summarise a) recent data on the pathogen population in relation to the rest of the UK and mainland Europe b) knowledge on the sources of primary inoculum responsible for outbreaks each year and c) the status of research on updating of the Smith period criteria for improved decision support. In each case we will focus on the practical messages to the potato industry in northern Britain.

INTRODUCTION

Management of the potato late blight disease, caused by *Phytophthora infestans*, remains a great challenge for the industry. Growers are generally constrained in their use of blight resistant cultivars and thus, at the start of each season, need to focus on managing sources of primary inoculum and making good decisions on the timing of fungicide applications for effective crop protection. Populations of *P. infestans* have changed in recent years and this continues to have implications for growers (Cooke *et al*., 2012). Such populations displaced the previous types and, while there is some debate on the evolutionary drivers (Mariette *et al*., 2015), an almost complete displacement indicates some selective advantage to the new forms compared to those found a decade ago (Cooke *et al*., 2012). Research at The James Hutton Institute continues to track the change in *P. infestans* populations *via* the AHDB Potatoes Fight Against Blight (FAB) campaign with scouts providing samples from potato blight outbreaks across the country. Pathogen isolation and DNA fingerprinting since 2003 has formed a valuable database that is being mined to answer several key questions on pathogen population structure and the nature and origin of primary inoculum initiating the regional late blight epidemics each season. Data on British crops are being extended with samples taken in the European industry-sponsored Euroblight programme (www.euroblight.net).

Growers and advisors need robust data on pathogen activity to decide the optimal timing of their fungicide applications. This is currently based on reports of outbreaks *via* FAB and Smith Period reports *via* the Blightwatch web site. The Smith Period criteria were defined empirically in the 1950s as a measure of blight activity and spread (Smith, 1956) and it is widely recognised that they should be updated to a) accommodate the new pathogen populations and b) provide more specific data on blight infection in line with the needs of precision agriculture. At The James Hutton Institute we are currently examining the predictive power of the Smith Criteria across more than 2000 late blight outbreaks recorded in the FAB database in light of

Met Office, Smith Period data, and scrutinising the humidity and temperature criteria for an updated set of criteria to improve the decision support tools available to growers (Skelsey & Cooke, 2013).

In this paper we describe, briefly, the materials and methods and recent results from the three late blight research projects described above with emphasis on the outputs that relate to crops in Northern Britain.

MATERIALS AND METHODS

The FAB campaign, funded by AHDB Potatoes, resulted in between 68 and 300 late blight outbreaks being reported and sampled each growing season from 2003 to 2014. Late blight infected plant material collected by FAB scouts were sent to Fera (Sand Hutton, York, UK) and forwarded to Dundee in potato tubers. Isolates, mycelium from the colonised potato tubers or material, pressed onto Whatman FTA cards was genotyped using a previously described 12-plex simple sequence repeat (SSR) assay (Li *et al*., 2013). Data was processed in Excel spreadsheets to define the common clonal genotypes and uploaded, along with latitude and longitude data to the Euroblight database (www.euroblight.net). Isolates that do not match those of the dominant clonal lineages are grouped in a 'catch all' miscellaneous category (termed 'Misc' or 'Other'). Once recorded in the database the location and genotype data was visualised spatially.

Information on almost 2000 FAB late blight outbreaks, each defined by the first section of the UK postcode was collated into a single dataset and examined in combination with regional data on the Smith Criteria kindly provided by the UK Met Office. The datasets were combined, defined according to nine GB regions, and analysed in the commercial software package MATLAB R2015b (The MathWorks, Inc., Natick, Massachusetts, United States). The relationships between each reported outbreak and the dates of full Smith Period alerts in the same region were examined.

RESULTS

The risk of potato blight was high in the 2014 potato growing season with early pathogen activity translating into many outbreaks reported via the FAB campaign (267 outbreaks resulting in >1000 samples). The population of *P. infestans* in British crops was dominated by a few clonal lineages (Fig. 1) but some regional variation was observed. The location of each reported outbreak and the lineage present can be seen on interactive maps on the Euroblight web site hosted at the University of Aarhus, Denmark (http://euroblight.net/pathogen-characteristics-and-host-resistance/sampling-sites-and-genotype-maps/). The 13_A2 lineage that comprised 78% of the GB population in 2008 (Cooke *et al*., 2012) declined to 28% in 2014 whilst the 6_A1 lineage increased from 12 to 60% over the same period. In 2014, the frequency of 13_A2 was lower in Scottish outbreaks than those in England and Wales (Fig. 1). Another feature of the population was the higher frequency of 'Misc' isolates sampled in Scotland (22%) compared to those in England (2%) and Wales (0%). A finer spatial analysis of the population substructure in Scotland indicated a dominance of the 6_A1 lineage in the 36 outbreaks sampled in Dundee area postcodes while the 20 outbreaks sampled in the Aberdeenshire and Moray regions had a higher proportion of the 'Misc' genotypes with relatively low levels of 6_A1 and 13_A2 (Figure 2).

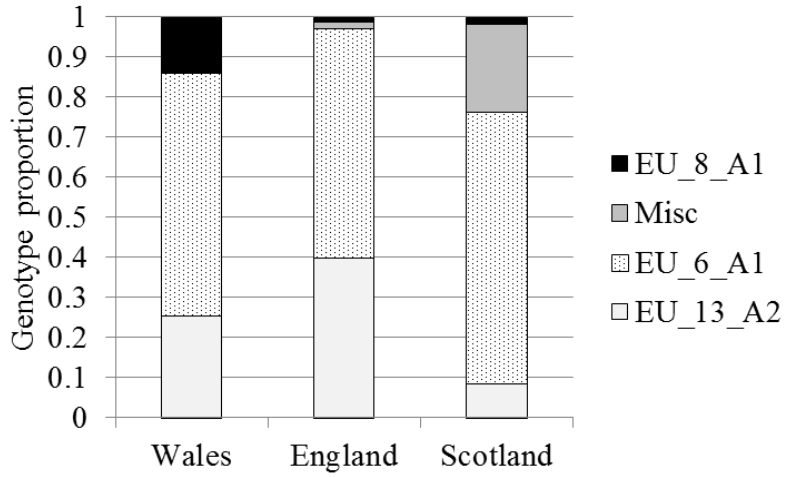

Figure 1. Proportion of different genotypes of *P. infestans* sampled from late blight outbreaks in British crops in 2014.

Smith Period alert data from three regions in northern Britain indicated that 38-42 % of outbreaks were preceded by a full Smith Period alert within 7 days of the outbreak report (Table 1). This figure increased to 58-68% and 63-79% for 14 and 21 days, respectively. These data are for a full Smith Period defined as 'two consecutive days in which the temperature does not fall below 10°C and there are at least 11 hours on each day when the relative humidity is above 90%'. Days having between 10 and 11 hours of high humidity result in 'near criteria' and 'near period' reports but the temperature criteria remains fixed at 10°C.

DISCUSSION

Recent data indicates that populations of *P. infestans* in northwest Europe, for example, Northern Ireland (Cooke, 2015), France (Mariette *et al*., 2015) and Britain (Cooke *et al*., 2012) are dominated by relatively few clonal lineages. In contrast, populations in the Nordic (Sjöholm *et al*., 2013) and Baltic states (Runno-Paurson *et al*., 2010) are highly diverse with almost every isolate being genetically unique. In the Netherlands, a mix of clones and highly diverse isolates has been reported (Li *et al*., 2012). The populations of *P. infestans* sampled in Britain in 2014 confirm the prevalence of three clones; 6_A1, first reported in the UK in 2004, 13_A2, reported in 2005 and 8_A1, reported as early as 1995 (Cooke *et al*., 2012). Their frequency indicates that that they remain well-adapted and aggressive under GB conditions. The data also indicates that the primary inoculum that initiates late blight each growing season is predominantly from local sources such as volunteer potato tubers or outgrade piles. The dominance of 6_A1 across the DD postcode region (Figure 2) suggests a significant early source of primary inoculum that spread rapidly during the wet conditions conducive to blight from late June into July (Figure 2). Growers thus need to be aware of the importance of managing primary inoculum.

The differences observed between the population sampled from crops in the Dundee and Angus postcodes and those sampled in Aberdeenshire and the Moray coast are interesting.

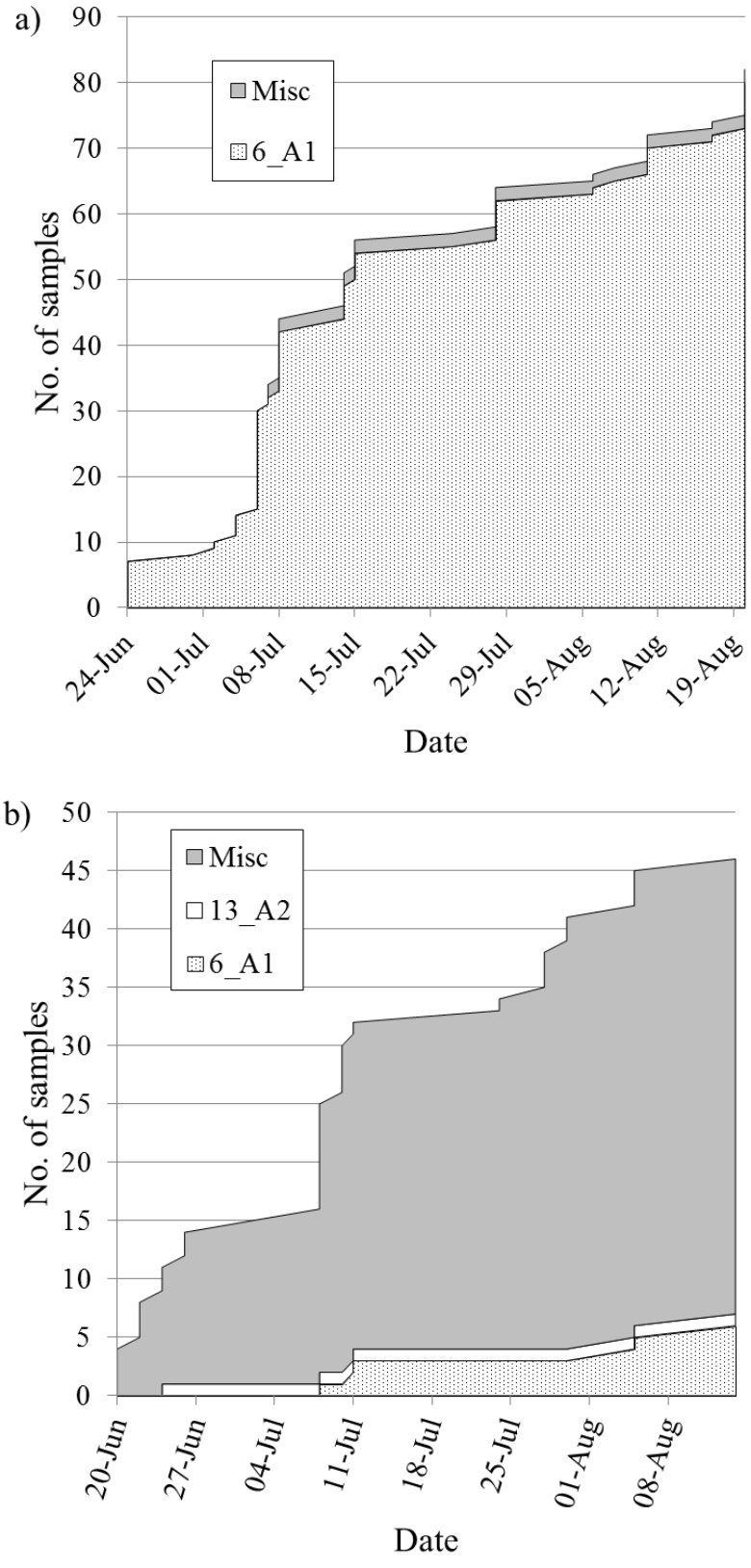

Figure 2 Cumulative total of *P. infestans* genotypes from late blight outbreaks sampled over the 2014 growing season in a) DD postcode districts including Angus, Dundee and northern Fife b) AB and IV postcodes including Aberdeenshire and Moray.

Table 1 Relationship between potato late blight outbreaks and preceding Smith Periods (SP) in Northern Britain from 2003 to 2014. The number of potato late blight outbreaks reported as part of the AHDB Potatoes FAB campaign and the total number of Smith Periods reported in MetOffice data for each region are shown in the upper two rows.

Region		East Scotland	North East England	North West England & North Wales
Number of potato late blight outbreaks reported		678	148	255
Number of SP recorded		4,434	3,788	4,282
Percentage of outbreaks preceded by SP alerts within;	7 days	38	44	42
	14 days	68	61	58
	21 days	79	72	63

The pathogen diversity in the more northerly region is consistent with a sexually recombining population in which long-lived soil-borne oospores act as primary inoculum. However, oospores as primary inoculum are generally considered to be more problematic when rotations are short (Yuen & Andersson, 2013) whereas crop rotations in this region are typically 4-7 years. Further detailed studies on the genetic diversity of these populations (Kamvar *et al*., 2015) and the aetiology of these outbreaks are required. Given the importance of this region as a source of seed potatoes it is important to note that none of the novel genotypes found in this region have, to date, been recovered again either within or beyond the region. This suggests these novel genotypes are not as aggressive and fit as the clonal lineages.

It was anticipated that every active blight outbreak would have been preceded by a full Smith Period alert; discrepancies were, however, observed which could arise under several scenarios. Firstly, due to anomalies between local weather conditions and the interpolated data from synoptic weather stations or the influence of micro-climate within the crop. Alternatively, blight activity outside of the Smith criteria is likely. Other studies have shown, for example, *P. infestans* infection below 10°C (Chapman, 2012). This finding supports the need for a new, fully validated, set of criteria to more accurately predict blight activity. We are examining the outbreak and Smith Period data in more detail to evaluate whether it was the temperature or humidity criteria that prevented full Smith alerts. In addition to this post hoc evaluation, detailed experimentation is underway with contemporary pathogen genotypes to more precisely define the durations of temperature and humidity that result in *P. infestans* infection and spread. These findings will be integrated into a modelling framework to upgrade the decision support system available to British growers (Skelsey & Cooke, 2013, Skelsey *et al*., 2009).

ACKNOWLEDGEMENTS

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MOSAIC VIRUS INFECTION AND THE OCCURRENCE OF GROWTH CRACKING IN TUBERS – ARE SOME POTATO CULTIVARS MORE PRONE THAN OTHERS?

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Summary: Infection of potato plants by viruses such as *PVA* and *PVY* may cause mosaic symptoms on the leaflets of the growing plant. Results of a previous investigation in 2004 showed that there was an association between mosaic virus symptoms and the occurrence of growth cracking in tubers. However, there was evidence from the investigation and from SASA's collection of virus infected cultivars that some are more prone to growth cracking than others, when infected with virus. Nearly a decade after this initial investigation, potato growers in Western Washington, USA were experiencing more issues with growth cracking, particularly in cv. Chieftain, and suspected virus infection (*PVY*) was the cause. The investigation detailed in this paper compared the incidence of tuber growth cracking in cvs Chieftain and Maris Piper when infected with PVY^N or PVY^O. The results clearly show that cv. Chieftain was far more prone to tuber growth cracking when plants were infected with *PVY* than cv. Maris Piper.

INTRODUCTION

Infection of potato plants by viruses such as *Potato virus A (PVA)*, *Potato virus V (PVV)*, *Potato virus X (PVX)* and *Potato virus Y (PVY)* may cause mosaic symptoms on the leaflets of the growing plant. Results of an investigation carried out in 2004 (McCreath & Carnegie, 2008) showed that there was an association between mosaic virus symptoms in plants and the occurrence of growth cracking in tubers. The association of growth cracking with mosaic symptoms did not seem to depend on any specific virus as it occurred with *PVA*, *PVV* and PVY^N. This finding was the first record to indicate that infection by the mosaic causing viruses may affect tuber quality by rendering tubers unmarketable because of growth cracking. However, there was evidence from the investigation and from SASA's collection of virus infected samples at Gogarbank, covering over 50 commonly grown cultivars, that some may be more prone to growth cracking than others when infected with virus.

Nearly a decade after this initial investigation was carried out, ware potato growers in Western Washington, USA were experiencing more issues with growth cracking, particularly in cv. Chieftain and they suspected virus infection (*PVY*) was the cause. Chieftain, a red North American cultivar, is grown widely in Western Washington, for the fresh table market.

Following a visit to America in 2013 to discuss the findings of an association between mosaic virus symptoms in plants and the occurrence of growth cracking in tubers an investigation was initiated in Scotland to determine if some cultivars are more prone to growth cracking when infected with mosaic virus. Cvs Chieftain and Maris Piper were used in the investigation; both

cultivars are susceptible to *PVY* (*PVY*^{O} and *PVY*^{N}). Chieftain was chosen due to the increased incidence of growth cracking observed in Western Washington, with *PVY* infection thought to be the cause. Maris Piper was chosen as the comparison cultivar because it is the dominant cultivar grown in the UK and, although it is susceptible to *PVY* infection, observations from tubers harvested from SASA's collection of virus infected cultivars suggest that it is not prone to tuber cracking as a result of virus infection.

MATERIALS AND METHODS

Preliminary investigation - 2013

In March 2013, two samples of tubers (cv. Chieftain) were sent over from Skagit County, Washington State, USA. Both samples were of approximately 20 tubers, with one sample having distinct growth cracks while the other sample had no growth cracking. The tubers were received into the UK Potato Quarantine Unit at SASA and tested for the presence of virus by TaqMan and RT-PCR. This preliminary investigation was to ascertain whether there was virus present in the cracked tubers.

Production of virus infected tubers in the glasshouse - 2013

Pathogen free (virus free) microplants of cvs Chieftain and Maris Piper, originating from the Nuclear Stock Unit at SASA, were grown in universal containers containing Murashige & Skoog medium. On 1 August 2013 when the microplants were 4 – 5 cm tall they were transplanted into 9 inch pots containing compost and allowed to establish in the glasshouse. There were approximately 30 plants of each cultivar.

On 1 October 2013, approximately 20 virus free plants of each cultivar (Chieftain and Maris Piper) were inoculated with virus. Virus infected leaf material was collected from reference plants in the glasshouse infected with either *PVY*^{N} or *PVY*^{O}. The leaf material was ground up with buffer to produce individual sap solutions containing each virus. Four leaflets were inoculated per plant comprising the two pairs of primary leaflets immediately next to the terminal leaflet. Prior to inoculation the leaflets were dusted with carborundum powder. Inoculation was achieved by pipetting 200 µl of sap, containing virus, onto the leaflets and rubbing the sap solution over the surface. A few minutes after inoculation the leaflets were rinsed with water to remove the carborundum. Approximately 10 plants of each cultivar were inoculated with each virus while an equivalent number of plants of each cultivar remained un-inoculated as virus free control plants.

On 5 November 2013, a sample of 4 leaflets was collected from the middle of the stems of each plant and tested for the presence of virus by ELISA.

The plants were allowed to mature in the glasshouse and senesce naturally before the tubers were harvested from the individual plants on 19 December 2013. All plants that had been successfully inoculated with either *PVY*^{N} or *PVY*^{O} (confirmed by ELISA as virus infected) were harvested. Any plants that had been inoculated with either *PVY*^{N} or *PVY*^{O} but were confirmed free of virus by ELISA were scrapped. The non-inoculated 'healthy control plants' of cvs Chieftain and Maris Piper, and which were confirmed free of virus by ELISA, were also harvested.

Field trial - 2014

The tubers harvested from the individual glasshouse plants of each cultivar (Chieftain or Maris Piper) and treatment (virus free or infected with either *PVY*^{N} or *PVY*^{O}) were bulked per cultivar/treatment. On 24 April 2014 the tubers were planted in a field trial at SASA's Gogarbank Farm, laid out as a randomised complete block design with 5 replicate blocks. Each cultivar/treatment plot comprised one drill of 10 tubers with each tuber spaced approximately 30 cm apart. A guard drill of virus free tubers was planted on either side of each plot. Each plot was 3 m in length with a 2 m gap between each of the 5 replicate blocks.

The trial was monitored for mosaic symptom development on the growing plants. On 28 July 2014 and 15 August 2014 leaflet samples were taken from each plant and tested for the presence of virus by ELISA.

The trial area received a standard blight and aphicide programme throughout the growing season. The haulm was desiccated using diquat on 27 August 2014. The trial plots were harvested by fork in September with the progeny of each plant harvested into a separate paper bag. The tuber samples were examined later in store and the number of tubers affected by cracking was counted in each sample. The tuber samples from all 10 plants per plot were bulked and the total weight of tubers was recorded.

Statistical analyses

The data from the field trial was analysed using GenStat for Windows 16th edition. The analyses of the proportion of cracked tubers per plot and proportion of plants producing at least one cracked tuber used binomial generalised linear mixed models with canonical functions. The analysis of the weight of tubers produced per plot used a normal linear mixed model. Replicate was treated as a random effect and the treatment structure as fixed. Estimates for the dispersion parameter were made when over-dispersion was indicated.

RESULTS

Preliminary investigation - 2013

Results of the virus testing of the tuber samples of cv. Chieftain sent over from Skagit County, Washington State, USA revealed that all tubers, apart from two from the non-cracked sample, were infected with *PVY*. Testing of the tuber samples by RT-PCR confirmed that the main *PVY* strain present was N:O / N-Wilga.

Production of virus infected tubers in the glasshouse - 2013

The success rate of inoculating plants with virus (as confirmed by ELISA) was high. All plants that were inoculated with *PVY*^{O} were successfully infected and all plants inoculated with *PVY*^{N} were successful except for three plants of cv. Chieftain. No growth cracking was observed on any of the tubers harvested from the virus inoculated plants in the glasshouse.

Field trial – 2014

All plants derived from tubers from virus inoculated plants in the glasshouse developed mosaic symptoms on the foliage. Virus testing of the field plants during the growing season confirmed the presence of either PVY^N or PVY^O. No mosaic symptoms were observed on the field plants derived from tubers from the un-inoculated healthy control plants in the glasshouse and virus testing of the field plants confirmed the absence of virus.

The percentage of tubers affected by growth cracking for each treatment is shown in Figure 1 and the percentage of plants producing at least one cracked tuber is shown in Figure 2. The growth cracks were healed fissures, of varying depths, in the surface of the tuber and most, characteristically, ran from the rose end of the tuber to the heel end. Cv. Chieftain showed strong evidence (p-value <0.001) for differences between the virus free and virus infected treatments in the proportion of tubers affected by cracking but the evidence was weaker for cv. Maris Piper (p-value 0.055). Cv. Chieftain was clearly more affected by growth cracking than cv. Maris Piper when plants were infected by virus. On average around 60% of tubers from virus infected plants of cv. Chieftain were affected by growth cracking with more than 90% of plants having at least one cracked tuber. However, cv. Maris Piper had more plants with one or more tubers with growth cracking in the absence of virus infection. The strain of *PVY* did not significantly affect the proportion of cracked tubers for either cultivar or the proportion of plants producing at least one cracked tuber.

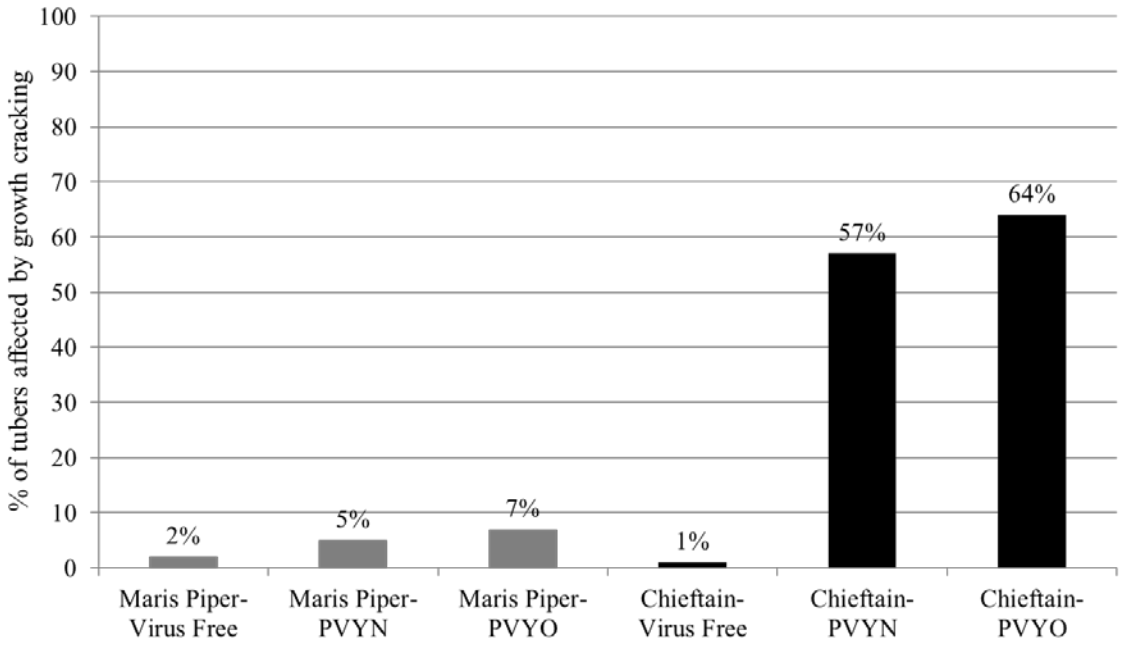

Figure 1. The percentage of tubers affected by growth cracking

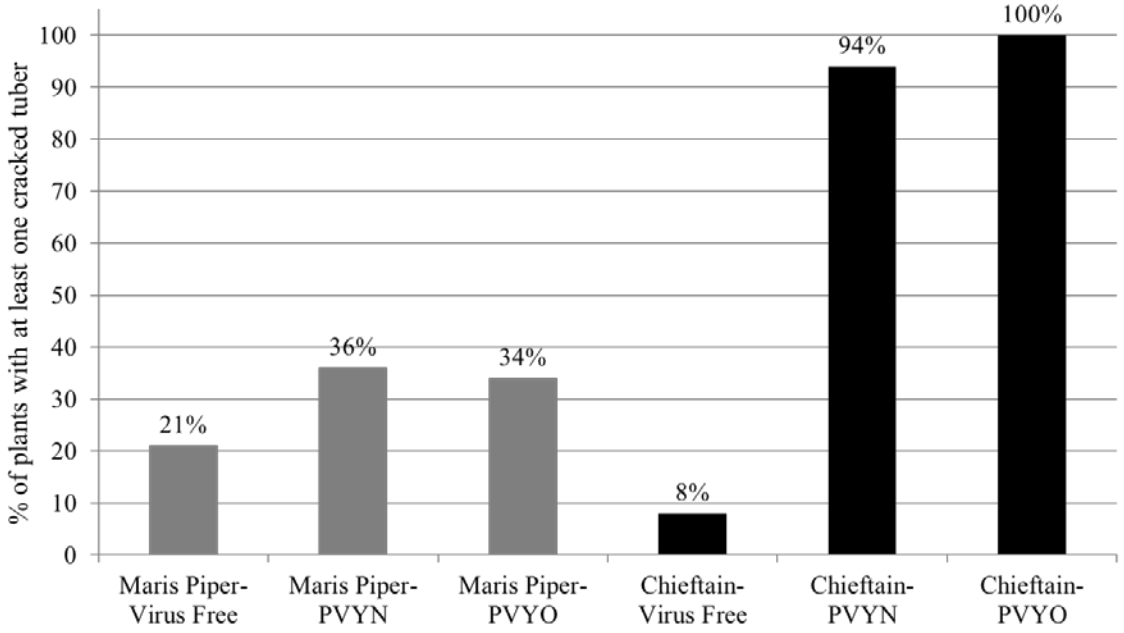

Figure 2. The percentage of plants with at least one cracked tuber

Virus infection had a sizeable effect on the weight of tubers produced by each plant (Figure 3; p-value <0.001). Cv. Maris Piper showed strong evidence for differences between the treatments (p-value <0.001) and there was also some evidence for differences between the treatments for cv. Chieftain (p-value 0.011). There was evidence for a difference between the two strains of *PVY* (p-value 0.003) and for an interaction between the strain of *PVY* and cultivar (p-value 0.020). Whilst cv. Chieftain lost similar levels of yield to both PVY^N and PVY^O, the yield of cv. Maris Piper was affected to a greater extent by infection with PVY^O.

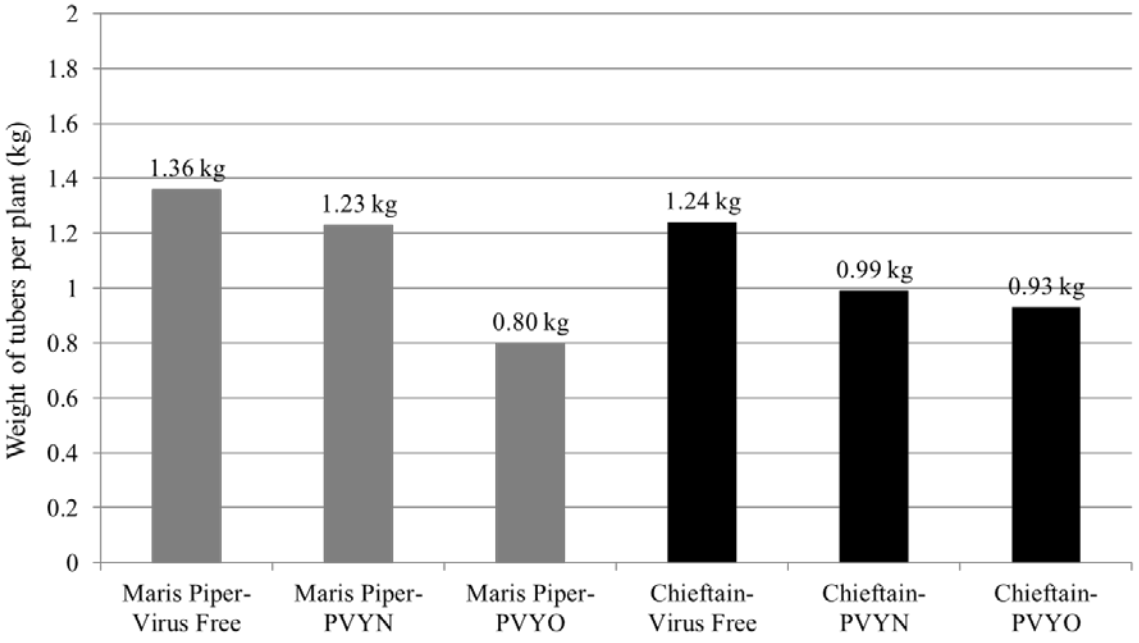

Figure 3. The mean weight of tubers produced per plant (kg)

DISCUSSION

The results clearly show that cv. Chieftain was far more prone to tuber growth cracking, when plants were infected with *PVY*, than cv. Maris Piper. This confirms observations from SASA's collection of virus infected samples at Gogarbank, covering over 50 commonly grown cultivars, that some are more prone to tuber growth cracking than others. No growth cracking was observed at the primary virus infection stage in the glasshouse. This is consistent with the earlier study (McCreath & Carnegie, 2008), which showed that growth cracking was much more prevalent with plants showing mosaic symptoms than with those symptomlessly infected i.e. more prevalent in plants with secondary infection (derived from planting a virus infected tuber) than with primary infection (foliar infection during the current growing season).

A higher proportion of virus free plants of cv. Maris Piper had at least one cracked tuber, compared with cv. Chieftain and in addition there was little difference between the virus free and virus infected treatments. The growth cracking observed in cv. Maris Piper may, therefore, have been due to another cause. Tuber cracking is generally regarded as a physiological response to changes in the growing conditions e.g. water availability. The growth cracks in the two cultivars were slightly different in their expression; on tubers of cv. Chieftain they were generally shallow and completely healed over, indicating they had formed early in the development of the tuber, whereas in cv. Maris Piper they were deeper and not completely healed over, indicating they had formed later in the growing season.

Infection of plants by PVY^N or PVY^O resulted in a lower tuber weight per plant for both cultivars. The yield from cv. Maris Piper was reduced to a greater extent by infection by PVY^O while cv. Chieftain lost similar levels of yield to both strains. These differences may be due to symptom expression in the plant having an effect on yield. During the growing season it was noted that in cv. Chieftain both strains of *PVY* gave a very similar mosaic symptom in the foliage whereas with cv. Maris Piper, PVY^O infection appeared to have a more severe effect on the growth habit of the plant and possibly, therefore, having more of an effect on tuber yield.

This study confirms both an association between mosaic virus infection and the development of tuber growth cracking and that some cultivars are more prone than others. If virus infected plants in a crop produce more tubers with growth cracks this would have an effect on marketable yield and, therefore, have a financial impact on the profitability of the crop.

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ASSESSMENT OF THE SPREAD OF PYRETHROID RESISTANT *SITOBION AVENAE* IN THE UK AND AN UPDATE ON CHANGES IN THE POPULATION STRUCTURE OF *MYZUS PERSICAE* IN SCOTLAND

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Summary: The aphids *Myzus persicae* and *Sitobion avenae* are vectors of non-persistent potato viruses. Preventing virus accumulation in seed crops usually requires aphid control. Intensive insecticide spraying has resulted in some aphid populations evolving resistance mechanisms and *M. persicae* has been very successful in doing this. More recently, UK populations of *S. avenae* have acquired pyrethroid resistance. Microsatellite markers have been used to study the genotypic composition of the Scottish *M. persicae* for more than twenty years and the population shows extreme reduction in genetic variation.. Here we present an update on the changes in the *M. persicae* population structure to the year 2015. In addition, we examine the UK population structure of *S. avenae* and demonstrate pyrethroid-resistant individuals are mainly composed of one clonal type.

INTRODUCTION

In this study we use microsatellite markers to examine the genetic population structure of two aphid species *Myzus persicae* and *Sitobion avenae.* Whilst the two species have different life histories and life cycles in the UK, both are important vectors of viruses. Although *S. avenae* does not colonise potatoes it is capable of virus transmission as it moves over the crop probing plants.

Treatment with insecticides helps reduce the virus spread by killing or disorientating aphids (Fenton *et al*., 2014). However, this has resulted in the selection of insecticide resistance with *M. persicae* having successfully overcome several classes of chemical insecticides (Bass *et al*., 2014). More recently, in 2011, control failures of *S. avenae* were reported on cereal crops in England. These were the result of a mutation in the sodium channel gene (termed kdr) that is known to confer resistance to pyrethroids (Foster *et al*., 2014). Bioassays confirmed that these populations had a resistance factor of ~40x to lambda-cyhalothrin and molecular diagnostics demonstrated that all the resistant aphids carried the mutation L1014F in the heterozygous form SR (Foster *et al*., 2014). These clones may be less resistant than aphids carrying the homozygotic form, RR, which have not yet been found in the UK or elsewhere. Possessing insecticide resistance can give a clone a selective advantage; however, these resistance mechanisms may also carry fitness costs suffered potentially during times of stress (Foster *et al*., 1999).

The *M. persicae* population in the UK has been wellcharacterized with some clones being highly successful and others having only short term success (Fenton *et al*., 2005; Kasprowicz *et al*., 2008). The UK population of *S. avenae* has also been studied previously with a widespread successful UK clone being reported (Llewellyn *et al*., 2003). Ecological factors influencing the success of such clones are not fully understood. Conducting genotypic analysis of suction trap caught material and relating the findings to the aphid's distribution and insecticide resistance properties aids the understanding of their population dynamics and agroecology. This is of great value when developing sustainable management strategies. In this study we report the distribution and frequency of the kdr mutation in UK suction trap collected samples of *S. avenae* and examine the level of genetic variation in the resistant and sensitive population using high resolution DNA markers. In addition, we examine the current genotypic structure of the Scottish *M. persicae* population and discuss possible factors that have favoured the expansion and decline of some genotypes.

MATERIALS AND METHODS

Myzus persicae

The data set consists of thousands of individual aphids sampled over Scotland in a twenty year period (1995-2015) collected from potato (*Solanum tuberosum*),oilseed rape (*Brassica napus*) and from 12.2m high suction traps. DNA was extracted and microsatellite loci amplification analysis and visualisation carried out (Malloch *et al.*, 2006). Six microsatellite loci were used: M35, M40, M49, M63, M86 and myz 9 (Sloane *et al*., 2001).

Sitobion avenae

S. avenae were collected from 15 UK suction traps in 2013 and 2014. DNA was extracted from over 1500 individuals (>900 from England and >600 from Scotland) and tested for the presence or absence of the kdr mutation using a PCR based allelic discrimination assay Malloch *et al.,* 2014. Aphids could be scored as sensitive (SS) or resistant (SR, RR). A subset of the DNA was genotyped using five microsatellite markers chosen on the basis of their resolution: Sm10, Sm12, s16b, Sm17 and saΣ4 (Simon *et al*., 1999; Llewellyn *et al*., 2003; Wilson *et al*., 2004).

RESULTS

Myzus persicae

The *M. persicae* population in Scotland is composed of a limited number of clones or multi-locus genotypes (MLGs). The ancestral population (1995-2012) has been dynamic with various MLGs expanding and subsequently collapsing, most probably in response to changes in environmental factors including changing insecticide usage (Malloch *et al*., 2012). From 2001-2007 clones resistant to dimethyl carbamates entered the UK (MACE clones). Most of these MACE genotypes expanded rapidly but then disappeared in a process known as clonal turnover (Kasprowicz *et al*., 2008). Between 2007 and 2012 two MACE genotypes (O and P) appeared in large numbers but unlike previous MACE clones these did not decline at first. It was thought these genotypes had better genetic backgrounds and did not suffer from resistance fitness costs

and had the physiological adaptations required to survive local conditions. Representatives of clones O and P were also found across Europe (Fenton *et al*., 2009). Clones which are capable of multiplying to very large numbers, over many seasons, and over a large geographical area are known as aphid super-clones. Super-clones O and P dominated the UK population from 2007-2012 and insecticide sensitive super-clones (I and J) which were common from 1995-2001 declined rapidly. However, our results show that contrary to expectation, numbers of the super-clone O have recently declined and three new clones (S, T and U) have appeared (Figure 1).

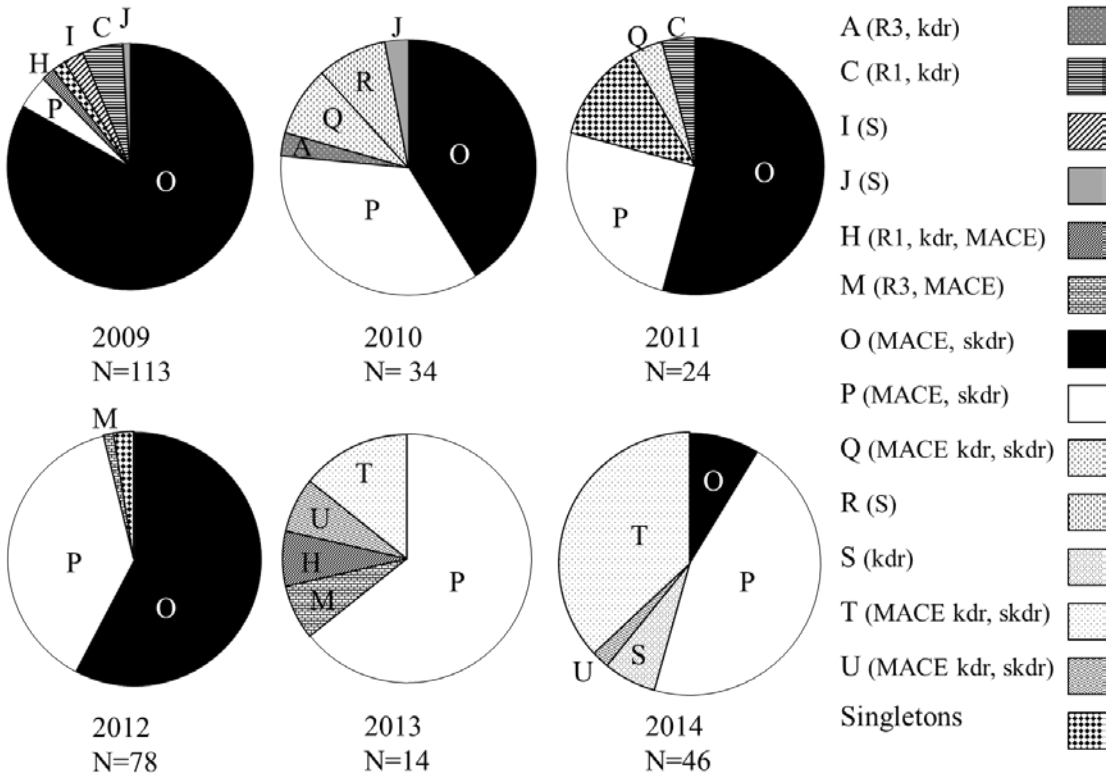

Figure 1. Changes in the composition of the Scottish *M. persicae* population based on samples caught in suction traps from 2009-2014

Sitobion avenae

Genotypic analysis of *S. avenae* suction trap samples was carried out in 2013 and 2014 and results show that whilst there is considerable diversity in the UK population some common genotypes are found. The most common genotype SA3 contained a mutation in the sodium channel gene, L1014F, which confers resistance to pyrethroid insecticides (kdr; Foster *et al.* 2014). The frequency of this resistance was determined by carrying out a PCR assay (Foster *et al.* 2014) on a large sample of *S. avenae*. The frequency was found to have remained stable in England between 2013 (30%) and 2014 (29%) but showed a dramatic rise in Scotland from 9% in 2013 to 29% in 2014.

kdr was mainly associated with one genotype, the SA3 clone (Figure 2), but in 2014 two individuals with non-SA3 genotypes were found to be associated with L1014F. The mutation

may be recombining into new genetic backgrounds or arising independently. If this is the case, these new genotypes could also have a selective advantage and are likely to increase. In order to determine what has happened, a further study has been carried out in 2015.

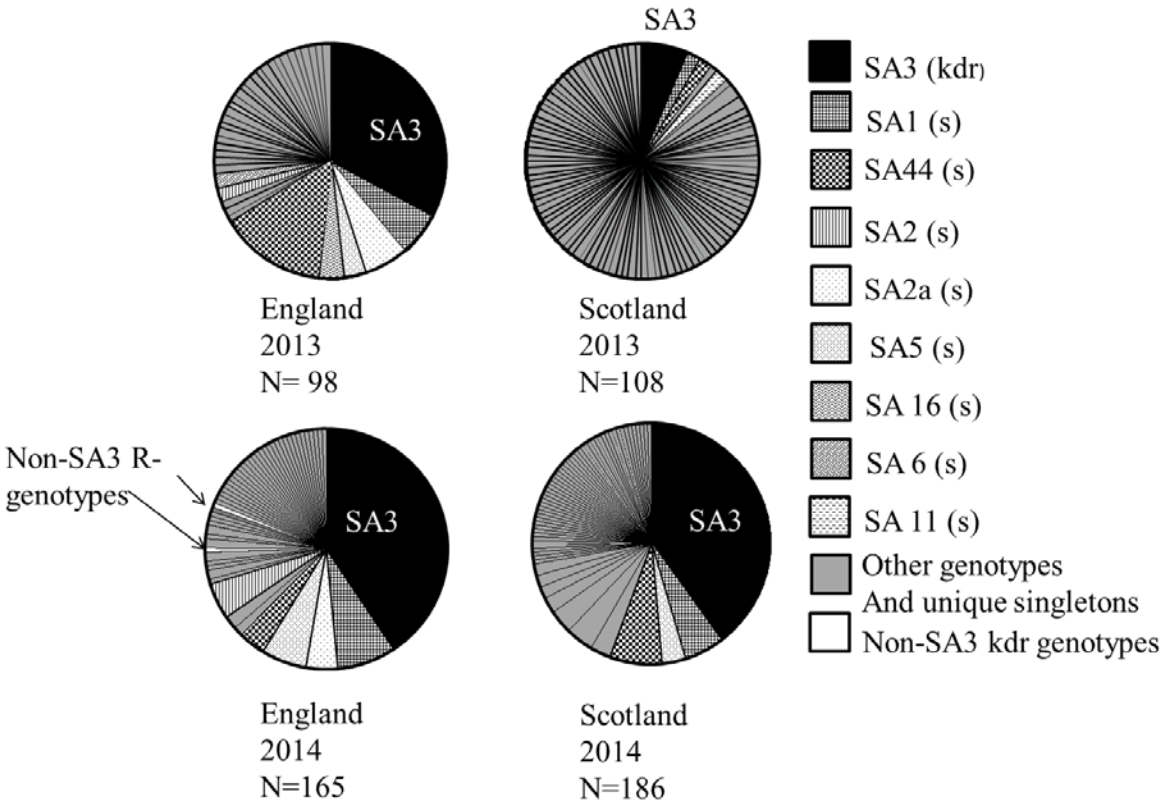

Figure 2. Comparison of the changes in the genotypic composition of the population of *S. avenae* based on samples caught in suction traps in England and Scotland from 2013-2014

DISCUSSION

M. persicae and *S. avenae* probably have different life histories in the UK because climate, host range and the availability of the winter host play a role. In winter *S. avenae* is able to lay eggs on British plants such as cocksfoot grass (*Dactylis glomerata*). This allows this species to reproduce sexually and overwinter as cold-hardy protected eggs. In contrast, the primary host for *M. persicae* is peach (*Prunus persica*) which is not readily available in the UK. Correspondingly, it has been suggested that only a few clonal genotypes persist over winter *via* asexual reproduction on secondary hosts. These differences in life habits have resulted in very different genotypic population structures in the UK with *M. persicae* having a very limited number of successful clonal genotypes, and the *S. avenae* population consisting of numerous genotypes which are the result of recombination. However, it is clear that *S. avenae* can also produce lineages that overwinter as clones.

Myzus persicae

Analysis of the Scottish *M. persicae* population in 2014 shows a recent decline in the super–clone genotype O. This genotype, like genotype P, has persisted in the UK for a number of years and has all the characteristics required for success, including resistance to pirimicarb and pyrethroids without any apparent negative fitness costs. The genetic background appears to be well suited to survival during the cold wet winters experienced in the UK. The reasons for the decline of clone O in 2013 and 2014 are unknown. The total number of *M. persicae* caught in the trap in Scotland was below average in 2013 (SASA, 2013) and the small sample size may have limited the interpretation of the results. Analysis of the population caught in the Scottish traps in 2015 will reveal if this decline in genotype O is continuing. Non-MACE genotypes: C, I and J made a brief return in 2009 and it is possible that this resulted from the ban on the use of neonicotinoid seed coatings on oilseed rape. This might allow insecticide sensitive non-MACE genotypes that once dominated the population during the period 1995- 2001 to use the crop as a possible winter reservoir. However, this does not appear to have happened as genotypes C, I and J have not been detected in the population since 2011. The decline in genotype O occurred at the same time as new clones S (kdr), T (kdr, skdr, MACE) and U (kdr, skdr, MACE) were detected. It has been suggested that new asexual genotypes arise from successive waves of colonising clones probably attributed mostly to plant imports (Fenton *et al*., 2009). We may be witnessing the next wave of colonisation or possibly even the development of the next super-clone. Alternatively, genotypes S, T and U may be subject to clonal turnover where they will eventually fall below the level of detection in a similar way to clones N, Q and R. Continued monitoring will allow us to determine the fate of clones O and P and the success of the new genotypes in a changing environmental background.

Sitobion avenae

In contrast to *M. persicae*, the diversity of the *S. avenae* population has been shown to increase in northerly latitudes (Llwewellyn *et al.*, 2003). We have identified a number of persistent common genotypes in the *S. avenae* population (SA1, SA3, SA44, SA2A, SA5 etc.). These were present in 2012 (results not shown), 2013 and 2014 suggesting that these genotypes are not recombining and are therefore likely to be reproducing asexually. One of these asexual clones (SA3) has been shown to carry pyrethroid resistance and given the selective advantage this clone would have it is perhaps to be expected that its frequency has increased. As with previous studies (Llewellyn *et al.*, 2003), our 2013 results show that the diversity of the more northerly Scottish *S. avenae* population was greater than the English population.
Two non-SA3 genotypes containing the SR mutation were identified in 2014. This indicates the possibility that the mutation may be capable of moving into, or evolving in, new genetic backgrounds. Continued monitoring of the UK *S. avenae* population in 2015 will determine if this finding is significant. If resistant clones are capable of sexual reproduction this raises the possibility that the RR genotype could occur resulting in a potential increase in resistance to pyrethroids.

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SULFOXAFLOR : A NEW INSECTICIDE FOR THE CONTROL OF APHID SPECIES IN POTATOES

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Summary: ISOCLAST™ Active (sulfoxaflor), is an insecticide developed for the control of sap feeding pests in a wide range of crops. Sulfoxaflor, a member of a new chemical class of insecticides, the sulfoximines, has been discovered by and is proprietary to Dow AgroSciences. GF-2626 (120 g a.i./litre sulfoxaflor SC) is being developed for use in a range of crops, including potato, for the control of aphid species. Laboratory testing has indicated a robust lack of cross resistance to multi resistant *Myzus persicae* (Peach potato aphid) or pyrethroid resistant *Sitobion avenae* (Grain aphid). Sulfoxaflor applied at 24 g a.i./ha was studied in sixteen trials across Europe and demonstrated significantly higher control than the comparison treatment, lambda-cyhalothrin. Sulfoxaflor achieved 76.7% mean control throughout the 21 day trial period, compared to 56.4% for lambda-cyhalothrin. Data supports the use of GF-2626 as an effective insecticide to control *M. persicae* regardless of resistance status and will provide an invaluable addition to UK potato growers for use within integrated pest management strategies.

INTRODUCTION

Aphid species and other sap feeding insects, especially those in the order Hemiptera are among the most economically damaging insect pests in the world. As well as direct feeding losses they are vectors for virus transmission. Within the potato crop, *Myzus persicae* (peach-potato aphid) and *Macrosiphum euphorbiae* (potato aphid) most frequently transmit *Potato leaf roll virus* (PLRV), *Potato Virus A* (PVA) and *Potato Virus Y* (PVY). Yield loss from aphid damage in the ware potato market in England and Wales was estimated at 1-2% (AHDB 2009); however, losses due to downgrading and failures in the seed sector from virus infection are far more damaging (and second only to losses from potato cyst nematodes) and were estimated to be 25% in 2007 (AHDB 2009). The Scottish seed area in 2014 was 11,433 hectares and SASA (2014) reported incidences of mosaic virus of 5%. In the absence of effective aphid control programmes or certified virus free seed the potential yield losses as a consequence of PLRV and PVY infection have been quantified as ranging from 60-80% (Lung'aho *et al*., 2007).

The active ingredient Isoclast™ Active (sulfoxaflor) is the sole member of a new chemical class of insecticides, the sulfoximines. Discovered and developed by Dow AgroSciences, sulfoxaflor was granted active substance approval by the European Food Safety Authority [EFSA] (18th August 2015). Sulfoxaflor is effective against a wide range of sap-feeding insects including those of importance in potato crops; *M. persicae*, *M. euphorbiae* and potato leafhoppers (*Empoasca* spp.*).* Product evaluations for use within the European Union are ongoing to support uses on a range of crops including potatoes, brassicas, cereals, pome and stone fruits (Harris & Mezei, 2014). Sulfoxaflor exhibits good activity to members of the order Hemiptera but much less activity to the other orders.

Mode of Action

Zhu *et al.,* (2011) reported that initial observations on the effects of sulfoxaflor on *M. persicae* showed excitatory symptoms such as tremors, followed by paralysis and mortality, suggesting that the sulfoximines act via the insect nervous system. Upon further analysis, sulfoxaflor was subsequently found to have an interaction with insect nicotinic acetylcholine receptors. Many studies have established that sulfoxaflor exhibits complex and unique interactions with the insect nicotinic acetylcholine receptor (nAChR) agonists that are distinct from those observed with neonicotinoids. Sulfoxaflor is a high-efficacy nAChR agonist with low affinity for the imidacloprid binding site (Sparks *et al*., 2013). Due to its mode of action and unique properties it is classified as the sole member of the Subgroup 4C (IRAC, 2014). Sulfoxaflor kills insect pests both on contact and through ingestion to provide knockdown and residual control. Studies have shown that sulfoxaflor displays translaminar movement (moves across the leaf surface) when applied to foliage and confers systemic activity via the xylem, protecting parts of the plant not covered with spray solution. Sulfoxaflor exhibits good stability within the plant, providing pest control for approximately 14 days post application (Sparks *et al*., 2012).

Resistance

M. persicae is the most economically damaging aphid pest of potato and has developed resistance to a range of insecticides (IRAC, 2014). Sulfoxaflor has shown a broad lack of cross resistance in many sap feeding insect strains resistant to carbamates, organophosphates, pyrethroids and neonicotinoids (Sparks *et al.,* 2013). Currently no reported evidence of resistance in the field to sulfoxaflor has been reported, though in two recent studies (Cutler *et al*., 2013; Bass *et al*., 2015) laboratory data were shown to prove slight cross resistance between sulfoxaflor and neonicotinoids in *M. persicae* samples containing the R81T target site mutation. Both studies reported sulfoxaflor as the most active insecticide having the lowest resistance factor among the tested IRAC Group 4 insecticides. These results provide evidence that sulfoxaflor controls *M. persicae* populations expressing R81T mutation under field conditions and only expresses moderate numerical increase in tolerance when compared to imidacloprid. Our research proves that sulfoxaflor can be used as an effective insecticide to control *M. persicae* regardless of its resistance status. As a consequence of insect resistance and regulatory pressure on older chemistry (OP's and carbamates), sulfoxaflor will be a useful rotation partner with other insecticide compounds, enhancing insect resistance management (IRM) strategies. The sulfoximines are nicotinic acetylcholine receptor (nAChR) agonists which are unique and specific in comparison with other nAChR agonists. Much research has been conducted in this area and allowed IRAC to conclude that sulfoxaflor (4C) is distinct from neonicotinoids (4A). Laboratory and field studies have shown that sulfoxaflor exhibits a robust lack of cross-resistance in populations of insects resistant to many other chemical classes, including the neonicotinoids.

Safety to bees and non-target arthropods

When tested in laboratory studies of adult honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) via contact or oral routes, the toxicity of sulfoxaflor was classified as acutely toxic. When applied in semi-field tunnel studies to mimic use conditions, sulfoxaflor showed no long term effects on brood development or colony strength. Measurement of bee foraging activity indicated little difference between untreated plots and those treated with sulfoxaflor. No reduction in overall bee colony health has been established when sulfoxaflor was used according to the product label.

Sulfoxaflor was tested in standard laboratory assays against a range of beneficial non-target arthropods, such as predatory mites, parasitoid wasps, lacewing, lady bird beetles and spiders. In the laboratory assays, only the aphid parasitoid wasp (*Aphidius rhopalosiphi)* demonstrated significant sensitivity to sulfoxaflor. However, in field studies, sulfoxaflor had no significant impact on seasonal population levels of the tested species, including populations of parasitoid wasps. Sulfoxaflor exhibits rapid soil breakdown and the plant and soil metabolites of sulfoxaflor have no insecticidal activity.

MATERIALS AND METHODS

Data are presented from sixteen trials conducted between 2008 and 2014 within Europe (UK, France and Germany) to study the control of a range of aphid species in potatoes. There were no significant control differences between the various species of aphid on potato and therefore it was considered appropriate to combine the data. The test species were *M. persicae*, *Macrosiphum euphorbiae* (potato aphid), *Aulacorthum solani* (foxglove aphid) and *Aphis nasturtii* (buckthorn aphid). All trials had 4 replicates, randomised in a complete block design with plot sizes ranging from 9 to 36 m^2. Spray application volumes ranged from 200 to 400 litres per hectare. Sulfoxaflor at the proposed rate of 24 g a.i./ha was compared to the pyrethroid, lambda-cyhalothrin (7.5 g a.i./ha). The aphids were counted at or one day prior to application on samples of 10-60 leaves, with populations of 3 to 417 aphids per leaf recorded. The same areas of the plant were counted at different dates after application: knock down effect at 1, 2-3 days, residual effect at 6-8, 13-14 and 20-21 days after application. The efficacy or % Control for each treatment was then calculated with Henderson and Tilton formula (Henderson and Tilton, 1955):

$$\%Control = \left(1 - \frac{nTreated_a \times nUntreated_b}{nUntreated_a \times nTreated_b}\right) \times 100$$

where $nTreated_a$ is the number of insects in the treated plots after the treatment; $nUntreated_b$ is the number of insects in the untreated plots before the treatment; $nUntreated_a$ is the number of insects in the untreated plots after the treatment and $nTreated_b$ is the number of insects in the treated plots before the treatment. The use of the correction with the untreated is an option recommended in trial series analysis when the magnitude of the response variable in the untreated varies greatly from trial to trial (Madden and Paul, 2011).

%Control values were analysed with a linear mixed model for trial series with repeated measures:

$$\%Control_{ijkl} = \mu + Treatment_i + Time_j + Trial_k + Treatment \times Time_{ij} + Treatment \times Trial_{ik} + Time \times Trial_{jk} + Treatment \times Time \times Trial_{ijk} + Block_{l(k)} + e_{ijkl}$$

with observations normally distributed: Treatment, Time and Treatment×Time modeled as fixed effects and the rest of the factors modelled as random effects. Correlation between observations at different assessment times, within each experimental unit, was modelled with autoregressive covariance structure. (Stroup, 2012).

Linear mixed model assumptions (normality and homogeneity of variance) were evaluated with graphical inspection of the residuals. Comparisons between treatments for the trial series, at each assessment time and averaged across the whole time of the experiment, were performed with the appropriate contrasts from the global model specified above. Statistical analysis was performed with SAS Proc GLIMMIX (SAS, 2011).

RESULTS

Observed values of % Control of aphid species (*M. persicae, M. euphorbiae, A. solani and A. nasturtii*) from a single treatment of sulfoxaflor at 24 g a.i./ha applied in May to July from trials throughout Europe are presented in Figure 1. Least squares means estimated with the mixed model are included in Table 1 and Figure 2. The analysis across trials indicates that sulfoxaflor was as effective as the pyrethroid, lambda-cyhalothrin at the 1 day assessment timing. From 2-3 days to 21 days sulfoxaflor achieved significantly higher control than lambda-cyhalothrin.

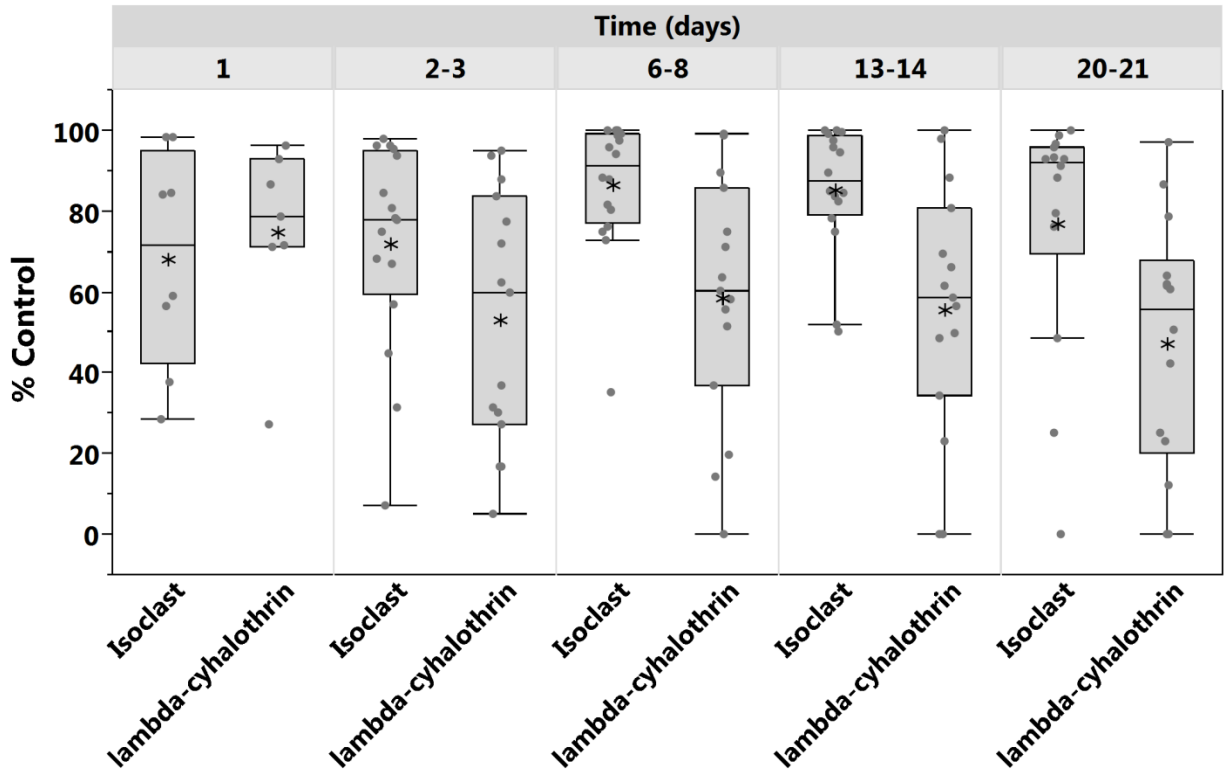
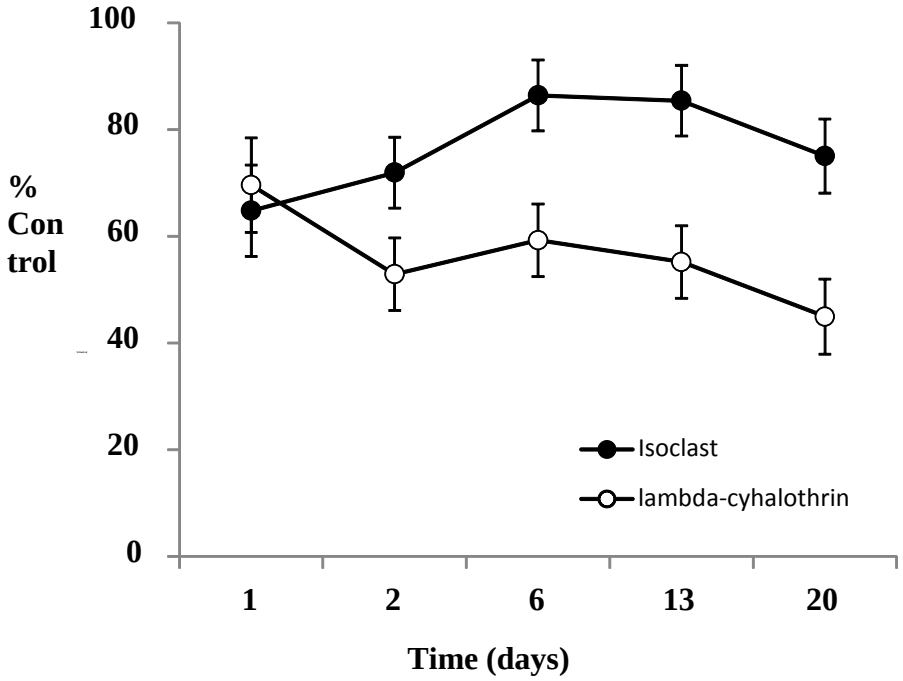


Figure 1 Observed values of % Control of aphid species (dots represent trial means, stars represent treatment means). 16 trials, 2008-2014

Table 1. % Control of aphid species by sulfoxaflor (Isoclast) and lambda-cyhalothrin from a single summer application, 2008-2014.

	%Control (least squares mean±std error)					
Days after application	1	2-3	6-8	13-14	20-21	Average 1-21
Number of trials	8	16	16	16	16	16
Isoclast 24 g a.i./ha	64.79±8.55 a*	71.91±6.64 a	86.40±6.64 a	85.41±6.64 a	75.03±6.95 a	76.70±5.17 a
Lambda-cyhalothrin 7.5 g a.i./ha	69.58±8.87 a	52.89±6.79 b	59.25±6.79 b	55.18±6.79 b	44.95±7.02 b	56.37±5.31 b



*Values not connected by same letter in each column are significantly different. (Tukey's HSD; p=0.05)

Figure 2. % Control of aphid species: comparison of sulfoxaflor (Isoclast) and lambda-cyhalothrin from a single application (lsq mean ± std error).16 trials, 2008-2014.

DISCUSSION

Trial data comparing single applications showed that sulfoxaflor applied at 24 g a.i./ha achieved significantly higher control of a range of aphid species compared with the pyrethroid lambda-cyhalothrin and when applied at a single application timing. Equivalent efficacy was demonstrated at the 1 day after application assessment (64.8% vs 69.6% from sulfoxaflor and lambda-cyhalothrin respectively) and thereafter sulfoxaflor achieved significantly higher control from the 2 to 21 day assessment period. The systemic action and longevity of control were clearly demonstrated with control around 80% recorded for sulfoxaflor from 6 to 21 days. During the same time period, lambda-cyhalothrin achieved not only a lower level but also a declining level of aphid control (59.3 to 44.9%). That sulfoxaflor demonstrates a consistently high level of control over an extended period has the potential for achieving high levels of virus control for potato growers. At the time of writing, no virus reduction data were available from treated potato crops; however data are available for cereal crops infected with barley yellow dwarf virus (BYDV), where following control of the virus infected aphids with sulfoxaflor significant yield increases, compared to the untreated, of 9-172% in autumn treated winter wheat were observed (Harris & Mezei, 2014). Through high levels of aphid control, sulfoxaflor has the potential to reduce virus infection.

CONCLUSIONS

Sulfoxaflor has demonstrated itself to be an effective product for the control of aphids on potato crops. It demonstrated effective residual activity over a two to three week period and has the potential to provide potato growers not only with a high level of aphid control but also a means of reducing the economically damaging consequences of virus infections in both the

ware potato crop and also the highly important seed potato crop. Controlling species resistant to neonicotinoids, pyrethroids, carbamates and organophosphates, whilst also exhibiting low toxicity to pollinators and beneficial insects, sulfoxaflor provides growers with a key tool for the control of aphid species and a useful rotation partner in insect resistance management programmes.

[TM] Trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

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THE POTATO CYST NEMATODE: NATIONAL DISTRIBUTION OF MITOTYPES

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Summary: In the UK, two species of PCN occur, *G. rostochiensis* and *G. pallida*, and are responsible for losses to the industry of £50/annum (Defra, 2010). Widespread cultivation of cultivars such as Maris Piper which has resistance (*H1*) against *G. rostochiensis*, has been highly effective in controlling this species (Barone *et al.,* 1990). However, this has inadvertently led to the increase in prevalence of *G. pallida* (Minnis *et al.,* 2002). Cultivars with high levels of resistance to *G. pallida*, such as Innovator, have only recently become available and their impact on controlling *G. pallida* remains to be determined. Currently the spread of *G. pallida* in Scotland is of increasing concern for the protection of seed land.

INTRODUCTION

The genetic variation of *G. rostochiensis* and *G. pallida* populations found in the UK relates to the presumed historical introductions of small proportions of the total genetic diversity found for these species in South America (Plantard *et al.,* 2008). To date, only *G. rostochiensis* pathotype Ro1 has been found in the UK and hence the use of cultivars with the *H1* gene have been effective in suppressing this species. However, phenotypically distinct populations of *G. pallida* are found in the UK and have been designated as pathotypes Pa1 and Pa2/3 (Kort *et al.*,1977; Phillips & Trudgill, 1998, Blok & Philips, 2012). The potential for UK *G. pallida* pathotypes to differ in their ability to overcome different sources of resistance indicates that cultivar choice may be important in a control program employing resistance. Managing PCN with resistance is an increasingly important option due to the introduction of recent restriction in nematicide applications (Regulation (EC) No 1107/2009). Thus, recent work has focused on developing new diagnostics that will enable rapid and accurate means to distinguish PCN genotypes in field populations to monitor their composition and to assist in cultivar choice (Hoolahan *et al.,* 2012; Mimee *et al.,* 2015). Monitoring field populations is essential to determine whether resistance is effective and durable.

Science and Advice for Scottish Agriculture (SASA) undertake annual preplant PCN tests from soil samples taken from seed and ware land by extracting DNA from soil floats and applying a DNA diagnostic assay to determine the presence of PCN and the species. These annual

collections of DNA samples yielding positive results for *G. pallida* (collectively ~ 1000) provide a unique resource for monitoring the distribution of PCN in Scotland and for further interrogation of the diversity within the species. We have used a region of mitochondrial DNA that is descriptive of three main mitotypes of *G. pallida* present in the UK, and have adopted a Metagenetics approach to perform sequencing and the simultaneous analysis of all samples (Eves-van den Akker *et al.,* 2015).

Mitotypes, based on maternally inherited mitochondrial DNA, provide a proxy for detecting different introductions of *G. pallida*, a pest which originates from S. America. Using this marker, we describe the distribution of mitotypes of *G. pallida* across Scotland at the resolution of individual fields. Interestingly, most fields contain a single mitotype, one fifth contain a mix of at least two mitotypes, and small minority (<3 %) contain a mixture of all three mitotypes. However, populations within mixed fields are highly heterogeneous. The relative abundance of each mitotype can vary across an order of magnitude. In addition, local areas within mixed fields are dominated by certain single types, and may suggest a complex underlying "pathoscape" within each field. This study provides a method for rapid, accurate, quantitative and high throughput typing of over 1000 samples simultaneously and demonstrates the potential of metagenetics for monitoring the distribution of pests and pathogens in the field.

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INVESTIGATING THE EFFECT OF ISOTHIOCYANATES ON *GLOBODERA PALLIDA* HATCH UNDER *IN VITRO* AND *IN VIVO* CONDITIONS

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Summary: Potato cyst nematodes are a major pest of potatoes in the UK. Due to EU legislation restricting nematicide use, alternative methods of nematode control are required. Biofumigation is the use of isothiocyanates produced from *Brassica* tissue to suppress soil pests. In this study, the effect of isothiocyanates on *Globodera pallida* juvenile mortality and hatching has been investigated. Although several isothiocyanates increased juvenile mortality, the most effective was allyl isothiocyanate which caused 100% mortality at 25µl/L after 72hrs exposure. In addition, allyl isothiocyanate reduced hatch under *in vitro* conditions after 7 days exposure. During a glasshouse trial, 100µl/L allyl isothiocyanate was found to significantly reduce hatching by 61%, when compared to a control. This suggests that allyl isothiocyanate may be a good candidate for the control of potato cyst nematodes in a biofumigation programme.

INTRODUCTION

Potato cyst nematodes (PCN) are a major potato pest that can remain dormant in the soil for over 10 years in the form of cysts containing between 100–300 juveniles. Control of *Globodera pallida* and *G. rostochiensis* (PCN) traditionally relies on crop rotation, host resistance and nematicide use. Due to European Council regulation (EC) No 1107/2009 restrictions, increasing emphasis is being placed on the reduction of the use of pesticides due to concerns regarding the possible negative impact on the environment. Therefore a considerable amount of interest is being shown in alternative PCN control methods.

Biofumigation is the suppression of soil pests by volatile hydrolysis products released into the soil after incorporation of glucosinolate-containing plant tissues (Angus and Gardner, 1994). When the plant tissue is disrupted, glucosinolate hydrolysis occurs in the presence of the enzyme myrosinase releasing breakdown products such as isothiocyanates (ITCs), nitriles and thiocyanates (Fenwick and Heaney, 1983; Serra *et al.*, 2002). It is believed that the ITCs are the active hydrolysis product able to suppress soil pests. Previous data suggests that exudates from a range of *Brassica* spp. can be used as a potential control method for PCN (Brolsma *et al.*, 2014; Buskov *et al.*, 2002; Lord *et al.*, 2011; Ngala *et al.*, 2015; Serra *et al.*, 2002). The objective of this research was to assess the effect of ITCs on *G. pallida* juvenile mortality, under *in vitro* conditions, and PCN hatch, under *in vitro* and *in vivo* conditions. This will provide information leading to the development of an optimised biofumigation strategy for the control of PCN.

MATERIALS AND METHODS

Isothiocyanates

ITC solutions (Sigma-Aldrich) were made up to the desired concentration in distilled water. The isothiocyanates used in this study were benzyl (BITC), 2-phenylethyl (PEITC), methyl (MITC), allyl (AITC), isopropyl (IITC), ethyl (EITC), phenyl (PITC) and butyl (BUITC).

Toxicity Assays

In the toxicity assay, ~30 hatched *G. pallida* Pa2/3 juveniles were exposed to 2ml of one of eight ITC solutions (BITC, PEITC, MITC, AITC, IITC, EITC, PITC and BUITC) at three different initial concentrations (12.5, 25 and 50 µl/L). Four replicates of each treatment were performed in wells of a six-well suspension plate. Treated juveniles were stored in the dark at room temperature (20±1°C) for 72hrs, after which time juvenile mortality was determined. The nematode was considered dead when it was immobile and did not respond to stimuli in the form of pricking by a needle. The mortality percentage was calculated and compared to a water control. Data was analysed by one-way analysis of variance (ANOVA) using Genstat v14.

Hatching Assays

Five *G. pallida* cysts, previously soaked in water for 72hrs, were exposed to 2ml of one of four ITC solutions (BITC, PEITC, AITC and EITC) at three different initial concentrations (12.5, 25 and 50 µl/L) for varying time intervals of 1, 4 or 7 days. Cysts were then transferred to potato root diffusate (PRD) where hatched juveniles were counted over a 4 week period. Four replicates of each treatment were performed. Treated cysts were stored in the dark at room temperature (20±1°C) throughout. Overall hatch was calculated and compared to a water control and data was analysed by two-way ANOVA using Genstat v14.

Glasshouse Trial

Pot trials were set up under controlled glasshouse conditions with *G. pallida* cysts. Muslin bags containing five cysts each were placed in 2L pots with John Innes No.2 loam compost. Six ITC solutions, made up of three ITCs (AITC, BITC and PEITC) in combination of high (100µl/L) and low (5µl/L) concentrations, and a water control were incorporated into each pot in 50ml volumes which were sealed with plastic film for three weeks. The cyst bag was removed from each pot and the effect of the treatments on juvenile hatch was estimated as described above. Six replicates for each treatment were set up in a randomised block design.

RESULTS

Toxicity Assays

ITCs had varying abilities to cause *G. pallida* juvenile mortality after 72hrs exposure compared to a water control (Table 1). IITC had no effect on juvenile mortality. PITC had no effect on juvenile mortality at lower concentrations but was able to cause a significant increase in mortality at 50µl/L. BITC, MITC and BUITC had no effect on juvenile mortality at 12.5µl/L but were able to cause a significant increase in mortality at 25µl/L and 50µl/L. PEITC, EITC

and AITC increased juvenile mortality at all three concentrations. AITC was the most effective leading to 100% juvenile mortality at a concentration of 25µl/L and 50µl/L after 72hrs exposure.

Table 3. Juvenile mortality (%) after treatment with different ITCs for 72hrs. Control is a water treatment. Blank units indicate 0-50% juvenile mortality, light grey indicates 50-75% juvenile mortality and dark grey units indicate 75-100% juvenile mortality.

	Concentration (µl/L)		
ITC	12.5	25	50
BITC	42.7	61.4	95.9
PEITC	82.6	71.6	79
MITC	42.5	78.9	98.3
AITC	87.3	100	100
IITC	17.2	34.5	36.6
EITC	77.3	86.6	90.4
PITC	37.1	41.5	76.2
BUITC	44.5	56.9	57.7
Control		32.1	

Hatching Assays

Cysts exposed to BITC and EITC showed no significant differences in total hatch at any exposure time or concentration compared to a water control (data not shown). PEITC had no effect on juvenile hatch after 1 and 4 days exposure at all concentrations tested compared to a water control; however, after 7 days exposure, all concentrations increased juvenile hatch compared to a water control (Figure 1a). After 1 day exposure, 50µl/L AITC decreased hatch compared to a control (data not shown). In addition, AITC at 25µl/L and 50µl/L after 7 days exposure also decreased juvenile hatch (Figure 1b).

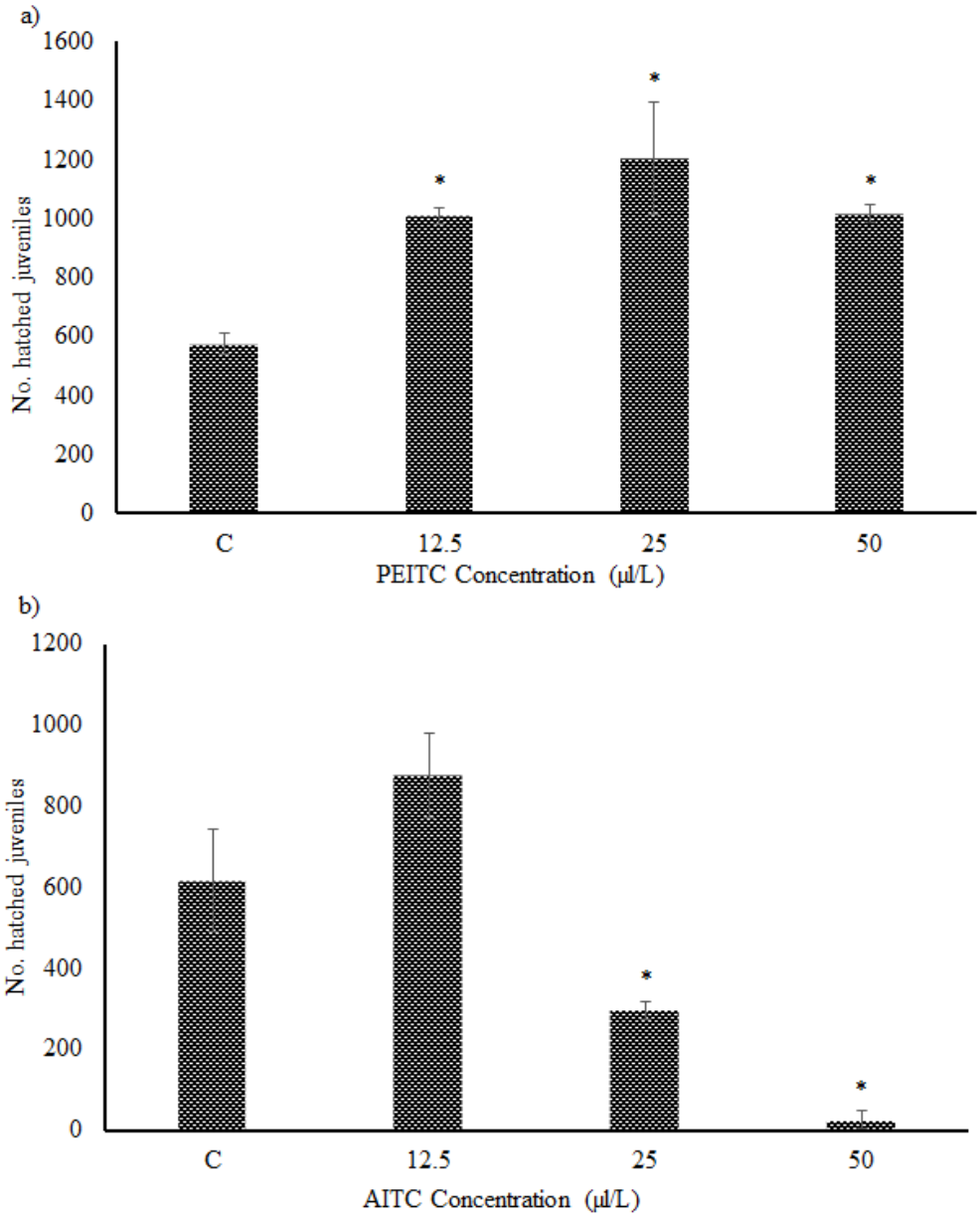

Figure 3. a) PEITC and b) AITC on G. pallida hatch after 7 days exposure Asterisk = significant differences (p=0.05) compared to the control.

Glasshouse Trial

Hatching assays completed after the glasshouse trial indicated that the only ITC able to reduce hatch was AITC (Figure 2). In addition, combining AITC with low concentrations of BITC and PEITC did not increase the ability of AITC to reduce *G. pallida* hatch.

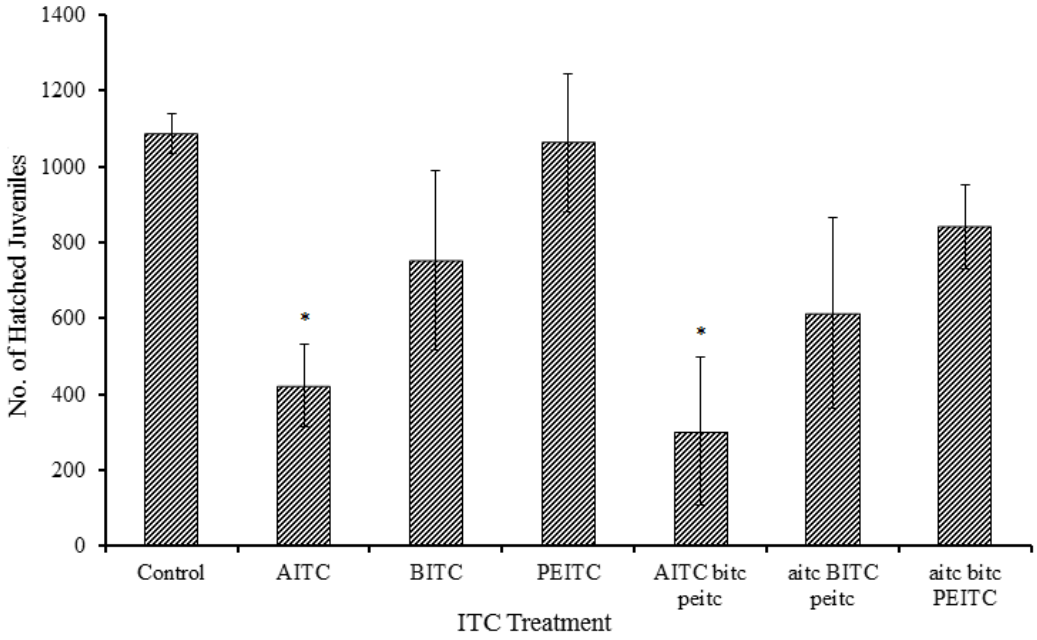

Figure 4. ITCs and G. pallida hatch under glasshouse conditions. AITC BITC PEITC = 100µl/L; aitc bitc peitc = 5µl/L. Asterisk = significant differences (p=0.05) compared to the control.

DISCUSSION

Isothiocyanates have varying effects on *G. pallida* juvenile mortality. Allyl isothiocyanate was the most toxic of the eight isothiocyanates tested as it greatly increased juvenile death at an exposure concentration of 25µl/L. PEITC in solution consistently caused juvenile mortality, which would suggest that it may be useful for reducing PCN levels but will not lead to 100% mortality. BITC, MITC, EITC and PITC increased in effectiveness as concentration increased, indicating that higher levels in the soil would be required for successful control. BUITC led to a low level of mortality compared to the other ITCs and IITC had no effect on mortality compared to a water control suggesting that these ITCs may not be suitable for PCN control.

Hatching assays indicated that BITC and EITC are not effective at suppressing juvenile hatch as neither had an effect compared to a control. This contrasts with the toxicity assay and suggests that encysted PCN are not affected due to the protective cyst. When cysts were exposed to high concentrations of AITC, hatch was significantly reduced after 1 and 7 days exposure. The ability of AITC to reduce hatch after certain exposure periods but not others is puzzling and requires more research. It may be due to the high natural variation within cyst content masking an effect, in which case further studies should include a larger number of cysts. PEITC concentrations which caused mortality in the toxicity assay appeared to increase hatch compared to a control after 7 days exposure. This increase is interesting and has also been noted during *in vitro* studies by Ngala *et al.* (2015) with *Brassica* spp. exudates. A potential explanation for this increase could be that PEITC may act indirectly on hatch by altering the permeability of the cyst wall, increasing the occurrence of hatching stimulants entering.

The glasshouse trial confirms that AITC is able to suppress juvenile hatch at high concentrations. In contrast to the hatching assay, PEITC had no effect on hatch in the glasshouse; this may be due to a reduced level of activity in the soil owing to increased headspace and an extended exposure period as ITCs are volatile and dissipate into the atmosphere over time. Combining ITCs in high and low concentrations had no effect on hatch compared to the ITCs on their own.

AITC may be a good candidate for use in a biofumigant PCN control programme. AITC concentrations used throughout this study are thought to be achievable in the field based on the glucosinolate profiles of different *Brassica* spp. (Lord *et al.,* 2011; Taylor *et al.,* 2014; Ngala *et al.,* 2014); however most research has focused on the glucosinolate concentration in *Brassica* biofumigants instead of the ITCs themselves so further investigation is required. In addition, ITCs vary in type and concentration both between and within *Brassica* spp. so research into selecting efficient crop lines must also be undertaken.

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MAPPING THE *H2* GENE TO A *GLOBODERA PALLIDA* PATHOTYPE PA1-RESISTANT POTATO CULTIVAR

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Summary: The potato cyst nematode (PCN) *Globodera rostochiensis* and *G. pallida* pose a threat to potato crops across Europe and the rest of the world. Discovery of the *H1* resistance gene which confers almost complete resistance to *G. rostochiensis* pathotype Ro1 was integrated into the cultivar Maris Piper and commercially deployed in 1966. Its successful repression of multiplication has unintentionally led to the selection for *G. pallida*. This selection has subsequently caused infestation in approximately 60% of all potato growing land in England and Wales. No one gene has been discovered which successfully confers resistance to all three European pathotypes of *G. pallida*. The *H2* gene is considered to be dominant and occur at a single locus within the potato genome. Both phenotypic and genotypic techniques were used and the data presented here shows the progress of the research.

INTRODUCTION

Nematodes are unsegmented roundworms which make up the phylum Nematoda, and are arguably the most widely distributed organism on the planet – occupying every ecological niche (Roberts *et al*., 2012). Most species are non-parasitic and free-living, while some parasitise animals, insects, and plants (Masler, 2013). A major target for parasitic nematodes is plants. They have evolved and diverged to parasitise across taxa and not only have a destructive effect on the plant itself but also on the potential crop yield.

The cyst nematodes are sedentary endoparasites which parasitise many cash crops causing huge yield losses. The most economically important cyst nematodes are considered to be those of the genera *Heterodera* and *Globodera*. From the genera *Heterodera, H. glycines* (soybean cyst nematode) and *H. schachtii* (sugar beet cyst nematode) are of economic interest but *H. avenae* is the most widespread, causing damage to wheat, barley, and oat crops in more than 50% of cereal growing land in Europe (Lilley *et al*., 2005).

The species *G. rostochiensis* and *G. pallida* are prolific and economically important parasitic nematodes of potato crops, having the ability to reduce total yield by 70% (Brown and Sykes, 1983, Greco *et al*., 1982). Damage to infected fields can be prolonged for extended periods of times (tens of years) due to the ability of the cysts to remain dormant in the soil.

When a biotrophic pathogen such as potato cyst nematodes (PCN) invades a host plant, the outcome is determined by whether the host resistance (*R*) gene recognises the pathogen/pest avirulence factor (*avr* gene) (Eitas and Dangl, 2010). In response to pathogen attack and the consequential release of effectors into the host, NB-LRR (nucleotide binding – leucine rich repeat) resistance proteins are key to the plant's defence response (Eitas and Dangl, 2010).
These NB-LRR proteins recognise effector proteins (directly or indirectly) and initiate effector triggered resistance.

A host plant can be referred to as resistant when the level of reproduction of the pathogen or pest is significantly below what would normally be expected for that plant. Kort *et al.* (1977) noted that a single resistance gene could not control all populations of *G. pallida* and so created a pathotype defining scheme; sorting populations based on their ability to multiply on resistant cultivars. In Europe, three pathotypes of *G. pallida* were defined (Pa1, Pa2 and Pa3) (Kort *et al*., 1977). The Pa1 pathotype is distinct from the Pa2/3 pathotypes and it is likely that it was introduced separately into Britain (Phillips and Trudgill, 1983) with introductions into Northern Ireland and Scotland (Blok *et al*., 1997).

Research carried out by Dunnett (1963) using wild diploid *Solanum multidissectum* found resistance which was effective against virulent Pa1 populations. This resistance gene was designated *H2*, and was found to be a major effect, dominant resistance gene (Phillips, 1994). The aim of the current research is to identify the *H2* gene in a resistant cultivar and map its location to the potato genome.

MATERIAL AND METHODS

PCN Inoculum

Cysts from the *G. pallida* (pathotype Pa1) population from The James Hutton Institute PCN collection were used. Viability of the cysts was tested by adding single cysts to 2ml of Potato root diffusate (PRD) in 12-well plates and left to hatch for seven days at 20°C. PRD was produced by cleaning the roots of a potato plant cv. Désirée with water to remove any excess soil and then placing the plant in a beaker with 250mL sterile distilled water for at least 2 hours. Following this, the liquid was filtered through Whatman paper and stored at 4°C. Fifteen cysts were aliquoted into individual glass vials and used for the resistance test.

Plant Material

A cross between susceptible cv. Picasso and resistant cv. P55/7 yielded 192 progeny plants. Cuttings taken from the plants were used in the first screening experiment. Tubers from 154 progeny and 12 control (Picasso, P55/7 and Désirée) plants were harvested and kept in a cold store (4°C) over the winter.

Screening Set-Up

Screen 1 – Summer 2014
Racks containing eight root trainers (4 chambers/root trainer) were filled with compost and alternating rows were inoculated with the Pa1 cysts. Cuttings from all 192 progeny plants were dipped in root growth hormone and planted in the infected wells. Cuttings were kept under plastic covering for the first week to increase humidity. Plants were left to grow for 8 weeks to allow suitable root systems to form and the cysts to hatch and juvenile nematodes to infect the roots. The experiment was replicated three times, with repeats two and three having randomised plant positions.

Screen 2 – Spring 2015
Racks of eight root trainers were filled with compost and alternating rows were inoculated with Pa1 cysts. Tubers from the Picasso x P55/7 progeny were taken from the cold store and allowed to sprout for one week prior to planting. One centimetre square pieces of tuber were cut and planted, sprout down, in infected wells. The experiment was replicated three times, with repeats two and three being randomised. Plants were left to grow for 8 weeks, uncovered.

Control Plants

Screen 1 – Summer 2014
Cuttings were taken from parent Picasso and P55/7 plants, re-potted and infected with Pa1 cysts as positive and negative controls respectively. Cuttings from the susceptible cultivar Désirée were also taken and infected with Pa1 as an additional positive control. Each control had four cuttings, repeated three times, with repeats two and three being randomised. Samples were left to grow for 8 weeks, uncovered.

Screen 2 – Spring 2015
Tubers were removed from cold store and allowed to sprout for one week prior to planting. One centimetre square pieces of tuber were cut and planted, sprout down, in infected wells. Each control had four tubers tested and repeated three times, with repeats two and three being randomised. Samples were left to grow for 8 weeks, uncovered.

Scoring of Females

At 8 weeks, the root trainers were opened to expose the root system. All visible females were counted for each side of the plant; the total was the sum of each side of the plant.

Data Analysis

Histogram results were generated using SigmaPlot 12.5. The "average number of females" data was generated using default settings.

RESULTS

Summer 2014

A total of 192 plants were analysed, P55/7 (resistant parent) gave an average of three females per replicate, but replicate one scored 4. Therefore the threshold for resistance was set at 4, with any plant with an average of <4 being designated resistant and an average count of >5 designated susceptible. Figure 1 shows the segregation of progeny plants. Fourteen plants were excluded from the analysis due to only one repeat out of three yielding any results. The segregation ratio for this material was 0.5:1 (resistant:susceptible) which differs from the 1:1 ratio which would be expected from a normally segregating dominant resistant gene bred from a heterogenic dominant and homogenic recessive tetraploid parent.

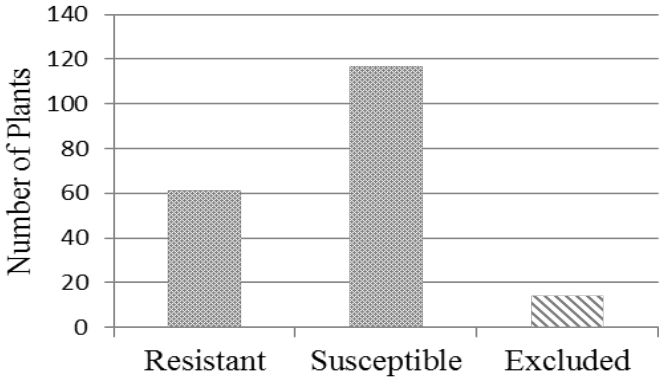

Figure 1. Genetic segregation of progeny plants from the Picasso x P55/7 cross. Based on the number of females present on root systems, plants were designated as either resistant (≤4 females) or susceptible (≥5 females). The excluded column contains fourteen plants which had data <2 replicates.

As the progeny plants did not segregate in the 1:1 ratio as expected, analysis was carried out on the data to analyse the distribution. A bimodal distribution would normally be expected from a cross of this type, but the results in Figure 2 shows a positive left skew; normally associated with the presence of multiple genes acting together

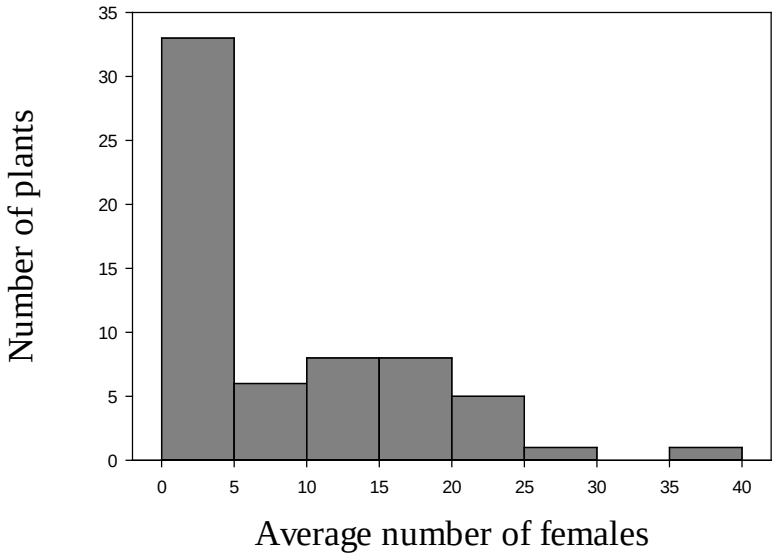

Figure 2. Distribution of 192 progeny plants from the Picasso x P55/7 cross. The expected bimodal distribution of samples is not present, but a positive left skew is observed.

Spring 2015

Too few plants in Summer 2014 gave results for all three replicates, making further analysis very difficult. The data which this experiment did yield was inconclusive, and so the screen was repeated to ensure the progeny from the Picasso x P55/7 could be designated as either resistant or susceptible with confidence. In spring 2015 the experiment was repeated using tubers produced by the progeny plants instead of leaf cuttings as in the first screen. Only 154 progeny plants from the original cross could be tested due to the failure of some plants to produce tubers, or tubers not sprouting once they were removed from the cold store. In line with the average for P55/7 (resistant parent), an average value of ≤ 1 female was required to be defined as resistant, lower than the threshold set in the first screening. A threshold of ≥ 18 females was set for susceptible plants based on the Désirée (susceptible control) average of 18.33. A much higher number of progeny plants were excluded from the analysis based on the more stringent threshold values which were used. The plants which were included in the analysis segregated in a 0.8:1 (resistant: susceptible) ratio, which is closer to the 1:1 ratio which would be expected from this cross (Figure 3).

As with the 2014 experiment, distribution analysis of the progeny plants was carried out. Figure 4 illustrates the positive skew in the data.

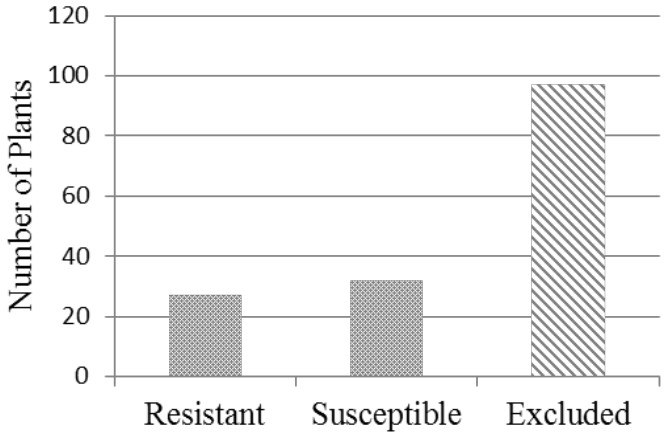

Figure 3. Segregation of progeny plants from Picasso x P55/7 cross. The threshold for resistance was <1 females and >18 females for susceptible, based on the average number of females across the three replicates. Plants which did not fall in either the resistant or susceptible bracket (95 plants) were excluded from the analysis.

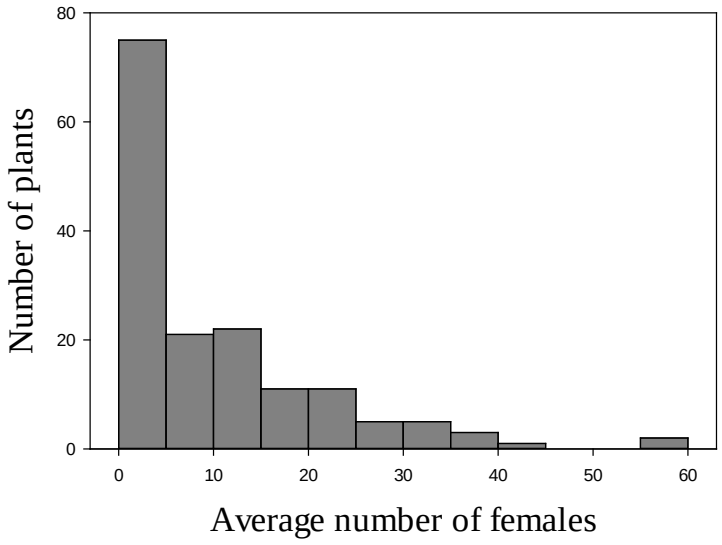

Figure 4. Distribution of 154 progeny plants from Picasso x P55/7 cross. Results differ from the bimodal distribution which would be expected from this cross, and show positive skewing to the left.

DISCUSSION

The experiment was repeated in Spring 2015 because of poor plant growth during the Summer 2014 screen. Using tubers increased the survival rate of the progeny plants and although this led to a drop in samples size (154 from 192 plants) more plants had data for all three replicates. This increase in results led to better data analysis and designation of plants as either resistant or susceptible.

The *H2* resistance gene is widely considered a single dominant gene (Blok and Phillips, 2012). It was however proposed by Dunnett (1961) that it was possible that *H2* resistance was controlled by multiple *R* genes. The change in segregation ratio from 0.5:1 to 0.8:1 (Figures 1 and 3 respectively) was helped greatly by an increase in progeny survival rate in the second screening experiment. The segregation ratio observed in the spring screening experiment highlight that the *H2* gene is indeed acting in a dominant manner.

Neither screen gave the expected distribution results. If these results are correct, it would mean that *H2* is not a single gene but rather multiple genes whose quantitive effect results in Pa1 resistance and would support the hypothesis put forward by Dunnett (Dunnett, 1961).

With the increased confidence which came with the Spring 2015 results, the next step in mapping the *H2* gene is carrying out gene enrichment. DNA from individuals designated as either resistant or susceptible will be pooled with DNA from both Picasso and P55/7 and sequenced on an Illumina platform. SNP (single nucleotide polymorphism) calling will subsequently be carried out and any true alternative alleles which are discovered between the resistant and susceptible pools will be taken forward and focused on as a potential candidate for the *H2* gene.

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MANAGEMENT OF PCN – THE POTENTIAL FOR NEW DIAGNOSTICS AND RESISTANT CULTIVARS

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Summary:
The production of cultivars highly resistant to *Globodera pallida* and reliable, cheap and fast diagnostic tools to assess PCN incidence in field soils are two key factors urgently required to improve management of PCN. In a field trial, four highly resistant cultivars: Arsenal, Innovator, Eurostar and Performer proved very effective in managing PCN. Levels of PCN control equivalent to 1% of the population increase on the susceptible cultivar Maris Piper were achieved. Planting Maris Piper with a standard nematicide programme resulted in PCN populations that were 30-fold greater than populations developing on these resistant cultivars. PCN population levels were assessed using three diagnostic methods: a traditional count of cysts and eggs; a PCR diagnostic developed by SASA using the float extracted from the soil; and the same PCR using isolated cysts. The results demonstrate the considerable potential of the PCR diagnostic for providing reliable assessments of field PCN populations.

INTRODUCTION

Scottish Agronomy (SA), in collaboration with John M. Stevenson Limited, approached SASA to estimate PCN populations for a series of field trials investigating the efficacy of PCN control options available to ware potato growers. Two trials were planned for Mill Field at Luffness in East Lothian for the 2014 season, a site chosen because of an established infestation of *Globodera pallida*. The first trial was a field evaluation of cultivars with high levels of resistance to *G. pallida* which are now available for commercial production in the UK; and the second trial investigated the efficacy of a range of incorporation methods for nematicide treatments (not covered by this paper).

In response to the introduction of the EU PCN Directive (EC, 2007), which came into force in 2010, SASA developed a novel high throughput polymerase chain reaction (PCR) diagnostic for statutory pre-crop PCN tests. This method has now been in routine use for 'qualitative' assessments of the incidence of PCN in Scotland for over five years. The SA field trial provided an opportunity for SASA to evaluate whether the PCR diagnostic could be used to provide a 'quantitative' assessment of PCN incidence. As SASA is also the UK centre for conducting National List assessments of new potato varieties, including assessments of resistance to PCN, the SA field trial also provided the additional benefit of comparing the performance of resistant cultivars in the field with their previously determined performance in controlled pot tests conducted as part of the National Listing procedure.

MATERIALS AND METHODS

Initial Investigation

Mill Field at Luffness was chosen for these trials, a 23.3ha field of which 12.2ha at the eastern side of the field was sampled in February 2014 prior to planting. The field was divided into 11 strips running parallel with the eastern field boundary. Each of these strips was subdivided east to west into four blocks (see Figure 1). The field was sampled according to the SASA protocol for the standard EU rate of 1500ml/ha using a 15ml corer to take approx. 100 cores/ha adopting a 'W' pattern to walk the 1ha strip. These samples were collected as 25 x 15ml cores from each block of just over ¼ha.

Trial Design

Following the initial investigation (Figure 1), parts 4a and 5a were selected for the resistant cultivar trial (and part 7c for the nematicide incorporation trial). These parts of the field were selected as having high populations of *G. pallida* with no *G. rostochiensis*. The trial was planted using a split plot design with seven cultivars and two treatments replicated over four blocks. The cultivars chosen with their National List/Independent Variety Trial scores for resistance to *G. pallida* pathotype 2/3 were: cvs. Maris Piper (2), Osprey (3), Royal (4), Arsenal (9), Innovator (9), Eurostar (8) and Performer (8). Eurostar and Performer were independently tested as part of this study. A score of 2 indicates susceptibility, whereas a score of 9 is indicative of a high level of resistance (relative susceptibility of c. 1%) (EC, 2007). Two treatments were used: untreated and nematicide treated (a pre-planting commercial application of Nemathorin® at 30kg/ha incorporated to a depth of 25cm). Each block of the trial was planted over four beds, with two beds treated and two untreated. The order of planting of the cultivars was randomised between blocks. Each plot was planted over 6 metres and two beds. Overall the cultivar trial consisted of 56 plots covering 86 metres and eight beds. Pre-planting (Pi) and post-harvest (Pf) PCN population assessments were carried out for each plot.

Field Sampling

The plot area in the cultivar trial was 3.6m by 6m. For both pre-planting and post-harvest assessments of PCN incidence, 100 cores were drawn from this relatively small defined area to produce a soil sample of 1200ml. This soil sample was then thoroughly mixed to produce three equivalent 400ml homogenised plot sub-samples, labelled with the suffixes, A, B or C.

Laboratory Analysis

PCN were extracted from dry soil samples using an automated cyst extraction system (carousel) based on traditional principles of sieving and flotation (Fenwick, 1940). The incidence of PCN within each of the A sub-samples was assessed by a visual count of cysts on the 'float' (debris) from the carousel, followed by egg/juvenile counts of the cyst contents. For the B sub-samples, the floats were scraped into Eppendorf tubes and analysed by PCR. For each of the C sub-samples, a visual count of cysts on the float was made and the cysts were placed into Eppendorf tubes and analysed by PCR; so for the C sub-samples the C_t value recorded was obtained without the presence of any non-PCN DNA that could potentially compete with the PCR reaction.

Visual examination of soil floats using low power microscopy was carried out to meet the requirements of the EU PCN Directive. Egg/juvenile counts were determined by crushing extracted PCN cysts and suspending the contents in 100ml of water. The average of six separate counts of eggs and juveniles per 1ml aliquot were then made using a Peters' counting slide. Molecular diagnoses were carried out using the high throughput polymerase chain reaction (PCR) method developed at SASA for pre-crop soil tests for PCN (Reid *et al*., 2010, 2015). A first PCR assay tests for the presence/absence of *Globodera* spp. and a second assay of positive first assay samples provides diagnosis to species. This method was used on the entire 'float' from the carousel (B sub-samples) and on the total extracted cysts (C sub-samples). In a real time PCR assay a positive reaction is characterised by accumulation of a fluorescent signal. The C_t (cycle threshold) values recorded relate to the number of cycles required for the fluorescent signal to reach a threshold level. If more DNA is present in the original sample, fewer cycles are required to amplify the fluorescent signal to reach the threshold. Therefore, C_t levels are inversely proportional to the amount of DNA in the sample.

In summary, the data provided by the laboratory analyses of the trial plot soil samples are cyst counts (sub samples A and C); egg-juvenile counts (A), C_t values from the float (B) and C_t values from the extracted cysts (C).

RESULTS

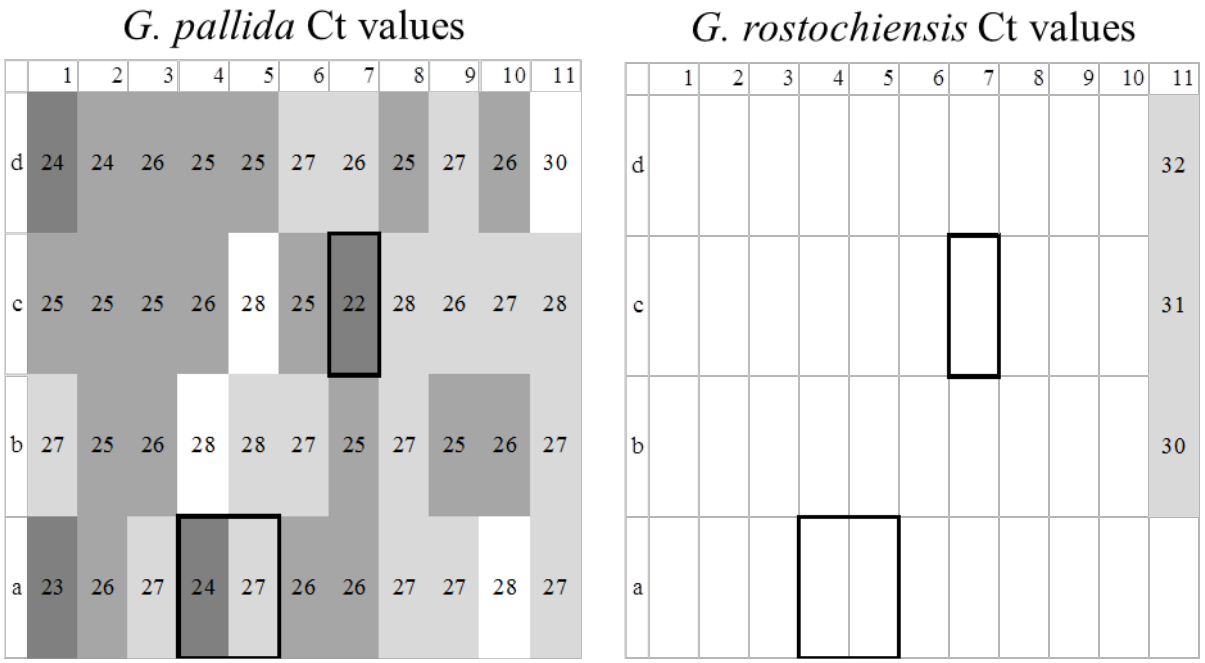

Figure 1. Initial investigation of PCN incidence in Mill Field to determine the site of the field trials. A 12.2 ha area of the field was divided into 11 strips (numbered 1 to 11), each sub-divided into 4 blocks (a-d). C_t values from PCR tests on the 'float' are presented for both species of PCN. Highlighted blocks 4a and 5a were chosen for the cultivar trial and block 7c for the incorporation trial.

The initial survey (Figure 1) revealed a widespread infestation of *G. pallida* throughout the field, with C_t values ranging from 22 in block 7c to 30 in block 11d. In contrast the distribution of *G. rostochiensis* was confined to blocks 11b to 11d. No *G. rostochiensis* was detected in the blocks chosen for the trial either pre-planting or post-harvest.

A summary of the results of the resistant cultivars trial in terms of changes in pre-planting (Pi – initial population) and post-harvest (Pf – final population) cyst counts and egg counts is presented in Table 1. Cyst numbers increased on the susceptible cv. Maris Piper and on cv. Osprey (resistance score 3). The nematicide treatment reduced the increase in both cyst and egg counts: for cv. Maris Piper the egg count increased over 50-fold in the untreated plots, an increase reduced to 15-fold on the treated plots. A similar effect was observed for cyst counts. For cv. Royal (resistance score 4), greater control of the PCN population was achieved on both treated and untreated plots. For the four highly resistant cultivars, cyst counts declined and with the single exception of cv. Eurostar on the untreated plots, so did the egg counts. For such highly resistant cultivars, the effect of the nematicide treatment on PCN control was not evident. Expressing the Pf as a percentage of the Pf for a susceptible cultivar (cv. Maris Piper) provides a value of relative susceptibility. For cvs. Arsenal, Innovator and Performer, this value for the untreated plots is consistent with a resistance score of 9, which equates to a level of PCN control equivalent to 1% of the population increase on a susceptible cultivar.

Table 1. The influence of resistant cultivars on the control of *Globodera pallida* as indicated by changes in cyst and egg counts in untreated and nematicide treated plots.

Untreated

Cultivar	Cysts (Pi)	Cysts (Pf)	Change	Pi (eggs/ml)	Pf (eggs/ml)	Pf/Pi (eggs/ml)	Relative Susceptibility
Maris Piper	123	336	213	3.0	158.9	53.7	100%
Osprey	132	267	135	5.8	125.7	21.6	40%
Royal	136	165	28	7.6	29.9	3.9	7.3%
Arsenal	151	119	-32	7.4	3.5	0.5	0.9%
Eurostar	111	101	-11	2.2	2.8	1.2	2.3%
Innovator	110	100	-10	6.4	1.9	0.3	0.6%
Performer	134	118	-16	5.5	1.6	0.3	0.6%

Treated

Cultivar	Cysts (Pi)	Cysts (Pf)	Change	Pi (eggs/ml)	Pf (eggs/ml)	Pf/Pi (eggs/ml)	Relative Susceptibility
Maris Piper	209	275	66	5.7	90.1	15.8	100%
Osprey	240	298	58	8.9	133.4	14.9	95%
Royal	204	199	-5	7.8	14.2	1.8	12%
Arsenal	160	157	-3	8.1	4.9	0.6	4%
Eurostar	170	148	-22	5.5	3.4	0.6	4%
Innovator	161	160	-1	6.7	4.2	0.6	4%
Performer	163	146	-17	7.8	3.4	0.4	3%

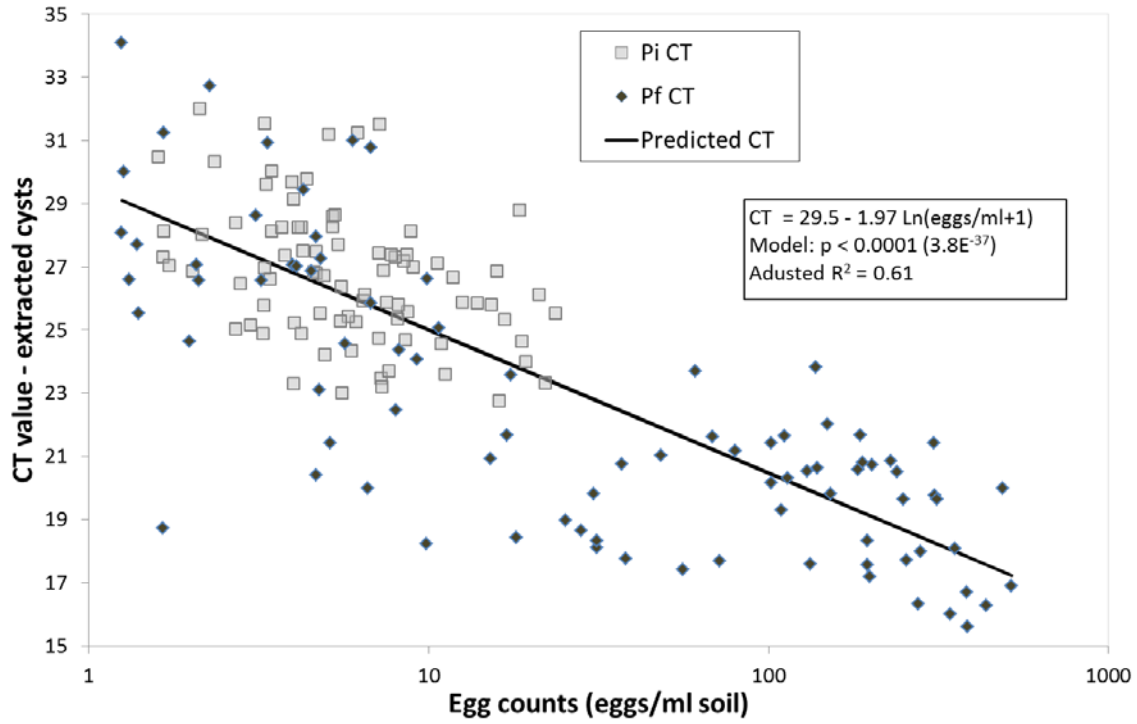

Figure 2. The relationship between the C_t values from extracted cysts (C sub-samples) and egg counts (A sub-samples).

Using the PCR diagnostic to obtain C_t values for both extracted cysts (C sub-samples) and cysts within floats (B sub-samples), highly significant relationships with egg counts were obtained (see Figure 2). These relationships were then used to provide an estimate of Pf egg counts from individual C_t values, and hence an estimate of Relative Susceptibility. These later values were converted into a resistance score for each cultivar (see Table 2). The results show a high level of consistency in terms of resistance scores calculated with identical scores obtained using direct egg counts and the PCR method on the cysts within the float (B sub-samples). The only marked discrepancy was obtained for cv. Royal using PCR on extracted cysts.

Table 2. Field trial estimates of cultivar resistance scores to *Globodera pallida* calculated using direct counts of eggs (A), PCR on extracted cysts (C) and PCR on cysts within float debris (B). A mean of these three scores and the resistance score from National List/pot tests is shown. Scores are only presented for data from the untreated plots.

Cultivar	Egg Count (Sub-sample A)	Extracted Cysts (Sub-sample C)	Cysts & Float (Sub-sample B)	Mean Score	Pot Test Score
Maris Piper	2	2	2	2	2
Osprey	3	2	3	3	3
Royal	6	2	6	5	4
Arsenal	9	8	9	9	9
Eurostar	8	7	8	8	8
Innovator	9	9	9	9	9
Performer	9	8	9	9	8

DISCUSSION

The cultivation of highly resistant cultivars had a major impact on the PCN population dynamics, with all four cultivars limiting PCN multiplication to around 1% of the increase achieved on susceptible cv. Maris Piper. The trial also demonstrated that the levels of control achieved with a nematicide treatment are far less effective in terms of PCN management: *G. pallida* still increased 15-fold on the Nemathorin® treated plots, albeit below the > 50-fold increase on the untreated plots. The effect of the treatment on PCN populations in combination with a highly resistant cultivar was negligible. In another aspect of this trial, no clear benefit of treatment in terms of yield was recorded. As resistance scores of cultivars are assessed on reference populations of PCN under controlled conditions in 1-litre pots, it is very encouraging to confirm that these highly resistant cultivars perform in a very similar manner when challenged with field PCN populations under field conditions.

The use of the three different methods to assess the incidence of *G. pallida* populations in the field trial plots has clearly demonstrated the potential to use the PCR method as a quantitative assay. Whilst a highly significant relationship between the PCR C_t value was obtained using cysts extracted from the float (Figure 2), a better relationship was obtained without extracting the cysts, but only for post-harvest samples (Pf) (data not shown). The relationship for pre-planting samples without extracting the cysts was poor. Why this should have been requires further investigation. However, given the amount of laboratory time required to complete cyst and egg counts, compared with the rapid and therefore much cheaper PCR method, the latter method has great potential to provide potato growers with much needed information on PCN infestations. As highlighted by the initial survey of Mill Field the standard PCR method provided a reliable assessment of the incidence of both species of PCN throughout the field. Such a level of quantification of both species is not feasible with conventional egg counts.

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DEVELOPMENT OF A RELIABLE METHOD FOR DETECTING *ALTERNARIA* SPP. IN POTATO CROPS

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Summary: The fungal pathogens *Alternaria alternata* and *A. solani* have caused increasing damage to potato crops across Britain. Visual symptoms are difficult to distinguish on crops. A number of diagnostic tests have been proposed for these species but results have been variable. This poster describes the optimisation of 2 assays to distinguish between the species and how the tests can differentiate between species on spore tapes and infected leaf material.

INTRODUCTION

Infection of potato crops by *Alternaria* spp. is becoming an increasing problem for growers in Europe and also the UK (AHDB, 2014). Early blight, caused by the fungus *Alternaria solani* has been a problem in many warmer continents (Africa, Asia and North America) but is increasing in its geographical range (AHDB, 2014). *A. solani* also attacks tomatoes and other crops in the US (Weir *et al.,* 1998). The appearance of symptoms in crops has been related to hot, dry growing conditions and periods of leaf wetness. Control measures differ between these two species as resistance issues are a problem for both. Resistance to the SDHI fungicide, boscalid (Tracker®) has been reported for *A. solani* (Gudmestad *et al.*, 2013) and resistance to the strobilurin fungicide, azoxystrobin (Amistar®) has been also been recognised (Karaoglanidis *et al.,* 2011). Therefore, effective control relies on accurate detection. Control sprays are generally integrated into late blight spray programmes (AHDB, 2014). A number of unpublished and published primers were tested for the detection of both species. Leaf samples were collected from SRUC potato trial sites for testing and in addition extracted DNA from spore samplers at SRUC trial sites was tested.

MATERIALS AND METHODS

Validation of primers

Pure cultures of *A. alternata* and *A. solani* were obtained from Dr Jurgen Leiminger from the Technical University of Munich to provide positive samples for the PCR Quick-DNA^{TM} Universal Kit.

Plant Material

A number of samples were collected from SRUC potato trials at Auchincruive, near Ayr. Leaves exhibiting necrotic lesions were tested in the laboratory for the presence of *Alternaria* spp.

Spore sampler tapes

A Burkard seven day spore sampler was run at Auchincruive. 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 by Fountaine *et al.* (2007).

DNA Extraction

Genomic DNA from plant material was extracted using both REDExtract-N-Amp (XNAPS, Sigma-Aldrich) and Quick-DNA^{TM} Universal Kit (Zymo Research, Germany) according to the manufacture protocol. For every sample, the DNA concentration was measured with the NanoDrop (Thermo Scientific, Wilmington, USA).

PCR testing of extracted DNA

For genomic DNA extracted from plant material, standard PCR was carried out using two master mixes REDExtract-N-Amp PCR mix and VWR Red Taq DNA Polymerase master mix (VWR, Belgium). For REDExtract-N-Amp, 0.2µM primers (Table 1) and 4µl tissue extract was used in a final volume 20µl. Amplification of 119bp (*A. solani*) and 122bp (*A. alternata*) was performed in a Biometra TProfessional Standard thermocycler (Biometra GmbH, Germany) under the following conditions: 94°C for 3min, followed by 30 cycles at 94°C for 30sec, 60°C for 30sec, 72°C for 1min and 1 cycle at 72°C for 10min. A 25µl final volume reaction was performed for VWR Red Taq master mix with 0.2µl each of the primer (Table 1) The PCR conditions were 95°C for 2min; followed by 30 cycles at 95°C for 20sec, 60°C for 30sec, 72°C for 2min; with a final extension at 72°C for 5min. GoTaq® green master mix (Promega, USA) was used to amplify DNA extracted from spore tapes; each reaction contained 0.4µl of primers (Table 1) in a final reaction volume of 20µl. PCR conditions were: 95°C for 2min, followed by 35 cycles at 95°C for 30sec, 60°C for 30sec, 73°C for 1min and one cycle at 73°C for 5 min. Amplified genomic DNA was loaded directly onto a 1.2% agarose gel after the PCR reaction was completed.

Table 1. Primer sets used in *Alternaria* spp. testing

Target Species	Sequence (5' – 3')	Product size	Source
A.alternata	For:TGGAACCTCTCGGGGTTACA Rev:CTGATTGCAATTACAAAAGGTTTATGTT	122bp	Lees *et al.* unpublished
A. solani	For:GGTGTTGGGCGTCTTTTTG Rev:GCTAGACCTTGGGGCTGGA	119bp	Lees *et al.* unpublished
A. solani	For:TCCGTAGCTGAACCTGCG Rev:TGGGTTGGTCCTTGTGGTG	152bp	Leiminger et al.,2015

Table1 shows the primers used in this study. Primers were obtained from Dr Alison Lees (JHI) and Leiminger *et al*. (2015).

RESULTS

Table 2. *Alternaria* spp. results in spore tape samples (2012-13)

Year	Site	Species detected	Date of first positive result	No of positive tests
2012	Auchincruive	*A.alternata*	09-Aug-15	24
2012	Midlothian	*A.alternata*	29-May-12	5
2012	Lanark	*A.alternata*	-	0
2013	Auchincruive	*A.alternata*	18-Jul-13	26

The results indicate that *A. alternata* can be detected in the environment from May onwards. The site with potato trials did not produce positive samples until July. No spores were detected at the Lanark site. In 2012, the first detection of *Alternaria* took place in August. In 2013, spores of *A. alternata* were detected in mid July at the Auchincruive site.

Of the leaf samples tested from Auchincruive, 100% were positive for *A. alternata* in 2014 and 50% were positive for the same fungus in 2015. No *A. solani* was detected in either year. The PCR assays successfully detected DNA isolated from pure fungal cultures. No cross reactions were observed between assays.

DISCUSSION

The results from the primer testing showed that the primers from Dr Alison Lees and Dr Jurgen Leiminger were capable of detecting both species in our laboratory. Testing of leaf samples showed that symptoms which appeared at the Auchincruive trial site were produced by *A. alternata.* Testing of spore tapes showed that spores of *A. alternata* could be detected in summer at a number of sites in Scotland. The mean air temperature rose to over 20 °C in the week of the spore detection and the crops were wet in the morning. A similar pattern was seen in Auchincruive in 2013, where spore detection follows a steady rise in temperatures to over 25 °C. In comparison commercial spore tape samples in England showed detection of *A. alternata* in late June. *A solani* was detected in one site in England in 2013 but not in any site in Scotland.

The availability of a reliable test for the 2 species will help growers accurately identify the cause of symptoms in crops and adjust fungicide programmes accordingly.

ACKNOWLEDGEMENTS

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INVESTIGATION OF THE COMPOSITION OF *GLOBODERA PALLIDA* IN UK FIELDS

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Summary: Potatoes are a very important food crop, so controlling pests and diseases is essential and requires ongoing monitoring of populations of pests and diseases to ensure that appropriate control strategies are employed. The potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* cause significant economic losses each year in the UK. At the James Hutton Institute (JHI), populations of *G. pallida*, representing the pathotypes Pa1 and Pa2/Pa3, have been collected from different sites in the UK during more than 50 years. Using a particular molecular technique, Terminal Restriction Fragment Length Polymorphism (T-RFLP), we can distinguish between populations representing these pathotypes, and this method was used to determine the composition of introductions from different field populations. Interestingly, a change in the composition in populations collected from the same field was observed, with a more complex composition in the recently collected population, indicating a potential mixture of pathotypes.

INTRODUCTION

Potato is the 4th most important global food crop, with more than 6 million tons of tubers produced per year in the UK. Controlling pests and diseases of this crop is challenging, and requires ongoing monitoring of populations of pests and diseases to ensure appropriate control strategies are employed. The potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* cause annual losses in the UK estimated at £50M/year (DEFRA, 2010). Genetic studies have shown that there have been three distinct introductions of *G. pallida* from South America into Europe (Plantard *et al*., 2008, Hockland *et al*., 2012), however the distribution of these introductions has not previously been examined in the UK. Populations of PCN in the JHI collection were collected from different sites in the UK during a period of more than 50 years and include populations that represent *G. pallida* pathotypes Pa1, Pa2/Pa3 (Phillips and Trudgill, 1998). In order to determine the composition of introductions in different populations and to investigate if there are any novel introductions, a Terminal Restriction Fragment Length Polymorphism (T-RFLP) assay was used with individual PCN cysts. This T-RFLP assay is based on mitochondrial DNA, which is maternally inherited (Gibson *et al.*, 2007).

MATERIAL AND METHODS

PCN Populations:

The following *G. pallida* populations were used:

Table 1. PCN populations investigated in this study

Name of population and year multiplied or sampled	Sample type	Number of cysts or nematodes tested
JHI collection, samples collected >50 years ago		
Lindley[1] (2010)	Single cyst	20
Luffness[2] (2011) Field 1	Single cyst	8
Luffness[2] (2011) Field 1	Single nematodes	10
Luffness[2] (2012) Field 1	Single cyst	6
Pa1 (B) [2] (2011)	Single cyst	10
Farcet[1] (2010)	Single cyst	7
Halton[1] (2010)	Single cyst	8
Newton[1] (2010)	Single cyst	10
Bedale[1] (2010)	Single cyst	7
Recent field samples		
Luffness (East Lothian[2]) (2010) Field 1*	Single cyst	5
Luffness (East Lothian[2]) (2014) Field 1*	Single nematodes	10
Harper Adams (Shropshire[1]) (2011)	Single cyst	9
Harper Adams (Shropshire[1]) (2012)	Single cyst	8
East Lothian[2] 1 (2013)	Single cyst	8
East Lothian[2] 2 (2013)	Single cyst	6
East Lothian[2] 3 (2013)	Single cyst	7
Ash (Shropshire[1]) (2010)	Single cyst	2
Crow (Shropshire[1]) (2010)	Single cyst	2
Bourne (Lincolnshire[1]) (2013)	Single cyst	12
Legge (Norfolk[1]) (2013)	Single cyst	6
Chinn (Herefordshire[1]) (2010)	Single cyst	5
Spalding (Lincolnshire[1]) (2013)	Single cyst	10

[1] England, [2] Scotland, * Cysts were collected from the same field as in JHI collection

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP allows the study of the composition of PCN populations, based on PCR amplification of variants of mitochondrial DNA of the nematodes or cysts with dye-labelled PCR primers, followed by a digest with a restriction enzyme and separating and analysing the DNA fragments.

DNA Extraction:
Cysts: Individual cysts were selected under a low power microscope with tweezers and placed into a 1.5ml Eppendorf tube. Single cysts were crushed with a plastic pestle in 30µl of MicroLYSIS®-Plus buffer (Microzone) for 2-3 min, centrifuged at 13,000 rpm for 90 sec then the supernatant was transferred to a 0.2ml PCR tube and heated to 65ºC for 15 min, 96ºC for 2

min, 65ºC for 4 min, 96ºC for 1 min, 65ºC for 1 min, 96ºC for 30 sec, 20ºC hold, then stored at -20ºC.

Single nematodes: Individual cysts were put in a 12 well plate in tomato root diffusate at 20°C, until J2 nematodes hatched. Individual nematodes were then transferred to a 0.2µl PCR tube containing 20µl 1x PCR buffer (Promega) with 10mg/ml Proteinase K (Roche, P6556). A few sterile glass beads were added, and the nematodes were shaken for 30 sec in a shaker (RETSCH), then incubated for 3 cycles of 95ºC for 1min, 65ºC for 1 h, 15ºC for 2 min. The tube was then centrifuged at 13,000 rpm for 90 sec and the supernatant, containing the DNA of the single nematode, was transferred to a fresh PCR tube.

PCR:
The PCR reactions contained 1.5µl 10x HF buffer (Invitrogen), 0.6µl of each of the primers F3 mtDNA-222(FAM) (5'-ATT AGA CCG ATA AGT TTA CAC CTT G-3)' and SCMT4-8(HEX) (5'-GAC TAG GTC CAT CAA TCT GAA CC-3') (10µM), 0.6µl $MgSO_4$ (50mM), 1.0µl dNTPs (2mM), 0.6µl BSA (10mg/ml), 0.2µl Platinum Taq Polymerase (Invitrogen), 8.9µl H_2O and 1.0 µl DNA. These were heated to 94°C 2 min, 94° 30 sec 55°C 30 sec 68°C 60 sec for 35 cycles for plasmid template and 40 cycles with DNA extracted from single cysts and single juveniles, 68°C 10 min.

Digestion with Restriction Enzyme:
A 1µl aliquot of master mix comprised of 0.1µl MULTI-CORE®buffer, 0.8µl H_2O, 0.1 ul restriction enzyme Taq1 (Promega) per reaction was transferred to each well of a 96 well PCR plate and then 5µl fluorescent PCR product was added to each well, mixed, and briefly centrifuged. Reactions were digested for 4 h at 65°C and then frozen at -20°C or processed to the next step.

T-RFLP Electrophoresis and Evaluation:
A master mix comprised of 895µl formamide (Sigma) and 5µl ROX1000 marker (GeneScan™ 401098) was mixed and 9µl added to each well of a 96 well plate (AB600); then 1µl of the enzyme digestion was added to each well (depending on amount of DNA, a dilution of up to 1 in 100 was used). The plate was transferred to the JHI sequencing facility and run on an ABI micro-capillary gel (Applied Biosystems) with laser detection. The fluorescent reads were analysed by Genemapper software v3.7.

RESULTS

T-RFLP patterns from single cysts from 7 PCN populations from the JHI collection, and 13 recently collected field samples from Scotland and England were examined. Cysts from populations from the JHI collection generally produced more uniform T-RFLP patterns compared to the patterns of recently collected field cyst. Also the patterns from Lindley, Luffness and Pa1 from the JHI collection were different from each other. We observed a change in the composition in the Luffness population from the collection and that collected recently from the same field with a more complex composition in the recently collected population. The T-RFLP pattern associated with the Pa1 population was found in cysts from the recent Luffness Field 1 samples but not in the Luffness JHI collection samples.

Table 2. Results from T-RFLP analysis for different PCN populations for single cysts

Name of Population and year multiplied or sampled	**Number of Cysts**	**Number of T-RFLP patterns (no. of cysts with any pattern)**
JHI collection, samples collected >50 years ago		
Lindley (2010)	20	2 (19+1)
Luffness (2011) Field 1	8	2 (3+8)
Luffness (2012) Field 1	3	2 (1+2)
Pa1 (B) (2011)	10	1
Farcet (2012)	7	1
Halton (2012)	8	1
Newton (2012)	9	2 (5+4)
Bedale (2010)	7	2 (6+1)
Field samples		
Luffness (2010) Field 1	6	3 (2+3+1) **Note: T-RFLP pattern differs from Luffness cysts of the JHI collection**
Harper Adams (2011)	8	3 (2+2+4)
Harper Adams (2012)	7	3 (1+3+3)
East Lothian 1 (2013)	8	1
East Lothian 2 (2013)	6	3 (3+2+1)
East Lothian 3 (2013)	6	4 (2+2+1+1)
Ash (2010)	2	2
Crow (2010)	2	2
Bourne (2013)	12	2 (11+1)
Legge (2013)	7	3 (5+1+1)
Chinn (2010)	5	2 (4+1)
Spalding (2013)	10	2 (7+3)

To test if there is leakage from the paternal mitochondria (in case this is different from the maternal), we performed a T-RFLP assay on DNA from individual juvenile nematodes from one cyst from Luffness Field 1 from the JHI collection and the field. So far we have not observed differences of individual nematodes within a cyst.

DISCUSSION

These results indicate that current field populations of *G. pallida* in the UK typically are composed of more than one introduction from South America. It cannot be determined how this occurred as we did not have historical and new samples from the same fields in the UK, other than from Luffness. However, a comparison of JHI PCN collection samples which were collected >50 years ago, with those collected within <5 years suggests that the composition of field populations has become more complex over time, though we cannot discount that this change in composition has occurred during the propagation of the historical samples over time. This increasing complexity in field samples raises the possibility of hybridisation between the different genotypes of *G. pallida* that coexist in the same field and the potential for the generation of novel genotypes with new phenotypes. A reassessment of the phenotypes of current UK field populations is needed to ensure that resistance used in potato breeding

programs is suitable and will provide broad-spectrum and durable resistance for *G. pallida*. This is particularly relevant to ensure the durability of new potato cultivars with high levels of resistance to *G. pallida* which are now available in the UK.

Although only a limited number of individual nematodes were tested for potential leakage of paternal mitochondrial DNA, this was previously investigated (Hoolahan *et al*. 2012) and it would appear to be a relatively rare event.

ACKNOWLEDGEMENTS

Access to the Luffness site by Allan Stevenson and Geert Knottenbelt and the sites of other anonymous growers in East Lothian is acknowledged. We also acknowledge the provision of samples by Andy Barker and Jeremy Cartwright and the technical assistance of students and staff at The James Hutton Institute and Harper Adams University. This work was supported with a PhD studentship from AHDB–Potato and The James Hutton Institute received funding from the Scottish Government for this research.

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THE EFFECT OF INCREASED SOIL ORGANIC MATTER ON SEVERITY OF DISEASE CAUSED BY *RHIZOCTONIA SOLANI* AG3 ON POTATO

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Summary: The impact of incorporating a typical source of organic matter (OM) used in Scottish Agriculture on potato disease caused by *R. solani* AG3 was investigated in a field trial. Increasing soil OM in plots which had been infested with *R. solani* prior to planting, resulted in delayed emergence (11 weeks cf. 7 weeks), and significantly lower tuber yields (3.2 cf. 18.6 kg/plot, in high and low OM plots respectively). Additional soil OM increased the incidence (45% cf. 19%) but not severity of black scurf on progeny tubers in plots infested with *R. solani*, but had no effect on emergence and tuber yield in un-infested control plots. Therefore, in this study, the main effect of increased soil OM was to delay emergence when soil was infested with *R.solani*, and this delay had a subsequent impact on tuber yield. The results are discussed in relation to additional studies looking at the impact of soil OM on *R. solani* survival in soil through a typical rotation.

INTRODUCTION

The incorporation of organic matter into field soil has been reported to both increase and decrease severity of disease caused by soil-borne pathogenic fungi; see review by Noble (2011). This is reportedly due to differences in the composition of the OM which may possess antagonistic properties and thus repress disease, or alternatively provide a substrate for increased pathogen growth. In this study we investigated the impact of increasing soil organic matter, with a typical source used in Scottish Agriculture, on crop emergence and subsequent yield and disease on progeny tubers in field soil infested with *R. solani* AG3 (hereafter referred to as *R. solani*).

MATERIALS AND METHODS

Field Trial

A field was selected which was naturally very low in OM; 0.2 g OM per kg of soil. A split plot design was created, with high and low OM areas of the field as main plots. A high level of soil OM was created with the incorporation of cattle manure mixed with barley straw (approximately 35 t/ha) which increased the OM to 0.5 g per kg, which whilst being referred to as high, is in fact, still relatively low. Within each main-plot, plots were either infested with *R. solani* or un-infested (control), there were four replicate blocks.

To infest plots with *R. solani*, sclerotia were prepared by inoculating potato dextrose agar (PDA) plates with a single isolate, AG3PT Rs08 (Fera collection), which were then incubated in the dark at 20°C for 6 weeks. Sclerotia were removed from plates using a scalpel blade, and then left to air dry at room temperature for two days. Batches of 5 g sclerotia mixed with vermiculite were spread along each of the prepared drills in the infested treatment plots, seed tubers were then placed along the drill in direct contact with the inoculum. The inoculum was incorporated into the soil at planting depth when the seed potatoes were mechanically covered. No inoculum was added to the soil in the control (un-infested) plots. Mini-tubers of the cultivar Markies were used (no official resistance rating available, but considered relatively susceptible to black scurf). Twenty five tubers were peeled and assessed individually for *R. solani* contamination using real-time PCR, which confirmed absence of infection. Each plot consisted of a single row of 13 plants with a guard at either end, surrounded by a guard row. Irrigation and crop management were as for a commercial crop. Emergence was recorded at intervals of 2 to 3 days. Mid-season (14 weeks after planting) a single plant per plot was carefully dug up and the number of stems, stolons and pruned stolons recorded. At final harvest, the surface area of black scurf on progeny tubers was recorded using the black scurf severity key from Woodhall *et al*. (2008); 0; no sclerotia present, 1; less than 1% of the tuber surface area covered in sclerotia, 2; 1 to 10%, 3; 11 to 20%, 4; 21 to 50% and 5; 51% or more.

Soil samples

Soil samples were collected by taking the top 10cm of soil from at least 100 points in a W-shape across the selected portion of the field to give a total of approximately 1 kg. Two sets of soil samples were taken prior to the trial being planted, the first was taken to establish that the trial area had no detectable inoculum, the second to ensure that no inoculum was introduced with the addition of organic matter into the split plot design. A week after planting, a soil sample was taken from each of the plots (consisting of a bulk of 25 cores). Similarly, soil samples were taken from each plot during the growing season (14 weeks after planting) and immediately after the final harvest.

To establish if the incorporation of additional OM into the soil affected the persistence of inoculum, soil samples (a bulk of 100 cores) were taken from each of the two main-plot areas 10 and 18 weeks post-harvest.

Real-time PCR: quantification of soil and tuber inoculum

Tuber peel and soil DNA extractions were carried out according to the methods of Cullen *et al.* (2001) and Brierley *et al.* (2009) respectively and the amount of *R. solani* AG3 DNA detected using the assay of Lees *et al.* (2002) was expressed as ng DNA /ml tuber sap or pg DNA /g soil.

RESULTS

Emergence and mid-season sampling

Within 5 weeks of planting the control treatments in both the low- and high-OM plots had reached over 95 % emergence. The time taken to reach near complete emergence was progressively longer in infested plots with low OM and high OM respectively, with the later

taking over 11 weeks from planting to reach 90% emergence (Figure 1). The number of main and secondary stems and the total number of stolons per plant did not differ significantly different between treatments. No stolon pruning was observed in plants from the control treatments: however, the proportion of stolons which had been pruned was significantly higher ($P<0.05$) in plants from infested plots with high OM than in plants from infested plots with low OM (0.45 and 0.11 respectively); data not shown.

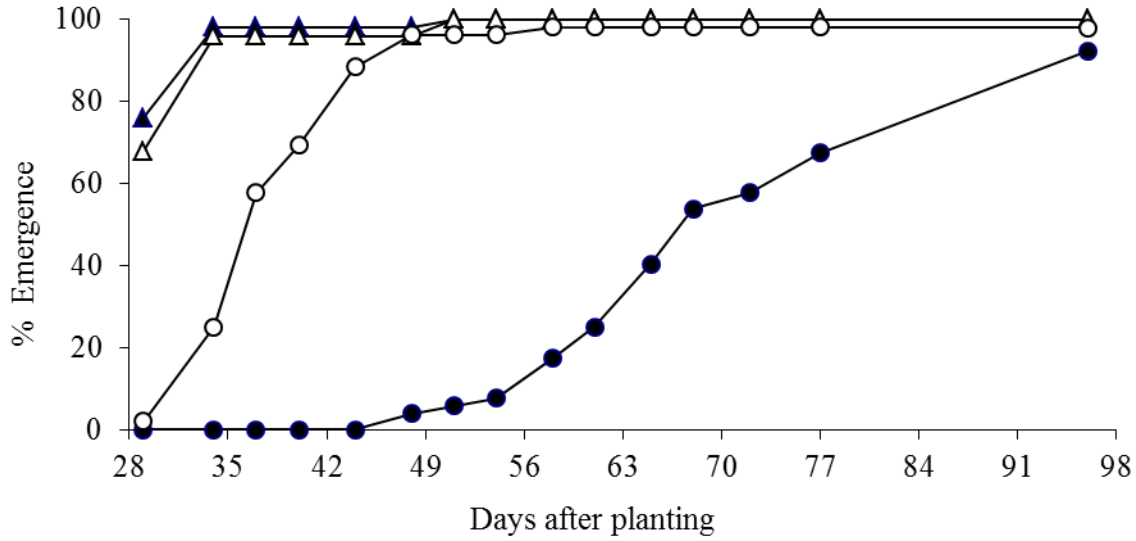

Figure 1. Percentage of plants emerged over time (days after planting) in un-infested control plots with low OM Δ and high OM ▲, and *R. solani* infested plots with low ○ OM and high ● OM. Mean of four replicate plots per treatment.

Tuber disease and yield

Black scurf (both incidence and severity) on progeny tubers was negligible in control (un-infested) plots (Figure 2A and B). Progeny tubers from high OM plots which had been infested with *R. solani* had a significantly ($P<0.05$) higher incidence and severity of black scurf than control plots (Figure 2). Additional soil OM significantly increased the incidence (45% cf. 19%) but not severity of black scurf on tubers grown in plots infested with *R.* solani (Figure 2).

The yield of tubers was significantly lower in treatments which had been infested with *R. solani* compared to control plots ($P<0.05$); however, the yield reduction was much less from infested plots with low OM than from plots with high-OM (Figure 3), reflecting the difference in time taken to reach near complete emergence between the treatments. The reduced tuber yields from infested plots were associated with variations in tuber size distribution (Figure 4); such that progeny from infested plots in the high-OM treatments were fewer in number and predominantly in the smaller (<45 mm) size class, whilst tubers from the infested plots in the low OM treatments were more numerous and predominantly in the 45-65 mm size class. The tuber size distribution from un-infested plots with low and high OM were very similar (Figure 4) with the majority of tubers being in the 65-85 mm size class.

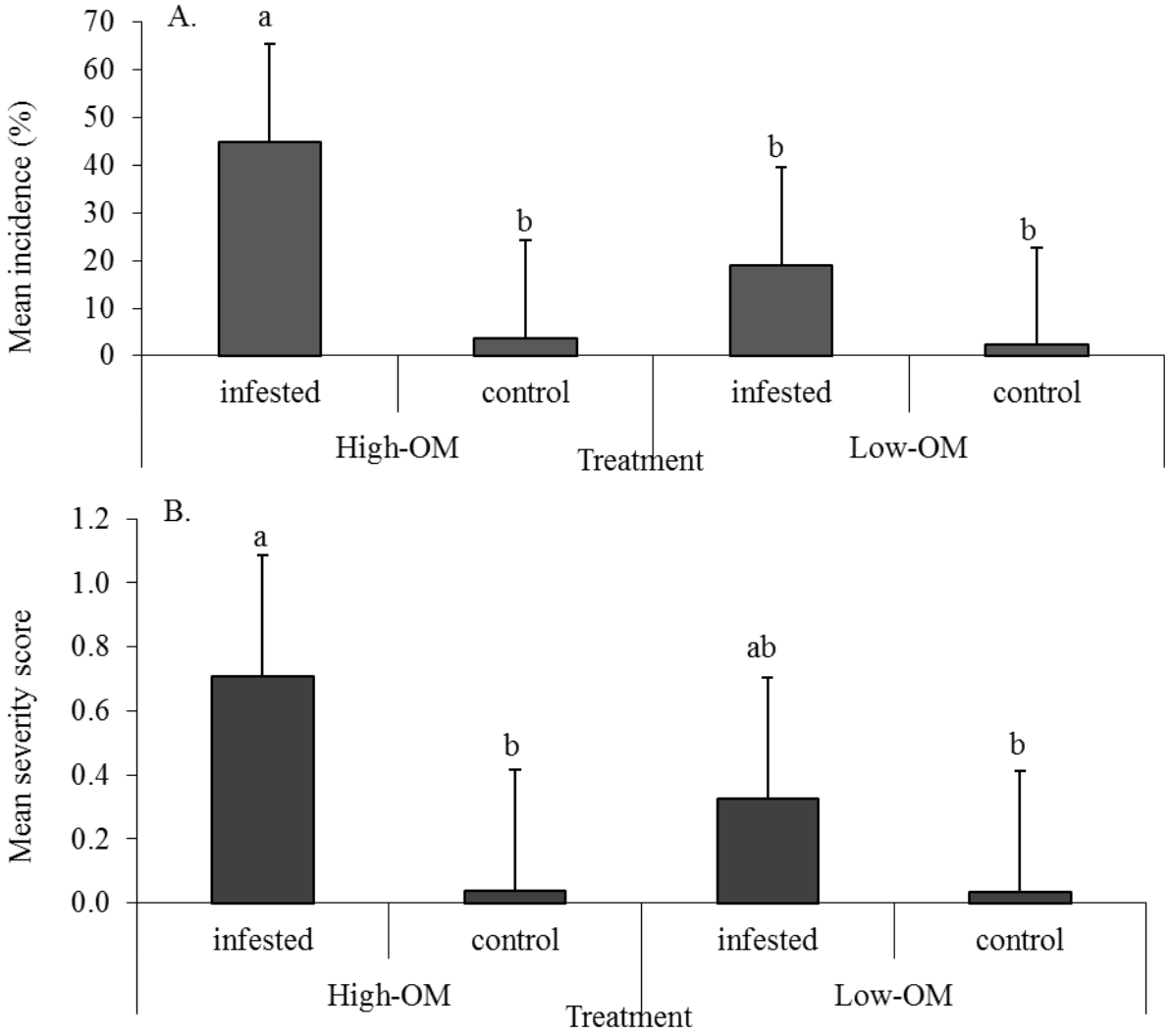

Figure 2. The effect of soil infestation with *R. solani* and the level of OM on A. the incidence and B. the severity of black scurf on progeny tubers at final harvest (mean of four replicates; bars represent lsd). Treatments not sharing the same letter are significantly different ($P<0.05$).

Soil samples

Inoculum was detected in very few soil samples on any of the sampling occasions. No inoculum was detected in the samples taken 1 week after planting or during the growing season. Immediately post-harvest, inoculum was detected in only a single plot, a high-OM soil amended with sclerotia.

In the soil samples taken from each of the main-plots in December and February after the trial was harvested, only a trace amount of inoculum was detected in two samples; in December this was in the low-OM main-plot, and in February in the high-OM main-plot. Therefore the results indicate that very low levels of inoculum (generally below the threshold of detection) were present in the soil both during the trial and in the months following harvest, but increasing the level of soil OM did not have an effect on the amount of inoculum detected.

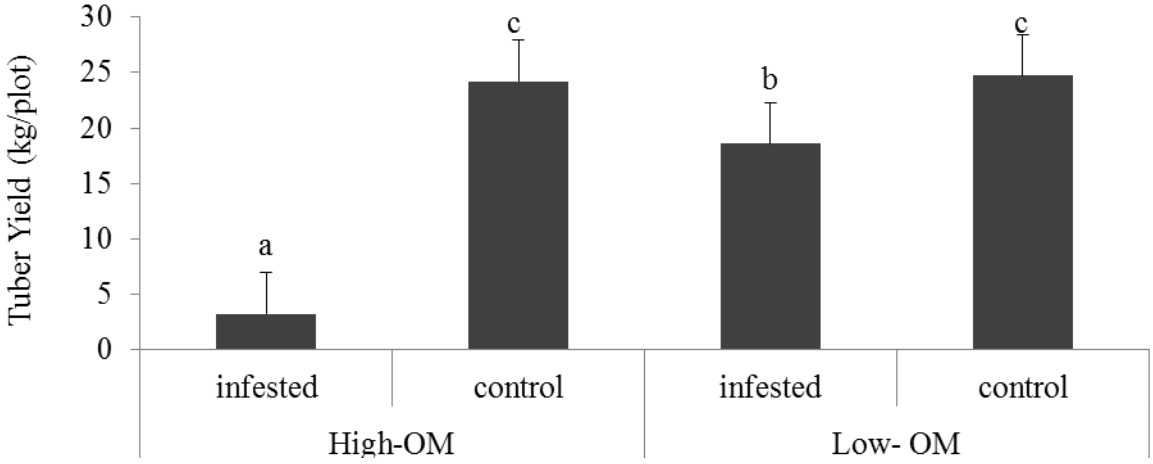

Figure 3. The effect of soil infestation with *R. solani* AG3 and the level of OM on the yield of tubers (kg per plot) at final harvest (mean of four replicate plots; bars represent lsd). Treatments with different letters are significantly different (P<0.05).

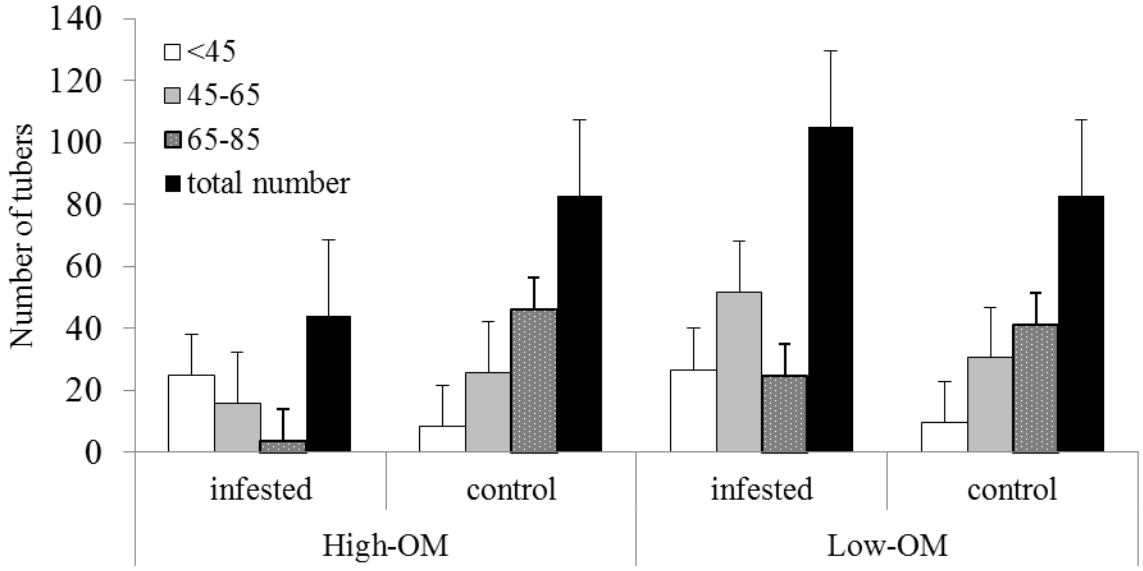

Figure 4. The effect of soil infestation with *R. solani* and the level of OM on the number of tubers in three different size grades (<45, 45-65 and 65-85 mm) at final harvest (mean of four replicate plots; bars represent lsd).

DISCUSSION

Early infection of newly emerging stolons and stems with *R. solani* can result in lesions, referred to as canker, and in more severe infections this results in girdling and pruning. Infection results in a delay in emergence and generally results in a decrease in tuber yield (Carling *et al.*, 1989) and can cause a greater number of non-target tuber sizes (Simons and Gilligan, 1997). The delayed emergence, evidence of stolon pruning and reduced yield in

infested treatments in this trial are in agreement with symptoms of early season infection. The effects were exacerbated in plots into which OM had been incorporated. This indicates the additional OM acted as a food source for the inoculum source used in this trial, and subsequently increased the severity of infection symptoms. The yield reduction in the high OM infested plots was quite severe, but had the tubers been left in ground longer they may have continued to bulk, thereby decreasing the apparent impact on yield.

The addition of soil-borne *R. solani* inoculum caused black scurf symptoms on progeny tubers, although the severity was generally low even in the infested high OM plots (mean severity score 0.7 on a scale of 1 to 5 of increasing severity). However, detection of inoculum in plots was rare, even shortly after the incorporation of sclerotia into the soil and post-harvest. Brierley *et al*. (2014) found that when black scurf developed on progeny tubers (mean severity equivalent to a score of 2) in a field crop, the soil remained infested with *R. solani* at detectable levels 5 months after harvest, but no inoculum was detected when soil was re- sampled 7 months later, indicating that inoculum is either short lived in the soil, or survives at levels below the threshold of detection. The findings reported by Brierley *et al*. (2014) are part of a long term study looking at the introduction of soil-borne pathogens of potato, including *R. solani*, into uncontaminated soils via contaminated seed and how this impacts on progeny disease and amounts of detectable inoculum in future years. This research will also investigate the impact of incorporating municipal compost into field soils on the introduction and persistence of soil-borne potato pathogens through a six year rotation.

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THE RELATIVE EFFECT OF SEED AND SOIL-BORNE INOCULUM ON DRY ROT IN POTATO

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Summary: The relative importance of seed- and soil-borne inoculum of three *Fusarium* species (*F. coeruleum, F. sambucinum* and *F. avenaceum*) was investigated in a glasshouse pot trial carried out in 2014 using cultivars Asterix and Saturna. Clean seed tubers were inoculated with either low or high inoculum suspension (low: 10^2 conidia ml^{-1}, high: 10^5 conidia ml^{-1}) and planted in compost to which either a low or high inoculum suspension had previously been incorporated. *F. sambucinum* caused significantly ($P<0.05$) larger rots than the other two *Fusarium* species. There was no effect of seed inoculum level on tuber rot severity for any of the *Fusarium* species. *F. sambucinum* soil inoculum (low and high levels) resulted in significantly more severe rots than control soils ($P<0.001$), whilst only high levels of *F. avenaceum* soil inoculum increased severity of tuber rots compared to controls ($P<0.05$). *F. coeruleum* soil inoculum had no effect on rot severity.

INTRODUCTION

Fusarium dry rot is one of the most important storage diseases affecting potato (*Solanum tuberosum L.*) and affects almost all commonly grown potato cultivars (Leach and Webb, 1981). Although the importance of *Fusarium* dry rot has declined over many years due to effective control measures the disease can potentially cause huge losses with up to 60 percent of tubers affected (Secor and Salas, 2001). In the UK and in the Nordic countries, the most common pathogen is *F. coeruleum* (Libert) Sacc. (Bjor, 1978; Olofsson, 1976; Peters *et al.*, 2008; Seppänen, 1983; Thomsen *et al.*, 2014). Other important species include *F. avenaceum* and *F. sambucinum* (formally known as *F. sulphureum*) (Peters *et al.*, 2008; Thomsen *et al.*, 2014). *Fusarium* pathogens infect through wounds on tubers caused mainly during handling at planting, harvesting and grading. Adams and Lapwood (1983) investigated the transmission of inoculum in the field and found that both *F. sambucinum* and *F. coeruleum* were transmitted from seed to progeny tubers. A study by Leach (1985) found that cut seed tubers inoculated with *F. sambucinum* resulted in high levels of *Fusarium* dry rot in progeny tubers, whilst increasing the *F. sambucinum* population in soil near the plant, and naturally occurring low levels of *F. coeruleum* in soil resulted in low levels of contaminated progeny tubers. In order to implement effective disease-management strategies for *Fusarium* dry rot it is important to understand the relative contribution of different inoculum sources in causing disease. The aim of the present study was to investigate the relative importance of seed- versus soil-borne inoculum of three species of *Fusarium* (*F. coeruleum, F. sambucinum* and *F. avenaceum*) in causing dry rot in two potato cultivars; Asterix and Saturna.

MATERIALS AND METHODS

The relative importance of seed- versus soil-borne inoculum of three different *Fusarium* species (*F. coeruleum, F. sambucinum* and *F. avenaceum*) at different inoculum levels was investigated in a pot trial in 2014. Cultivars Asterix and Saturna were used.

Inoculum preparation

Inoculum of each *Fusarium* spp. was prepared using the following method. Two isolates of *F. avenaceum,* 3 isolates of each of *F. sambucinum* and *F. coeruleum,* were grown on Synthetic Nutrient Agar (SNA) in 9cm^2 Petri dishes at approx. 18°C for four weeks. All isolates were UK isolates from potato. An inoculum suspension consisting of a mix of two or three isolates of each species was made by scraping the fungal colonies from the plates into sterile distilled water (SDW). The concentration of macroconidia was quantified with a haemocytometer and adjusted to 10^5 conidia ml^{-1} (high) or 10^2 conidia ml^{-1} (low).

Seed and soil inoculation

One day before planting, seed tubers of cvs Asterix and Saturna were surface sterilized in 0.5% sodium hypochlorite and rinsed twice in sterile water before being wounded with a sterile device consisting of four spikes (each with a diameter of 1 mm) in a quadratic square (20 mm on each side) as described by Heltoft *et al*., (2015). Tubers were then inoculated with 20 µl of either a high (10^5 conidia ml^{-1}) or low (10^2 conidia ml^{-1}) conidial suspension or water only in each of four wounds per tuber.

The low and high conidial suspensions were added (30 ml/6 l pot) to batches of James Hutton Institute compost (Invergowrie, Dundee), hereafter described as soil. Infested soil was mixed thoroughly by hand before filling 6 litre pots. Control treatments had an equivalent volume of water added.

For each *Fusarium* species, seed tubers of each cultivar inoculated with either low or high inoculum suspension were planted in soil to which either a low or high inoculum suspension had previously been incorporated. Control seed and soil inoculum treatments were included. There were three replicates for each treatment arranged in a randomised block design within the glasshouse. Plants were grown for 5 months prior to progeny tubers being harvested.

Harvest, incubation and rot measurements of progeny tubers

Harvested progeny tubers were wounded as described above (without the addition of inoculum). Tubers were placed in covered plastic trays and incubated in experimental storage rooms (10°C and 95% relative humidity) for eight weeks. After storage, the rots on the wounding site of the tubers were measured. Rots were assumed to be conical for the purpose of analysis. Therefore, the volume of the rot was recorded and calculated using the formula: Volume = $1/3\pi hr^2$, where r is half the width of the rot and h is the depth of the rot.

Statistical analysis

Statistical analysis was carried out using Minitab® version 17.2.1. The data was modelled by general linear model (GLM). Disease severity was measured as dry rot volume caused by the

individual *Fusarium* species. Disease severity was modelled with cultivar, seed inoculum level and soil inoculum levels as explanatory variables with three random replicates. Outliers were removed according to the residual plot. The data was tested for significance of the main effects and interactions. Differences of means were tested by Tukey's multiple comparison test.

RESULTS

The results in Table 1 show the volume of rot in progeny tubers after eight weeks storage. Overall, *F. sambucinum* caused significantly larger rots than the other *Fusarium* species. Dry rotting in treatments infected with *F. sambucinum* and *F. avenaceum* was more severe in Asterix than Saturna, but *F. coeruleum* had no effect on the severity of dry rotting in either cultivar. Soil infested with *F. sambucinum* and *F. avenaceum* resulted in significantly larger rots on tubers of both cultivars compared to control soil whilst there was no significant effect on tuber rotting when soil was infested with *F. coeruleum*. Seed inoculum had no significant effect on rotting severity for any of the *Fusarium* spp.

Table 1. Volume rot (cm^3) in progeny tubers after storage. Standard error of the means are given in parentheses.

	All *Fusarium*	*F. sambucinum*	*F. coeruleum*	*F. avenaceum*
F. sambucinum	3.6a *(0.7)*	-	-	-
F. coeruleum	2.1ab *(0.4)*	-	-	-
F. avenaceum	0.6b *(0.005)*	-	-	-
	*			
Asterix	3.2a *(0.5)*	5.3a *(0.7)*	2.9 *(0.2)*	1.2a *(0.3)*
Saturna	1.0b *(0.3)*	1.9b *(0.3)*	1.1 *(0.4)*	0.1b *(0.05)*
	**	**	n.s	**
Soil: high	3.3a *(0.6)*	6.5a *(0.7)*	2.4 *(0.5)*	1.1a *(0.05)*
Soil: low	2.9a *(0.4)*	3.4a *(0.4)*	3.5 *(0.1)*	0.3ab *(0.4)*
Soil: control	0.1b *(0.01)*	0.1b *(0.1)*	0.2 *(0.02)*	0.1b *(0.005)*
	**	***	n.s	*
Seed: high	3.3 *(0.5)*	4.0 *(0.5)*	4.7 *(0.5)*	1.3 *(0.4)*
Seed: low	1.6 *(0.3)*	3.5 *(0.5)*	0.8 *(0.1)*	0.4 *(0.06)*
Seed: control	1.4 *(0.4)*	3.3 *(0.7)*	0.5 *(0.2)*	0.3 *(0.05)*
	n.s	n.s	n.s	n.s

n.s=not significant ($p \geq 0.05$), *$p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Values within a column followed by different letters are significantly different with Tukey's test ($p < 0.05$).

DISCUSSION

F. sambucinum caused significantly larger rots than the other *Fusarium* species. In other studies, *F. sambucinum* was also considered as a more aggressive species than *F. coeruleum* and *F. avenaceum* (Esfahani, 2005; Peters *et al*., 2008; Wastie *et al*., 1989). Our study showed that increasing amounts of *F. sambucinum* soil inoculum increased dry rot severity. In another study, naturally low soil levels of *F. coeruleum* resulted in less *Fusarium* dry rot development of *F. coeruleum* in the progeny tubers indicating a relationship between soil inoculum levels and *Fusarium* dry rot development in the progeny tubers (Leach 1985).

Adams and Lapwood (1983) demonstrated that infected seed could result in infection and subsequent development of dry rots in progeny tubers; they found that whilst *F. coeruleum* was most readily transmitted from rotting seed tubers to progeny tubers, rather than from seed with symptomless infection, *F. sambucinum* was transmitted from highly contaminated seed to progeny. There were no differences between seed treatments for any of the *Fusarium* species in this study which may indicate that the inoculation of tubers was not successful; however, this may also be attributed to cultivar resistance. The effect of the *Fusarium* species on the severity of rots on the two cultivars is reflected in the cultivar resistance ratings. Although no ratings are available for *F. avenaceum*, both cv. Asterix and cv. Saturna are relatively resistant to *F. coeruleum;* rated 8 and 6 respectively (on a scale of of 1-9 where 9 is resistant), and whilst cv. Saturna also has moderate resistance to *F. sambucinum* (6), cv. Asterix is relatively susceptible (4) to this *Fusarium* species.

This study showed that different levels of soil inoculum influence the *Fusarium* dry rot development in progeny tubers after a period of storage and that cultivar resistance should be considered an important component of disease control.

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DEVELOPING AN RT-PCR ASSAY FOR THE IDENTIFICATION OF PSYLLID SPECIES

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Summary: Psyllids are a diverse group of phloem-feeding insects, of which a few species have been found to transmit plant pathogens that are responsible for huge economic losses. *Candidatus* Liberibacter solanacearum (CaLsol) is a psyllid-vectored pathogen that infects solanaceous and apiaceous crops. As the causal agent of zebra chip disease in potato, it has led to economic losses due to unmarketable produce throughout Central and North America and New Zealand. European haplotypes of CaLsol have been found to primarily infect apiaceous crops. There are thought to be more than 90 species of psyllid in Britain; however, this estimation is tentative as species are frequently misidentified. We describe the prerequisites for the development a real-time PCR diagnostic assay – detailing the criteria used to determine target species and the molecular methods for species discrimination, including the use of non-destructive DNA extraction and DNA sequencing.

INTRODUCTION

Psyllids (Psylloidea), also known as jumping plant-lice, are a group of ca. 3800 described species of phloem-feeding insects belonging to the suborder Sternorrhyncha, which also comprises of the aphids, white flies and scale insects. Some species are vectors of the bacterium *Candidatus* Liberibacter solanacearum (CaLsol), the causal agent of zebra chip disease in potato (Secor *et al*., 2009). Infected tubers are unmarketable as they display dark stripes that intensify when fried. Zebra chip was first reported in 1994 in Mexico, after which it spread throughout most of Central and North America through the native psyllid vector, *Bactericera cockerelli*, which was later introduced to New Zealand, along with the bacterium and disease (Munyaneza, 2015). CaLsol primarily infects solanaceous crops, although haplotypes found in Europe were found to primarily infect apiaceous crops, and in carrots lead to leaf curling, shortening of carrots and abundance of secondary roots (Munyaneza *et al*., 2010; Teresani *et al*., 2014; Nissinen, 2014).

Over 90 species of psyllids have been reported in Britain (Ouvrard, 2015), of which an estimated 31 species occur in Scotland, although these estimates are unreliable due to the lack of a centralised repository and the likelihood of species misidentification. Identification to species-level requires specialist knowledge as some species lack taxonomically useful features (especially in females), and morphological characters and colouration can vary within adults of the same species. Although CaLsol has not been reported in Britain, at least one of the known

psyllid vectors of CaLsol in carrots, *Trioza apicalis*, has been recorded in England, albeit at low numbers.

Accurate species identification is paramount to corroborate the psyllid fauna of Britain, therefore allowing the detection and monitoring of potential vectors of CaLsol. By combining classical taxonomy with DNA sequencing, we aim to develop a set of species specific primers and probes for use in a real-time PCR diagnostic assay to allow the accurate and rapid identification of a set of target psyllid species. The project is currently underway; an overview of the criteria used to determine target species, and the molecular methods for species discrimination are given in the following sections.

TARGET SPECIES SELECTION

A total of 33 target species were chosen using a set of criteria to determine whether they could be potential vectors of CaLsol or other significant diseases which could impact the British agricultural industry (Table 1).

Table 1. The list of target psyllid species included in the molecular assay. Criteria are not mutually exclusive and species are categorised under the primary criteria for their inclusion.

Criteria	Species	
1. Known/suspected vectors of CaLsol	*Trioza apicalis* *Bactericera cockerelli* *Bactericera trigonica*	*Bactericera tremblayi* *Bactericera nigricornis*
2. Species on apiaceous/solanaceous plants in the Britain & other European countries	*Aphalara avicularis* *Aphalara freji* *Aphalara maculipennis* *Craspedolepta innoxia*	*Diaphorina lycii* *Trioza anthrisci* *Trioza laserpitii* *Trioza flavipennis*
3. Species on other significant plants (e.g. crop border weeds)	*Aphalara exilis* *Aphalara pauli*	*Aphalara purpurascens* *Trioza rumicis*
4. Species in the Britain associated with Liberibacters/Phytoplasmas	*Cacopsylla melanoneura* *Cacopsylla affinis* *Cacopsylla ambigua* *Cacopsylla pyri*	*Cacopsylla pyricola* *Cacopsylla pyrisuga* *Cacopsylla peregrina* *Cacopsylla nigrita*
5. Other potentially significant species	*Bactericera acutipennis* *Bactericera albiventris* *Bactericera curvatinervis* *Bactericera maura*	*Bactericera salicivora* *Bactericera silvarnis* *Bactericera substriola*
6. Regionally common non-vector species	*Trioza remota*	

The assay will include all species that are known and suspected vectors (*viz*. those that have been associated with the bacterium in the literature) of CaLsol (*Criterion 1*). We include

psyllid species that have been reported on apiaceous/solanaceous plants that grow in Britain and other European countries as these species could potentially transmit the bacterium to apiaceous/solanaceous crops (*Criterion 2*). Target species are those that have been found on other significant plants, such as common crop border plants or weeds, as psyllids in close proximity to the crop may facilitate the transmission of CaLsol (*Criterion 3*). To allow the monitoring of other significant psyllid-vectored diseases, we include all psyllids that have been implicated in the transmission of other liberibacters and phytoplasmas (*Criterion 4*). The list also comprises of a subset of species in the *Bactericera* genus, as they occur in the same genus of four out of the five known/suspected vectors (*Criterion 5*). An additional species that poses a low risk but is common throughout Britain was included to allow future monitoring of population dynamics and to improve our understanding of general psyllid migration (*Criterion 6*).

MOLECULAR ANALYSIS

To assure that psyllids have been accurately identified prior to sequencing, a non-destructive DNA extraction method was used to maintain voucher specimens, allowing the cross-reference of morphology and DNA sequence, for example, in the case of cryptic species complexes. We used a variation to the non-destructive DNA extraction method in Percy (2003). Instead of creating an incision in the abdomen, the psyllid was pierced in the thorax and abdomen with a 0.14mm stainless steel headless pin. The specimen was processed using the DNeasy Blood & Tissue Kit (QIAGEN) with the following modifications to the standard protocol. After lysing the sample overnight, the exoskeleton was retrieved from the buffer and stored in 95% ethanol and 5% glycerol at 4°C. DNA was eluted in 50ul of elution buffer.

Samples were sequenced at two regions, the *cytochrome oxidase I* (COI) region using LCO1490 and HCO2198 primers (van de Vossenberg, 2013), and the *internal transcribed region 2* (ITS2) and partial regions of the rRNA 28S and 5.8S genes were amplified using CAS5p8sFcm and CAS28sB1d (Peccoud *et al*., 2013). Use of an additional nuclear barcoding marker in combination with the mitochondrial COI has been recommended to avoid issues arising from mitochondrial introgression (van Nieukerken *et al*., 2012). The following species have been successfully sequenced at the COI region; *Diaphorina citri, Trioza remota, T. albiventris, T. anthriscii,* and *T. apicalis*. The following species have been sequenced at the ITS2 for this project; *Bactericera cockerelli, B. albiventris, B. curvatinervis, Cacopsylla pulchra, C. brunneipennis, C. melanoneura, D. citri, T. remota,* and *T. albiventris.* The ITS2 region appears to be highly variable amongst psyllid species and is therefore ideal for species specific primer design. Its variability has been used to discriminate the *C. pruni* complex, revealing the existence of two biologically significant species (Peccoud *et al*., 2013).

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MAPPING *PASTEURIA* DISTRIBUTION IN SCOTLAND

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Summary: *Pasteuria* spp. are natural bacterial antagonists of many of the most economically important plant parasitic nematodes (PPNs). Parasitism of PPNs by *Pasteuria* spp. results in a reduction of root feeding and sterilisation of the host. The relative diversity and distribution of these bacterial parasites in relation to nematodes in Britain is poorly understood. Detection and discovery of *Pasteuria* spp. has previously relied on random sampling of nematode communities or directed sampling of soils known to be nematode-suppressive. We are developing a sensitive and semi-quantitative PCR based metagenetic assay to expedite detection and recovery of *Pasteuria* spp., and assess the influence of environmental factors on their survival.

ABSTRACT

Pasteuria spp. are gram positive, spore forming bacteria known to infect nematodes and water fleas (*Daphnia magna*). The host range of the genus *Pasteuria* is broad and they are globally distributed (Chen & Dickson, 1998). There is considerable interest in application of *Pasteuria* cultures in agriculture as a means of reducing pesticide use. The life cycle of *Pasteuria* spp. in PPNs begins with attachment to the nematode cuticle in the soil matrix which has an immediate suppressive effect, hindering movement and root invasion (Davies *et al.,* 1991; Vagelas *et al.,* 2011). Once attached, the spores of *Pasteuria* may germinate immediately, forming a germ tube which penetrates the nematode cuticle and musculature (Imbriani & Mankau, 1977). It has also been observed that some isolates of *Pasteuria* may only germinate once the nematode has invaded the root and formed a feeding site (Davies *et al*., 2011; Mohan *et al*., 2012). Once the spore has germinated the replicative stages of the bacteria fill the nematode pseudoceolem if nutrition is adequate (Davies *et al*., 2011), although the nematode remains alive and may continue to feed (Imbriani & Mankau, 1977). The second suppressive action of *Pasteuria* spp. is the alteration of embryogenesis resulting in sterilisation, where the nematode produces freckled or granular lipid like droplets in place of viable eggs which are in turn infected (Davies *et al*., 2011). The replicative stage then undergoes sporulation forming thousands to millions of mature *Pasteuria* endospores, dependant on the developmental stage of the host infected, which are released back into the soil matrix upon degradation of the nematode (Davies *et al*., 2011).

Pasteuria population surveys have largely been reliant on direct observation of nematodes encumbered or filled with mature endospores, during large scale nematode population studies (Hewlett *et al.*, 1994; Ko *et al*., 1995; Franco-Navarro & Godinez-Vidal, 2008; Elekcioglu, 1995). Directed studies recovering *Pasteuria* spores and spore encumbered nematodes from

soils known to be suppressive to nematode populations have also been undertaken (Davies *et al.*, 1990). These survey methods are time consuming and directed recovery of new or interesting isolates requires indication of nematode suppression from existing density data showing specific decline at a site over time, or anecdotal indication of suppression (Davies *et al*., 1990; Noel & Stanger, 1994). Phylogenetic studies of the *Pasteuria* 16S rRNA gene have allowed the development of PCR based methods to detect and discriminate between isolates of *Pasteuria* (Atibalentja *et al*., 2000; Duan *et al.*, 2003; Mauchline *et al.*, 2010; Rao *et al.*, 2012). Such methods allow presence/absence detection of *Pasteuria* spp. from DNA extracted from soils or nematodes with variable sensitivity (Duan *et al*., 2003; Mauchline *et al*., 2010) although it is not apparent that these methods have as yet been applied to large scale ecological surveys in published literature.

We are currently developing a sensitive, semi-quantitative, and high-throughput metagenetic assay which will target *Pasteuria* spp. and their nematode hosts in Scottish soils. This approach builds on the phylogenetic 16S rRNA studies undertaken so far on the genus *Pasteuria* (Atibalentja *et al*., 2000; Duan *et al.*, 2003; Mauchline *et al.*, 2010; Rao *et al.*, 2012), taking advantage of advances in sequencing and multiplexing technology (Faircloth *et al*., 2012). This will be combined with recent advances in 18S PCR based nematode diagnostics (Porazinska *et al*., 2009; Sapkota & Nicolaisen, 2015) to give a comprehensive picture of the presence, diversity, and distribution of *Pasteuria* spp. in Scotland, their relationship with the environment, and with their hosts. The method developed may be used in future to direct detection and recovery of new and interesting isolates of *Pasteuria* which may parasitise nematodes of scientific or economic impact; or to study the relative ability of different soils to support *Pasteuria* spp. in climates where information is similarly limited.

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STUDIES ON A NOVEL IMMUNITY FACTOR FOR IMPROVED VIRUS RESISTANCE IN POTATOES

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Summary: Plant genomes (*Solanum tuberosum*, *Nicotiana benthamiana* and *Arabidopsis thaliana*) encode homologs of human Mx proteins, an essential and powerful component of the human innate immune system. These homologs are entirely uncharacterised but show similarities in structure and domains to the human Mx proteins. Microarray data shows two Mx homologs are upregulated upon infection in potatoes infected with PVY and their induction correlates with virus resistance. Like human Mx, the mechanism of resistance may have a broad mode of action and broad spectrum antiviral activity, possibly making the findings applicable to a multitude of crops. We aim to investigate the expression of the homologs during viral infection and in response to plant hormones, as well as study the antiviral activity of the homologs *in vivo*. If an antiviral activity of potato Mx is identified, this will provide a new marker for targeted resistance breeding.

Potatoes are the world's 3rd most important staple food crop and are of economic importance in Scotland. Seed potatoes grown in Scotland are exported to EU and more than 30 non-EU countries worldwide (SASA, 2015). Viruses cause decreased yield and quality of potato tubers and high-grade seed tubers must be substantially free from viruses. In recent years recombinant strains of the aphid transmitted *Potato virus Y* (PVY), some of which cause potato tuber necrotic ringspot disease, have increased in incidence (including in Scotland) making it the most important potato virus problem in most potato growing regions (Gray *et al*., 2010).

This project involves the study of a mechanism of resistance to viruses found in animals but not yet investigated in plants. In animals, proteins of the Mx family disrupt virus replication and are a powerful component of innate immunity providing resistance to a wide range of viruses (Haller *et al*., 2015). Human Mx (MxA) interacts directly with components of viruses that are essential for replication. The exact mechanism by which it acts is unclear; however, the current model involves multimerisation of MxA to form ring structures that bind viral components. The mechanism of action is dependent on the virus and can vary from direct binding of viral structures, thereby disrupting the association of other complexes, to the relocalisation of viral components in order to sequester and/or degrade them (Verhelst *et al*., 2013).

Plant genomes encode homologs of Mx proteins (~35% amino acid identity), which remain entirely uncharacterised (Hong *et al*., 2003). Preliminary analysis of the plant homolog protein sequences show that some of them possess similar domains to the Mx proteins in animals.

Using microarray data from potatoes infected with PVY, we have found that two Mx homologs are upregulated upon infection and their induction correlates with virus resistance.

Since the mechanism of resistance may have a broad mode of action in plants against a number of viruses and similar homologs have been found in other plants (e.g. *Nicotiana benthamiana* and *Arabidopsis thaliana*), the findings may be applicable to control viruses in other important crops. We will present a comparison of the potato, and other plant Mx homologs with their animal counterparts, preliminary data analysing the expression of potato Mx homologs in detail during virus infection and in response to different plant hormones, and testing their antiviral activity by overexpression and knock down. The results of this project may provide new information on an Mx-based resistance mechanism that may be used to devise novel breeding strategies.

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PATHOGENS ASSOCIATED WITH PIT ROT IN POTATOES

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Summary: A study was undertaken to determine the main pathogens associated with pit rot in potatoes. Until now, it has largely been considered that physiological factors or bacteria are the main causes of pit rots. However, this study provides evidence that fungal pathogens were the predominant pathogens isolated from pit rot lesions sampled from Scottish seed crops; bacterial pathogens were isolated less frequently, with pit rot lesions in 63% of the crops testing negative for *Pectobacterium* species.

INTRODUCTION

Pit rot is thought to occur when cool temperatures in storage halt progress of infection by one of a range of pathogens and the tuber tissue collapses (Harris, 1978). Pit rots in potatoes can vary from numerous small indented lesions a few millimetres across, to larger lesions up to 2-3 cm in diameter. In recent years there has been an increase in the prevalence of pit rot in Scottish seed stocks (SASA observation) and it has been speculated that this is may be due to the loss of sulphuric acid as a desiccant leading to diquat and pulverisation becoming the main haulm destruction methods, or changes in the pathogen populations responsible. Pit rot has commonly been attributed to bacterial infection (Wale *et al*., 2008) but fungal pathogens such as *Phoma* spp. and *Cylindrocarpon* have been frequently attributed to pit rot formation in diagnostic work done at SASA and also noted elsewhere (Wale *et al*., 2012). Hence, a survey of seed potato crops (PB and SE grades) displaying pit rot symptoms was undertaken over two storage seasons to determine the relative contribution of fungal and bacterial pathogens associated with pit rot symptoms in Scottish crops.

MATERIALS AND METHODS

Tuber selection

Samples of potato tubers displaying classic pit rot symptoms were collected from PB and SE crops throughout Scotland by an agronomist over a two year period and submitted to SASA for testing. Ten tubers from each sample were randomly selected. Prior to testing, the tubers were washed, surface sterilised in 1% sodium hypochlorite and thoroughly rinsed with water.

Fungal identification

A section from the leading edge of each rot was aseptically removed from below the epidermis

using a scalpel. Where tubers had multiple rots, each rot was sampled. The tissue samples were plated onto Potato Dextrose Agar supplemented with streptomycin and incubated at 21℃ for up to 14 days to allow development of fungal growth. Isolated fungi were identified using morphological characteristics.

Bacterial identification

For each lesion, a piece of tissue (~2mm^3) from under the lesion was placed into a 1.5ml Eppendorf tube to which 1ml of Extraction buffer was added (50 mM phosphate buffer, pH 7.0: 4.26g Na_2HPO_4 (anhydrous) and 2.72g KH_2PO_4 in 1.0 L of distilled water, adjust pH and sterilise by autoclaving at 121 °C for 15 min. Prior to use, add 0.5g Lubrol flakes (Nonaethylene glycol monododecyl ether, Sigma), 1.0g tetrasodium pyrophosphate and 50g Polyvinylpyrrolidone-40000 (PVP-40) and dissolve). The tubes were placed in a rack, secured with micropore tape and placed at an angle in a rotary incubator before shaking at 100rpm for 16-24 hours at 4°C. Positive controls were prepared by adding a loopful of *Pectobacterium atrosepticum* to 1 ml extraction buffer. A *P. carotovorum* positive control was also prepared using the same method. A negative control consisted of extraction buffer only. All liquid was pipetted from each tube, leaving any tuber material behind, and placed in clean Eppendorf tubes before centrifuging (10,000g; 4°C) to pellet any extracted bacteria. The supernatant was removed and the pellet was resuspended in 80µl Ringer's solution and 200µl glycerol before vortexing. The samples were then stored in a freezer at -80ºC.

To evaluate the bacterial composition of extracts, samples were defrosted before preparing a 10-fold dilution series in Ringer's Solution down to 10^{-3} dilution in triplicate, vortexing between dilutions. One hundred microlitres of each replicate was spread onto four Crystal Violet Pectate Medium (CVPM) plates per dilution. Half of the plates were incubated upside down at 25°C for development of *P. atrosepticum* and the other at 36°C to favour *P. carotovorum*. All of the controls were treated in the same way as samples.

The plates were checked for pitted colonies after 48 hours and any such colonies were picked off and subcultured onto Nutrient Agar plates and incubated under the same conditions. Identification of colonies resembling *Pectobacterium* spp. was established using PCR tests whereby a suspension (approximately 10^6 cells/ml) was prepared from a single suspect colony suspended in molecular grade water (Sigma). The suspension was mixed by vortex and 500µl was transferred to a 1.5ml Eppendorf and incubated in a hot block for 5 minutes at 100°C. Samples were then frozen at -20°C until PCR for *P. atrosepticum* was performed. The PCR mastermix consisted of 9.5µl molecular grade water; 12.5µl REDTaq Readymix (2x), 1µl Primer ECA1f (2.5µM); 1µl ECA2r (2.5µM) per reaction (De Boer and Ward, 1995) to which 1µl of the boiled cell sample was added. PCR conditions were 95°C for 5 mins followed by 40 cycles of 94°C for 30s, 62°C for 45s, 72°C for 45s and then a final step of 72°C for 8 minutes. PCR products were run on a 1.5% agarose gel and visualised under UV transillumination (λ=302 nm). Tests were also performed for *Dickeya* spp. using the method of Nassar *et al*. (1996). Pectolytic colonies which were not identified as *P. atrosepticum* or *Dickeya* spp. were presumptively diagnosed as *P. carotovorum* based on morphological appearance.

Table 1 Fungal pathogens and *Pectobacterium* spp. found in each pit rot sample (n=23 for fungal testing; n=19 for bacterial testing). The figures represent the total number of lesions in which each pathogen was found per 10 tuber sample. For *Pectobacterium* spp., H = heel ends, L = lesions (number represents the number of lesions affected per 10 tuber sample) and nt = not tested. The percentage of total (n) samples affected by each pathogen is shown.

Sample number	Fungal pathogens								*Pectobacterium* spp.	
					Phoma spp.					
	Cylindrocarpon	*Fusarium spp.*	*Colletotrichum coccodes*	*Polyscytalum pustulans*	All *Phoma* spp.	*Phoma exigua*	*Phoma foveata*	*Phoma eupyrena*	*Pectobacterium atrosepticum*	*Pectobacterium carotovorum*
1	5	2			2	1		1	nt	nt
2		1	15		5			5	nt	nt
3	1	1	8		1			1	pos H + 1 L	neg
4					4	1		3	1 L pos	neg
5	34				3	3			2 L pos	neg
6	3		34						3 L pos	neg
7					9		9		neg	neg
8	8								neg	neg
9	5		4		9			9	neg	neg
10					19		19		pos H +5 Ls	neg
11	18				6	4	1	1	1 L pos	neg
12					16			16	neg	neg
13	42	1	5						4 Ls pos	neg
14	29		9		6			6	neg	neg
15	1	1			24	9	2	13	pos H	pos H
16	6								neg	neg
17		4							pos H	pos H
18	10				1			1	neg	neg
19			1		1			1	neg	pos H
20	4	1			12	1	3	8	neg	pos H
21	7				2			2	neg	neg
22	10			4					nt	nt
23			6						nt	nt
% samples affected	65	30	35	4	70	29	22	56	42	21
Affected lesions per sample (mean)	12.20	1.57	10.25	4.00	7.50	3.17	6.80	5.15	2.43	N/A

RESULTS

Results are shown in Table 1. The most common pathogens found in the pit rot samples tested were *Cylindrocarpon* and *Phoma* spp., with 65% and 70% of samples affected respectively. *P. atrosepticum* was present in 7 crops (37%) when sampling pit rots, and 9 crops (47%) in total (when counting pit rot and heel end positives). *P. carotovorum* was not isolated from any pit rot lesions sampled, but was present in heel end tissue in 21% of stocks. Unexpectedly, *Colletotrichum coccodes* was isolated from 35% of samples, often as the sole pathogen detected.

Of the *Phoma* species isolated, *P. eupyrena* was most prevalent. When *Cylindrocarpon* was isolated in a stock, it was associated with the majority of lesions present, more so than for other pathogens. *Cylindrocarpon* averaged 12.2 contaminated lesions per 10 tuber sample, compared with *Colletotrichum* (10.25), *Phoma* species (7.5) and *P. atrosepticum* (2.43) per sample.

In four of the samples, pit rots had developed around powdery scab lesions. The predominant pathogen found associated with all of these lesions were *Cylindrocarpon* spp.

DISCUSSION

This study looked at the causes of pit rots in 23 tuber samples (of which 19 were tested for both fungi and bacteria). *Phoma* species were present in 70% of samples, with the most prevalent being *P. eupyrena*, followed by *P. exigua* and *P. foveata* (56%, 29% and 22% of samples respectively). Work by Wale (2012) showed a link with colonisation of potato haulms by *P. foveata* after the use of diquat and this strongly correlated with gangrene development on daughter tubers. Although *P. foveata* is generally a more aggressive pathogen, causing extensive rots with large cavities rather than 'pit rot' symptoms, all three *Phoma* pathogens can thrive in cool storage conditions and are frequently isolated together from infected stocks (SASA observation). Therefore, a similar pathway may be responsible for tuber infection caused by all three pathogens as that described by Wale (2012). Certainly, in the 1950s, *P. eupyrena* was seen much more frequently on dead potato haulms than *P. foveata* (Moore, 1959) and it is plausible that this is the mode of infection of tubers. The increased use of diquat as a haulm desiccant (since the loss of sulphuric acid) may have facilitated the higher number of pit rot infections seen in recent years.

A general survey of tuber dry rots undertaken in the late 1990s (Choiseul *et al*., 2007), showed that *P. eupyrena* was present in Scottish stocks for two of the three years studied, though the rate of infection within those stocks was relatively low compared to other fungal pathogens such as *P. foveata*, *Fusarium avenaceum* and *Cylindrocarpon*. In that study, *Cylindrocarpon* was highly prevalent, being found in around a third of stocks or more each year. In this present study, both *P. eupyrena* and *Cylindrocarpon* spp. were found at higher levels (56% and 65% respectively) and a more extensive survey of both pit rots and dry rots would be useful to indicate whether these pathogens are more prevalent than they once were.

In inoculation tests by Choiseul *et al*. (2007), the average width and depth of lesions caused by *P. eupyrena* (8.8mm wide; 5.5mm deep) and *Cylindrocarpon* (7.7mm wide; 7.6mm deep) were significantly lower than those caused by other pathogens such as *Fusarium spp* and *P. foveata* (14.6-29.4mm wide; 13.9-19.1mm deep). Similar sized lesions were observed during this study (data not shown) for these two pathogens, which may account for their higher prevalence in the 'pit rot' samples surveyed, compared with more aggressive *P. foveata* and *Fusarium* spp. pathogens whose symptoms generally extend beyond the size of those classed as 'pit rot'.

As to be expected, most of the pit rot lesions observed in this study had formed around lenticels, sometimes around wounds, but it is interesting to note that in more than a quarter of samples where *Cylindrocarpon* was isolated, rots had formed around powdery scab (*Spongospora subterranea*) lesions, suggesting that these lesions might act as a focal point for pit rot development, allowing pathogens to gain entry.

Unexpectedly, *Colletotrichum coccodes* was found in 8 stocks, sometimes within a considerable proportion of lesions. This pathogen is mostly considered to cause a skin blemish disease known as black dot, although Choiseul *et al.* (2007) noted its detection in rot samples. It is not clear whether *C. coccodes* was a primary cause of rotting in the current study (although in many lesions no other pathogens were isolated) but its potential to cause such rots is worthy of further study.

Whilst *P. atrosepticum* was found in 47% of the samples tested, only 7 of the 19 (37%) stocks had *P. atrosepticum* associated with pit rot lesions. Therefore, in 63% of the stocks, neither of the *Pectobacterium* species were isolated from pit rot lesions (despite heel end (vascular) *P. atrosepticum* infection being present in some of these stocks). Furthermore, tests detected that this pathogen was associated with fewer pit rot lesions, averaging only 2.43 lesions per stock, than some of the fungal pathogens. *P. carotovorum* was not found in association with any lesions although 21% of samples had heel end infections. This demonstrates that although the bacterial pathogens were present in some of the stocks tested, as vascular infections, they were associated with fewer pit rot type lesions than the fungal pathogens isolated. Further testing would be useful to determine whether bacterial pathogens could be considered less important as pit rot pathogens than fungal pathogens.

In summary, this study has demonstrated that fungal pathogens were associated with the majority of pit rot lesions tested, with *Cylindrocarpon* and *P. eupyrena* being the most commonly isolated pathogens, whilst bacterial *Pectobacterium* species were infrequently isolated from pit rot lesions, even when the bacterial pathogens were known to be contaminating the seed. This contradicts previous opinion that pit rot was largely attributed to bacterial infection caused mainly by *Pectobacterium* species. There would be value in further elucidation of the conditions favouring pit rot development for each pathogen and establishing the impact of haulm destruction practices on pit rot development in store.

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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
European and Mediterranean Plant Protection Organization	EPPO		
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			