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PRACTICAL IMPLEMENTATION OF ENVIRONMENTAL SCHEMES IN A COST EFFECTIVE AND SUSTAINABLE SYSTEM: A FARM CASE STUDY

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Summary: Pittarthie is a small 170 ha family farm in upland Fife. As a working farm operating on a very tight budget we have embraced a wide variety of environmental schemes over the past 20 years. Far from being detrimental to the overall profitability of the farm, the conservation, trial and experimental work compliments the more traditional side of the business. Implementing the schemes has provided some interesting challenges; some schemes seem overly complex, but generally there are workable solutions and some examples are provided here. Significant recycling and a low carbon footprint also help keep costs down and improve sustainability.

Apart from winning several awards, our success has been measured by the increase in the number and variety of birds and other wildlife on the farm, the return of spawning sea trout in the burn after a gap of more than 60 years and excellent water quality. There have been some educational spin-offs too. Moreover, the farm remains commercially viable, yielding additional marketable products such as charcoal.

INTRODUCTION

Pittarthie is a small family farm of 170 ha in upland Fife. The area is pretty bleak and unlike the large arable farms in other parts of the country we don't have big profits to put back in; on the contrary, Pittarthie Farm operates on a very tight budget. Predominantly a livestock farm, we have diversified over the years taking advantage of various environmental schemes to improve our operations, reduce our carbon footprint, assist in conservation measures, and produce other marketable commodities whilst complying with ever changing environmental legislation. It has required a lot of hard work and been very frustrating at times but ultimately immensely satisfying. More importantly, far from being detrimental to the overall profitability of the farm, implementation of these environmental schemes has been complimentary.

It is worth noting that 80% of what we do in terms of conservation measures, field trials etc. is sponsored or grant aided and would not be possible without the help of SNH (Scottish Natural Heritage), various Agri-Environment Schemes, the Fife Local Biodiversity Action Plan (LBAP), SEPA (Scottish Environment Protection Agency) the Forestry Commission and others. Even with the grants we are lucky to break even in cost terms but the overall benefits make it worth the effort.

In this paper I have very briefly summarised some of schemes used, the organisations we have worked with, and examples of the various conservation, energy efficiency, and innovative measures we have in place. I also touch on educational benefits and success

measures. I will provide more detail and examples in my presentation. These developments have not happened overnight and we are currently in year 20 of a 50 year plan!

SCHEMES AND PARTNERSHIPS

We have benefited from a variety of different environmental schemes over the years, some of which have now been superseded by new schemes. The most notable of these include the Scottish Governments' Rural Stewardship Scheme (RSS), the Countryside Stewardship Scheme (CSS), the SEPA Habitat Enhancement Scheme, and the Forestry Commissions' Woodland Grant Scheme (WGS). More recently the SRDP (Scotland Rural Development Programme) has been set up by the Scottish Government to help develop rural Scotland and we are hoping to be accepted onto this scheme.

In implementing the schemes we have worked with numerous different organisations including:

- Fife Council
- Fife Coast and Countryside Trust
- Fisheries Research Services (FRS) at Faskally, Pitlochry.
- Forestry Commission
- Farming & Wildlife Advisory Group (FWAG)
- Royal Society for the Protection of Birds (RSPB)
- Scottish Executive Environmental and Rural Affairs Department (SEERAD) which has now become Scottish Government Rural Payments and Inspections Directorate (SGRPID)
- SEPA
- SNH

In addition we have also had close links with various universities, colleges and agricultural advisors etc. including:

- Elmwood Agricultural College
- Frontier Agriculture
- St Andrews University
- Stirling University

Our experience with the various schemes has been a bit mixed. Some were comparatively straightforward and easy to deliver, others required a bit more creativity and innovation, whilst some appear very long winded, complicated and rigid which can be a barrier to the practicalities of implementation.

CONSERVATION/ENVIRONMENTAL PROTECTION

Conservation and protection of the environment is a key theme on the farm and apart from creating wildlife corridors we have taken an overview of the whole landscape so that a variety of measures can be integrated and linked on a long term basis. These include ponds and wetlands, which not only provide a habitat for wildlife but act as agri-nutrient filters over wide areas. Where possible watercourses have been de-canalised, bridges and fords have

been used instead of culverts, fish ladders and silt traps have been installed and other measures taken to control erosion via water margins. We have also created viable habitats for water voles.

Traditional woodland and hedge management, e.g. laying, pollarding and coppicing, have been reintroduced on the farm. There are some sacrificial woodland crops including goat willow and osiers. We use creative planting to make the most of the landscape and its history and where possible we try and take account of historic landscape use before overwhelming it with new planting schemes. SSSI sites on the farm have been regenerated using a combination of rehydration, scrub clearance and heather re-seeding.

A number of trials are always being carried out on the farm. These include the use of biofumigants, the trialing of seed mixes/crops to assess their suitability as global warming/climate change impacts become more apparent in Northern Areas, and various fence trials to find options that are friendly to wildlife yet keep livestock firmly in their place.

ENERGY EFFICIENCY

Maintaining a low carbon footprint and keeping energy costs down is very important to us. We recycle as much as possible, almost 100% on the trial areas, and strive to be carbon neutral, although we cannot of course control all emissions from the stock and silage is dealt with by contractors.

Wind and solar power is widely utilised. Both are used to power stock drinkers, a small field centre and bird friendly electric fencing. We also use solar and wind power for irrigation and managing/maintaining the wetlands and wetland bogs.

There are 15 year old hardwood plantings on the farm which are now used for charcoal production and further income is provided by thinning the woodland areas.

INNOVATION

In managing and regenerating the landscape we have had to be creative at times. Regeneration of the SSSI sites required re-seeding the area with heather and to do this we developed our own heather seed drills. We have also developed a variety of multi-sized seed drills for other purposes. Other engineering developments have included tree and hedge planting machines.

A key innovation has been the development of mobile watercress beds in the wetland area. The plants absorb phosphates and help limit nitrates produced elsewhere on the farm and the beds provide a good habitat for wildlife.

We try to avoid using pesticides and herbicides as much as possible but do need them occasionally, e.g. for dealing with caterpillars in Kale and for weed control. Very specific herbicides and/or glyphosate are used for the weed control. The glyphosate is generally used in sterile strips to deter invading weeds from the field edges rather than overspraying whole areas. This not only keeps down the cost but helps leave skylark nesting strips. Good rotations and sterile seedbeds are also key. When chemical use is necessary it is done from a quad bike or using low volume Nomix sprayers. Knapsack sprayers have been specially developed and adapted too. One scheme insisted on knapsack spraying of large areas to

comply with one of their regulations. This proved both time consuming and impractical but was overcome by building some broadcast nozzles for the knapsack sprayers.

There are steep banks on some areas of the farm but we were still able to seed them using a spraying seed technique together with an industrial starch medium helping the seeds stick to the bank and acting as a fertilizer when properly blended.

SUCCESS MEASURES

One of the key success measures is that farm remains productive although in cash terms it does not make huge profits, struggling to break even in some years.

In environmental and conservation terms there are a variety of measures that can be used, and these are all showing real benefits. These include:

- RSPB and in-house bird counts – the species and numbers of birds have steadily increased. Last year we had our first thousand bird flock of passerines. The farm also boasts the first "duck decoy" in Scotland for over a century which is enabling assessment of wild migratory birds such as mallards and widgeon to be tagged in order to gather population information.
- Fish and eel movements: sea trout now return to the burn to spawn for the first time in over 60 years. Elver and silver eel movements are also recorded.
- Presence of water voles and water rail; these endangered species are breeding on the site.
- There are two otter holts on the farm. Initially this caused problems as the otter eat the voles but the problem has now been solved using special fencing.
- Badgers; some problems with cattle licks and undermining of some of the fences necessitating new planting and other measures around the new setts. I am also concerned about TB.
- Good water quality is achieved in the burn with BMWP scores in excess of 1500.
- There is a marketable roe resource.
- Shoot game books.

As a consequence of all the hard work our efforts have won the farm a number of National Awards and it is regularly used for educational purposes and 'spreading the word'. Farm visits have been booked by several organisations including FWAG, SEERAD/SGRPID and SEPA. Others such as SNH, RSPB, LBAP and Universities are regular visitors and there are open days for other people.

CONCLUSION

We have demonstrated that by taking a holistic view of the farm and embracing environmental schemes as complimentary to traditional business, it is possible to meet environmental regulations and enhance biodiversity in a cost effective and sustainable way. It may require effort and innovation but you don't have to sacrifice large tracts of land. The seeds I sow today I hope my two sons will reap in the future.

UPDATE ON THE EU THEMATIC STRATEGY FOR PESTICIDES

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Summary: This paper outlines latest developments on the EU Thematic Strategy for Pesticides. The key elements of the Strategy have been adopted by the EU and the governments and administrations of the countries of the UK are in the process of determining how it will be implemented.

EU THEMATIC STRATEGY FOR PESTICIDES

Adoption of the Thematic Strategy for Pesticides meets a commitment made by the European Parliament and Council when adopting the 6th Environmental Action Programme to further reduce the impact of pesticides, particularly plant protection products, on human health and the environment.

The specific objectives of the Thematic Strategy are to:

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

The Thematic Strategy consists of four pieces of legislation, they are:

- a new Regulation concerning the placing of plant protection products on the market (replacing the current authorisation directive 91/414/EEC);
- a Directive on the sustainable use of pesticides (introducing for the first time substantial Community legislation on the use of pesticides);
- a new Regulation on pesticide statistics (which will be used to populate risk indicator models and processes);
- an amendment to the Machinery Directive 2006/42/EC setting standards for the new pesticide application equipment being brought to the market.

The two key elements of the Strategy are the Authorisation Regulation and Directive on sustainable use. This paper outlines the key features of this legislation.

Authorisation Regulation

The Regulation updates the regulatory framework for the placing of plant protection products on the market. The process for placing products on the market is essentially a continuation of existing practice: manufacturers' submitting data demonstrating the safety and efficacy of products containing a specific active ingredient to competent authorities; competent authorities' evaluating this information and providing details via the European Food Safety Authority to an EU Standing Committee; the Committee's approving (or not) the active substance for use in products; and then competent authorities' deciding whether specific uses of products meet safety and efficacy standards and can be authorised.

The new regulatory framework is, however, in some key aspects fundamentally different from the current regime:

- Firstly, by employing the use of hazard criteria. The existing regime is based on assessment of risk. Therefore, the intrinsic properties of a chemical (e.g. whether it is mutagenic, a reproductive toxin, endocrine disruptor, very persistent, bioaccumulative or toxic) do not necessarily preclude it from consideration for authorisation in a plant protection product if exposure can be contained within levels assessed as constituting an acceptable risk. Under the new arrangements the properties of a chemical will determine whether or not it can be considered for inclusion within an authorised pesticide.
- Secondly, by introducing the concept of 'substitution'. Under these arrangements authorisations for products containing active substances which meet the standard for approval but with a less favourable overall profile may be refused or withdrawn if safer alternatives are available.
- Thirdly, through the use of zonal applications. Under these arrangements the EC is divided into three zones within which applications for the authorisation of products may be made to one or more member States; and if to more than one, a lead member state evaluates the dossier on behalf of the others. All member States grant or refuse authorisations with the same conditions as the lead member State unless their specific national conditions justify alternative conditions of use or refusal.
- Finally, authorisations may be renewed, withdrawn or amended if they compromise compliance with the Water Framework Directive (WFD). The WFD is EU legislation that is designed to improve the quality of surface and ground water bodies, taking account of ecological and chemical quality. It introduces quality standards and a requirement to reduce or phase out emissions of a list of 33 priority and priority hazardous substances (of which three are pesticides) and provides that the level of purification required to produce drinking water supplies should be reduced. The Directive also requires member States to identify and set quality standards for substances identified as relevant to national conditions (5 pesticides have been identified).

There is much to commend in the new Regulation, including an increase in standards and improvement to the working of the regime, and the UK Government welcomed most of it. However, it opposed its adoption because the hazard criterion for potential human endocrine disrupters could compromise pest, weed and disease control, but in the absence of an impact assessment or of a definition of endocrine disruption on which one could be based, it was

impossible to be certain about these effects or to judge whether there would be any positive benefit to human health from this element of the proposals.

The effects of the Regulation are therefore not yet clear, although work to develop definitive provisions for endocrine disrupters is underway. Furthermore, the degree to which pesticide may compromise compliance with the WFD is difficult to predict with confidence. A recent modelling exercise conducted by the Environment Agency indicated that some 6% of groundwater bodies and 12% of surface waters in drinking water protected areas may be at risk of failing their WFD requirements. This may, of course, change as the way in which pesticides and land management practices evolve and scientific and technological knowledge develop.

Directive on sustainable use

The other key element of the Strategy is the Directive on sustainable use. The Directive sets out a framework for delivering sustainable use: requiring member states to develop a National Action Plan to reduce the risks associated with pesticide use and then listing a number of measures which will populate these plans (these include: initial and continuing training of users, distributers and advisors; controls on sales; inspection of application equipment; facility to require notification of spray operations; protection of water, amenity and conservation areas; and promotion of low-input management systems). The directive recognises that member states have a variety of regimes to control the use of pesticides (and to some extent the Directive 'cherry picks' the best of these controls). It also recognises that member states will have a variety of issues that they wish to address.

Generally, the UK is well placed to meet the challenges contained in the directive:

- The existing Government Pesticides Strategy, with its stakeholder groups looking at protection of human health, water and biodiversity, improving practice in the amenity and amateur sectors and looking to address the product availability issue provides the basis for developing a National Action Plan;
- Training such as that offered by the NPTC, BASIS and NRoSO provide an infrastructure to deliver initial and on-going training;
- Sales controls may need slight tightening;
- Testing of application equipment, the NSTS provides an infrastructure to test equipment on a five and then (from 2020) three yearly basis.
- Protecting water, providing advice to enable users to use products and application techniques that pose less risk to the aquatic environment and drinking water, use of mitigation measures to tackle pollution caused by spray-drift, run-off, or drain-flow and minimising user in higher risk amenity areas;
- Protecting amenity and conservation areas, through the availability of advice on how to minimise use;
- Use of low input pest, weed and disease management systems, through the development and provision of advice on the sort of measures which constitute such an approach and then having an infrastructure to ensure this is disseminated to and adopted by users.

It is important to recognise that the degree of risk arising from the use of pesticides will be assessed by way of a series of indicators. There is an expectation that risk will continue to decline over time so it should be anticipated that these controls will develop over time.

The UK Government supported adoption of the Directive which represents a proportionate strengthening of controls on the use of pesticides.

NEXT STEPS

The Government is currently consulting on the measures necessary to implement the Directive (as the Regulation is largely 'directly applicable' there is little scope for determining implementation measures). The implementation project will be guided by the principles that: there should be no reduction in the current levels of control and that the measures which are developed should be proportionate to the risks which are being addressed. Once the consultation is concluded Ministers will consider responses and determine approaches/measures. The Government will then develop any necessary legislation and/or agree new or enhanced packages of voluntary measures with industry bodies to deliver the required actions ahead of the implementation deadline of 25 November 2011.

CONCLUSIONS

The Thematic Strategy for Pesticides is a significant development in the way pesticides are regulated and controlled. The two key elements of the Strategy: a new Authorisation Regulation; and Directive on the sustainable use of pesticides, have the potential the impact fundamentally on the availability of pesticide products and the way they are used.

The Regulation raises the bar in terms of the degree of protection afforded by the regulatory risk assessment process and brings some welcome improvements to the functioning of the regime. Some of these improvements should help facilitate greater availability of products, but this will be countered by provisions relating to the use of hazard triggers, comparative assessment and substitution.

Similarly the Directive seeks to increase the degree of protection at the point at which pesticides are used. It introduces measures which are aimed at minimising use and improving the knowledge and practice of users, distributors and advisers. It is anticipated that development and increased use of integrated approaches will provide some counter to pressure on the availability of products.

The Thematic Strategy package and impact of legislation such as the WFD exerts a downward pressure on pesticide use. The UK government believes that the use of products should be minimised, but does not necessarily accept that the degree of risk is dependent upon the quantity of products used. The Strategy envisages a fundamental change in the way some users approach pest, weed and disease control. It is important that it is implemented in a way which enables society to continue to derive the benefits of responsible pesticide use whilst minimising the risk of adverse impacts.

THE FUTURE CENTRAL ROLE OF IPM IN EU CROP PROTECTION: HOW CAN ECOLOGICAL RESEARCH BE PUT INTO PRACTICE?

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Summary: Food security issues and associated drivers are discussed, including rapid human population increase, climate change, loss of biodiversity, reduction in per capita cropped land, water shortages and pesticide withdrawals. IPM offers a suite of crop- and region-specific solutions to address these pressures and is compatible with organic, conventional and GM crops. A global review for several major crops covering 26 countries indicates that IPM can reduce pesticide use and increase yields in most cases studied. Research at SCRI with public and private sector collaborators is developing pest-resistant cultivars, semiochemicals, pest monitoring tools, diagnostics and predictive models as components of novel 'IPM toolboxes' to help meet future food production needs in developed and developing countries.

INTRODUCTION

Humans have been farming for about 600 generations, with rapid changes in intensification occurring over the last 2-3 generations (Pretty, 2009). Food security is rising up the global agenda. We live in an era of unprecedented human population growth, concurrently facing global threats from climate change, economic uncertainties, rising energy costs and tougher crop protection regulations. The United Nations predicts that there will be more than 4 billion people living under water scarcity by 2050, up from 0.5 billion in 1995. In addition, global per capita cropland is less than half of the figure in 1961 and more than 900 million people are hungry, with the projected global population set to increase to around 9.1 billion by 2050 (Engleman, 2009).

Recent EU reviews of pesticides have eliminated over 60% of active substances, with major and minor crops being affected. The changes to 91/414/EEC in 2010, based on hazard estimates rather than risk assessment, will further reduce pesticides available to the U.K. In addition, tightened Maximum Residue limits legislation, new Sustainable Use and Water Framework Directives will further limit the use of remaining pesticides, particularly herbicides. These trends present a huge challenge to many sustainability issues, not least food production when challenged by weeds, pests and diseases causing major crop losses despite costly agricultural inputs (pesticides, fertilizers, fossil derived energy). Eurostat surveys (2007) show that despite having only 8% of the world's agricultural area, the EU is the world's largest producer, user and exporter of pesticides. In 2007, the global crop pesticides market was $33.19 billion, of which EU alone accounted for $10.42 billion. Despite these costly inputs of pesticides (insecticides, fungicides, herbicides), current global figures for crop losses still show that pests, diseases and weeds are reducing food availability and security considerably. For example, crop losses due to pests are reported in the order of 26-

29% for soybean, wheat and cotton, while losses for maize, rice and potatoes are in the order of 31%, 37%, and 40% respectively. In cotton, losses due to pests can be as high as 80% (Oerke, 2006). Despite a clear increase in pesticide use, crop losses have not decreased during the last 40 years, a period when the global human populations has increased by approximately 2.6 fold. To put crop losses due to insect pests into a human perspective, it is estimated that the amount of food that insects consume pre- and post harvest is sufficient to feed more than 1 billion people. By 2050 it is thought that we will need to feed an extra 3 billion people. At the same time it is likely that insects will surge in numbers, pest types and in migratory range due to climate change factors, as evidenced by insect fossil records during past periods of rapid climate change (Currano, 2009).

CAN IPM RESEARCH HELP IN A SECOND 'GREEN REVOLUTION'?

Several options are discussed in terms of increasing food production and agricultural sustainability. All have 'trade offs': For example, we could expand cultivation into new lands, but then risk further loss of biodiversity and associated ecological services, valued conservatively at around $33 trillion/year (Costanza *et al.* 1997). Of this figure, beneficial insects provide key pollinators and biocontrol of pest species, valued at £117x10^9 and $417x 10^9 per year respectively. The challenge, therefore, is to reduce crop losses due to pest species whilst conserving beneficial species i.e. species selective measures tailored to specific agro-ecosystems, within a policy driven framework of reducing pesticide usage and adverse impacts.

Biological control has been practiced for many centuries, particularly in poorer and warmer countries which have serious pest problems but generally can't afford expensive pesticide inputs. Modern research on the development and integration of several combined pest control measures (IPM) can be traced back to 1959 at the University of California, where Stern, Smith van den Bosch and Hagen published a seminal paper on the integration of chemical and biological control of the spotted alfalfa aphid. In this study they defined now widely used IPM terms including 'economic thresholds', 'economic injury levels' and 'general equilibrium levels'. These ideas later evolved to include ideas of IPM blending and harmonising several multi-faceted approaches, including breeding for pest resistance and use of semiochemicals. The aim is to combine these 'IPM tools' in an organised way and to optimise them in an 'IPM toolbox' packages via a detailed understanding of 'systems level' ecology. This agro-ecosystem approach involves knowledge of soil organisms, crop and non-crop plants, multiple herbivores, several guilds of natural enemies and sometimes super-parasites. The main challenge is to devise farmer-friendly 'IPM packages' which work together to reduce pesticide inputs and which also reduce selection pressure on any one part of the system (e.g. development of pest populations with resistance to pesticides or with the ability to overcome host plant resistance genes in crops). It is believed that additive or synergistic interactions between IPM tools can increase the durability of individual IPM components and thus the whole IPM package.

So does IPM really work at the farm level? In a recent survey of 62 international IPM projects covering 26 countries and 25.5 million ha of crops including rice, maize, wheat, sorghum, vegetables, potato, cotton and legumes, over 60% of the projects resulted in reduced pesticide inputs and increased yield. On average, yields increased by 40% and pesticides were reduced by 60% (Pretty, 2005), indicating a broad potential for IPM globally.

IPM RESEARCH AT SCRI: UNDERSTANDING UNDERPINNING AGRO-ECOLOGY.

Plant breeding for pest and disease resistance in IPM

The use of pest resistant cultivars is a key foundation of a durable IPM programme. At SCRI with MRS Ltd and other plant breeding groups, we develop soft fruit, cereals and potatoes with genetic resistance to key pests and pathogens including raspberry aphids, potato blight and barley yellow mosaic virus. New biotechnologies including marker-assisted selection methods, QTL analysis of complex genetic traits and improved plant transformation methods are helping to speed up the process of getting new pest-resistant crops onto the market. For many crops this typically still takes 10+ years, so long term planning and funding security is required. Reliance just on pest resistant crops, with typically one or few major resistance genes, inevitably produces strong selection pressure and crop protection failure. This is demonstrated by SCRI's long term research and breeding for raspberry aphid resistance (Birch *et al.*, 1994). Virulent biotypes of the large raspberry aphid, *Amphorophora ideai*, overcame the first resistance gene in c. 40 years but have overcome a replacement resistance gene in less than 10 years. We are thus at a 'tipping point' for some pests, where they can overcome single control measures (e.g. R genes) in a shorter time than scientists can create new solutions. To make matters worse, growing crops like raspberry under plastic tunnels means we have increased the window of attack from 2-3 months up to 10-11 months and also provided a more suitable climate for two other aphid pest species to thrive, *Aphis ideai* and *Macrosiphon euphorbiae*. On the positive side under protected cultivation, certain key predators including spiders and hoverflies are greatly enhanced compared to the open raspberry fields, opening up new opportunities for biocontrol as an IPM tool for soft fruit and other protected crops (Birch *et al*., 2008).

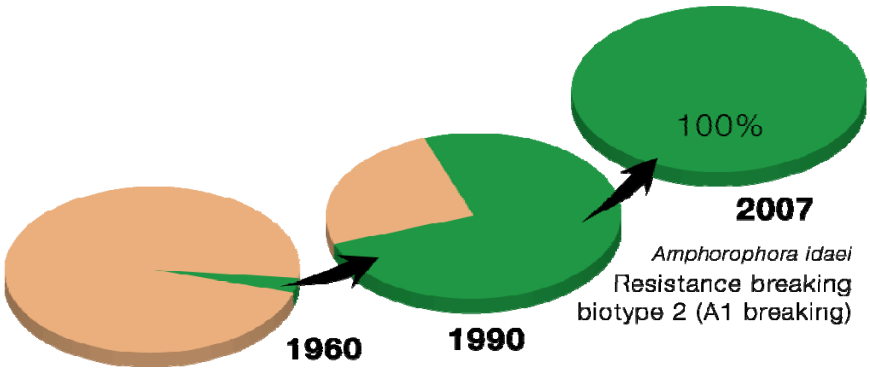

Figure 1. Effect of selection pressure on virulent raspberry aphid biotypes when a single control measure (major gene resistance) is used in a raspberry monoculture system (Birch *et al.,* 1994; 2004).

Use of semiochemicals in IPM

SCRI together with collaborative research groups at SAC, NRI and EMR have developed unique, multi-disciplinary approaches involving entomologists, phytochemists and biophysicists to identify novel pest attractants and repellents. For example, under a five year Defra Hortlink project, we have used our fundamental chemical ecology knowledge to develop and commercialise the first precision monitoring trap for raspberry beetle, in collaboration with Agrisense Ltd. The combined studies of floral volatile chemistry

(Robertson *et al.,* 1994), chemical ecology, insect behaviour and electrophysiology provide a way of using 'biomimicry' to fool the raspberry specialist pest into a trap that represents a giant raspberry flower in terms of key host recognition signals (colour and smell for this pest). This trap is now being successfully used in the UK, Norway, Sweden and France to fine-tune and reduce the application of selective insecticides to pest 'hot spots' in plantations, based on weekly catch thresholds (Birch *et al.,* 2004). In recent on-farm trials, this IPM approach, using raspberry beetle traps as monitoring tools, gave similar levels of crop protection as the farmers standard practice, using a currently recommended insecticide (Birch *et al.*, in prep). The same approach is now being developed for other types of pests in the UK and across the EU, via HDC, EU and TSB funding applications.

Optimising food webs for biocontrol in IPM

SCRI undertakes long-term studies on agro-ecosystems, focusing on raspberry (as a model perennial system) and several arable crops including oilseed rape, barley, beet and maize (as model annual crops within rotations). Hawes *et al.* (2007) used data from the Farm Scale Evaluations to demonstrate that in the arable systems studied, there was strong evidence of 'top down' control on herbivores by generalist predators and more specialist parasitoids. This effect differs markedly from results obtained from ongoing experiments comparing food webs in field-grown versus protected raspberries in polytunnels (Birch *et al*., in prep). Raspberries bred at SCRI provide a unique UK example of a crop plant with several types of genetic resistance to aphids. We find that these pest resistance genes provide a strong 'bottom up' effect on aphids, complemented by several key natural enemies including spiders, hoverflies and entomopathogenic fungi. These biocontrol agents operate in a 'top down' manner much more effectively on the protected crop than in the open field, thus helping to offset the large increase in three different aphid species under this warmer, season-long micro-climate in polytunnels.

Ecological engineering at field and landscape scales

Re-instating landscape diversity, connectivity and complexity can promote system functions including biocontrol and pollination. It is accepted that returning agroecosystems to a position of ecological balance in this way can reverse adverse effects of intensive agriculture and also promote natural pest regulation via biocontrol (Nicholls and Altieri 2004). This approach is generally consistent with IPM objectives and is the focus of ecological engineering strategies such as the habitat manipulation approach at farm level to promote conservation biocontrol (Jonsson *et al*., 2008) and the cultivation of crop mixtures at field level to decrease pathogen pressure and spread (Newton *et al*., 2009). However, when faced with the complexity of agroecosystems habitat-based ecological engineering is not a precise tool. The complexity of the system poses challenges for achieving predictable pest suppression. To overcome this demands a more focussed approach, in which detailed knowledge of the specific ecological processes and interactions are used to devise methods of ecological engineering that have a predictable impact on pest suppression over multiple seasons. This is easier to address for specific pest-crop combinations and underlies the concentration on the development of IPM tools that act within restricted components of the system. However, the challenge is to take a more general approach, assisted by mathematical models, that seeks to regulate or suppress a range of pests and diseases and operates under a range of conditions, as these are more likely to result in sustainable solutions. This can only be achieved with a deep understanding of the 'ecological machinery' of arable systems and the mechanisms linking pest populations to the arable environment. Habitat manipulation is a

key area of research: Field margins, shelter belts, 'green' dispersal corridors linking fragmented landscapes are all being investigated, using advanced spatio-temporal modelling linked to aerial/satellite imaging for remote sensing and 'ground truthing' via targeted sampling of prioritised functional biodiversity groups within agricultural landscapes. Sophisticated modelling approaches that incorporate spatial and temporal heterogeneity are also being developed at SCRI to test various 'ecological engineering' scenarios at field and landscape scales.

GLOBAL APPROACHES FOR OPTIMISING IPM

According to Heinrichs *et a.l* (2009) the main development needs to optimise IPM globally are: 1. *Problem identification* (current and future pests affected by crop management and climate change). 2. *Research activities* (ecological, agricultural, technical and socio-economic issues). 3. *Communication, extension and training* (on farm demonstrations, field days, farmer field schools). For these to have fastest impact, close collaboration between researchers and farmers in required. The SCRI LEAF Innovation Centre together with MRS Ltd provide a unique route for delivering IPM tools and packages to end-users via our 'research pipeline'. Our effectiveness is demonstrated by the commercialisation of pest and disease resistant crop cultivars, genetic markers for selecting novel pest resistance traits, diagnostic tests and pest monitoring traps linked to action thresholds, for greatly reducing insecticide applications. The demand from end users (consumers, growers, supermarkets, policy groups) is rapidly increasing in this area, reflected by rapidly increasing IPM-related funding from Hortlink, Technology Strategy Board, EU FP 7 and other agencies. We are optimistic that the long term, multi-disciplinary groundwork at SCRI will provide key IPM tools for the future. IPM is compatible with organic, conventional and GM crops (Birch and Wheatley 2005) and so has benefits to all sectors of crop production. This is essential in a century of urgent need for increased food security, biodiversity protection and agricultural sustainability under threat from climate change and global human population growth.

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THE SCOTTISH SOIL FRAMEWORK – NEW PERSPECTIVES

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Summary: The recognition that soils perform various roles and services in the natural, cultural and built environments is moving the debate on soil protection away from a 20th century view of soil as primarily a growth medium for biomass and food production towards a new paradigm for the management of soil as a carbon sink, environmental buffer and economic asset in its own right. The recently published Scottish Soil Framework will ensure that "soils are recognised as a vital part of our economy, environment and heritage, to be safeguarded for existing and future generations". The objectives of the Framework are to instigate a process by which key stakeholders will work together to achieve more effective management of our soils. This will ensure that not only soils themselves are protected but also that impacts on other aspects of the environment are mitigated (e.g. greenhouse gas loss from soils, flooding and pollution).

INTRODUCTION

Across the world, there are as many words to describe the soil as there are ways of using or making a living from soils. To most of us, soil is just mud or dirt; a planner might see it as an overburden or regulated waste, and a geologist will describe it as 'regolith'. For food producers, soil is the basis for their livelihoods. Soils are also features of the natural heritage, with their own intrinsic conservation potential and playing a part in the processes that form and alter the biological and geological diversity of our natural heritage. Yet, there is no simple definition of what a soil is or does. A soil can be defined by the sum of its constituents (air, fluid, solid, biomass), the functions it performs (e.g. basis of food and biomass production, support to biodiversity and habitats, environmental buffers) or the physical biological and chemical properties it exhibits (e.g. nutrient level, pH, compaction, contaminant level).

Effectively, soils are important to all because they are a valuable natural resource which lies at the heart of all life on Earth, providing and supporting key ecosystem services. But soils cannot be treated as a renewable resource. Natural soil formation is a continuous but slow process that may require millennia to achieve climax state. Soils, therefore, must be seen as a non-renewable and finite asset which is increasingly coming under pressure from anthropogenic activities and natural changes. Worldwide, there are many historical examples of unsustainable use of soil and the resulting widespread economic and societal impacts. In the 1930s in the USA, fertile topsoil was swept away in the 'Dust Bowl'; President Franklin D. Roosevelt then famously said that 'The nation that destroys its soil, destroys itself', and through the New Deal policy, encouraged a new approach to farming in order to avoid similar catastrophes. The recognition that soils perform various roles and services in the natural, cultural and built environments is moving the debate on soil protection away from a 20th

century view of soil as primarily a growth medium for biomass and food production towards a new paradigm for the management of soil as a carbon sink, environmental buffer and economic asset in its own right. Protection and sustainable use of soil resources must take account of the diversity of soil capacity, resistance and resilience to pressures. Healthy soils are key to many aspects of rural economic growth and management of natural resources. The development of a national soil protection framework is a first step to ensure that the sometimes conflicting demands on Scotland's soil resources can be assessed. The Scottish Soil Framework (Scottish Government, 2009a) will ensure that soils are able to adapt to the current and future challenges of a changing climate.

WHY DOES SOIL MATTER TO ALL?

Soils contribute to the provision of environmental, economic and societal goods and benefits. In recent years, the concept of soil quality, defined as 'the capacity of a soil to function within the ecosystem boundaries and interact positively with the environment external to this system' (Larson & Pierce, 1991), has helped to identify soil indicators and environmental standards for the assessment of state and pressures on soil. Conventionally, seven broad soil functions are recognised and used to describe the roles that soils perform: providing the basis for food, forestry and other biomass production, controlling and regulating environment interactions, storing carbon, supporting biodiversity, cultural and archaeological heritage, providing raw material and a platform for development.

In Scotland, centuries of sustainable use by land managers has kept our soils in relatively good health (Towers *et al.,* 2006). Viable crops, livestock and timber production have been connected to the natural fertility of our soils and the skills of our land managers to maintain and increase the properties of soils that further land productivity and economic benefits. Understanding the environmental impacts of farming practices is the first step towards keeping soil in good agricultural and environmental condition and maintaining healthy soil resources capable of delivering a wide range of environmental and ecological functions. Soil biological, chemical and physical quality is not only essential to crop and biomass production but is also crucial to the protection, restoration and enhancement of soil diversity, rare soil types and important soil functions. Protecting soil biodiversity is as important for preserving healthy natural habitats as for maintaining productive farmland (JNCC, 2007). Soil organisms actively modify soil structure and composition, and hence they largely determine soil function and affect plant growth and crop yields. Soils with high organic content (peat and organo-mineral soils) also act as carbon sinks and potential contributors to greenhouse gases when disturbed.

The wet and cold climate of Scotland, coupled with distinctive geology and landforms, has given rise to very diverse soils. Over 3000 Mega Tonnes of carbon are held in Scottish soils, equivalent to around 50% of the total UK soil carbon store (Scottish Executive, 2007). Often Scottish soils have moderately fine textured subsoil that can impede drainage periodically. Despite the majority of Scotland's territory being classed as rural, much of it remote, few soils in Scotland can be described as 'pristine' as little of the countryside has been untouched by human activities. Currently around 25% is used for arable crops and improved grassland, and a further 17% is under woodland. With only 17% of Scotland's soils being classified as prime agricultural land (LCA class 1, 2 and 3a), most land is only capable of supporting rough grazing. Changes to soils that affect their chemistry, physical properties and biological make-up are often irreversible.

Research undertaken in support of the development of the Scottish Soil Framework (Towers *et al*., 2006) and recent studies from the Scottish Government (e.g. Programme 3: Protection of the Nation's Soils (Scottish Government, 2008) and ECOSSE (Scottish Executive, 2007; Scottish Government, 2009b)) have shown that soils are now at risk from the direct impact of climate change and mitigation / adaptation measures (e.g. changes in land use, increased demands on land for energy production). Scottish soils are a sink of carbon equivalent to 25 times the size of UK above-ground vegetation, and, if degraded, could become a significant source of greenhouse gases and carbon loss. Soil organic matter is one of the key properties of soil helping to maintain soil fertility, structural properties and water holding capacity. Promoting good soil carbon status has always been a cornerstone of good agricultural practice (as an essential nutrient for plant growth and provider of good soil structure). Good soil carbon status is also a recognised contributor to wider environmental functions (i.e. improving soil water retention capacity, retention of pollutants and reducing erosion risk) and is now also becoming pivotal to the development of low carbon economy strategies (as a vital component of the global carbon cycle and local sinks and sources of greenhouse gases). Loss of soil organic matter is not just a threat to soil multi-functionality but is also an indicator of the impact of the changing climate.

SOIL PROTECTION – POLICY CONTEXT

Nowadays, most aspects of human activities are controlled and regulated, ensuring that their impacts on air, water quality and biodiversity are not detrimental to human health and the natural environment. But, until recently, soils have not gained equal recognition to water, air and other parts of the natural heritage in sustainable environmental management and protection policy. Awareness of the role of soils has grown in recent years from several international initiatives. The UN Millennium Assessment (MA) (2005) provides an assessment of the consequences of ecosystem change for human well-being and the scientific basis for actions needed to enhance the conservation and sustainable use of those systems. The MA does not directly focus on soil or land per se but considers the relationship between soils and the services they provide for human well-being and poverty reduction. Other UN conventions on Biological Diversity, Climate Change and Desertification also consider aspects of soil protection (Giger, 2006).

At European level, the publication in 2006 of the Thematic Strategy for the Protection of Soils (European Commission, 2006a, 2006b, 2006c) set the frame for high-level action on soil protection. The proposed Soil Directive sets out common principles for protecting soils across the EU. Within this common framework, EU Member States will be in a position to decide how best to protect soils and how to use them in a sustainable way in their own territories. The Commission also underlines the crucial role that soils can play in mitigating climate change, and has undertaken a review of existing information on the interrelations between soil and climate change (Schils *et al*., 2008).

The need for the sustainable use and management of soils is also implicitly recognised in many environmental policies. A holistic understanding of soil functionality in any location requires an accurate assessment of the current status of the soil, the drivers of change affecting the soil status (e.g. land use change and climate change), the threats/risks to soil (e.g. contamination, planning development, loss of biodiversity, coastal realignment and coastal squeeze) and the impact of stress and the response of the soil (linked to soil resilience and resistance).

SOIL PROTECTION IN SCOTLAND – FRAMEWORK FOR ACTIONS

Since the publication in 2006 of the EU Soil Thematic Strategy, there is more awareness of soils in policy development but it remains that at a strategic level, synergies and tensions between different policy areas - such as agriculture, forestry, renewable energy, flood management and rural development - often remain implicit rather than explicit. Past approaches to soil protection have been sectoral, with legislation and codes of practice developed in response to specific pressures (e.g. PEPFAA Code, Cross Compliance). With changes in land use and perceptions of soil values, come also new or increased threats and pressures and the need for a better understanding of the relationship between soil and environment to provide evidence to develop new policy.

The Scottish Soil Framework (SSF) published by the Scottish Government in May 2009 is a step towards a more systematic approach to soil protection. It was developed through concerted engagement between the Scottish Government and its main stakeholders. The SSF presents the Scottish Government's vision that 'soils are recognised as a vital part of our economy, environment and heritage, to be safeguarded for existing and future generations'. The development of the SSF has spanned two Scottish administrations and has to be seen as a national initiative responding to an increased awareness of the wider values of our soils and the pressures on them in a changing climate. The SSF was developed in parallel with the proposals for an EU Soil Framework Directive. However, the SSF is not intended as a mechanism for the transposition of any future EU Directive, nor is it linked to the development of new national soil legislation. The main aim of the SSF is to 'promote the sustainable management and protection of soils consistent with the economic, social and environmental needs of Scotland'. The SSF recognises that soils perform various roles and services in the natural, cultural and built environments but that most soils are managed specifically to optimise the delivery of one or two functions.

Evidence gathered to support the development of the SSF has also shown that most soils in Scotland are in generally good health. But it also identifies a range of local and national threats and pressures to soils arising from changing climate and land use policy. To address these issues, the SSF identifies 13 Soil Outcomes (SO) which will align with and support the Scottish Government purpose of increasing sustainable economic growth and the National Outcome to 'value and enjoy our built and natural environment and protect it and enhance it for future generations':

SO1 Soil organic matter stock protected and enhanced where appropriate
SO2 Soil erosion reduced and where possible remediated
SO3 Soil structure maintained
SO4 Greenhouse gas emission from soils reduced to optimum balance
SO5 Soil biodiversity, as well as above ground biodiversity, protected
SO6 Soils making a positive contribution to sustainable flood management
SO7 Water quality enhanced through improved soil management
SO8 Soil's productive capacity to produce food, timber and other biomass maintained and enhanced
SO9 Soil contamination reduced
SO10 Reduced pressure on soils by using brownfield sites in preference to greenfield
SO11 Soils with significant historical and cultural features protected
SO12 Knowledge and understanding of soils enhanced, evidence base for policy review and development strengthened
SO13 Effective coordination of all stakeholders' roles, responsibilities and actions

The Soil Outcomes cover all aspects of soil functionality and interactions with other components of the natural heritage. It is clear that achieving these Soil Outcomes can only be taken forward through joint efforts involving the key partners and stakeholders. The SSF, as published, cites 39 activities which will contribute to the delivery of the Soil Outcomes. Some activities relate to specific activities currently led by Scottish Government departments. Others are on-going or committed stakeholder-led projects and as such fall directly under the stakeholder's ownership.

THE WAY FORWARD

The SSF establishes a Soil Focus Group (SFG) whose role includes raising awareness of the Scottish Soil Framework, and providing a platform for liaison and pooling of resources and information amongst key delivery partners and stakeholders. The SFG will contribute to developing and building on the activities set out in the Scottish Soil Framework, identifying and prioritising additional actions where necessary. It is expected that the SSF will give all stakeholders access to more reliable data and evidence on soil status, threats and processes. This is essential to identify soils at risk from changing climate and land use. It will ultimately ensure a more effective delivery of advice for the sustainable management of soils. It will help to increase awareness of soil issues among the general public and organisations and to promote understanding of the role of soil in the delivery of natural heritage benefits and services. This should lead to a better integration of soil issues at early stages of policy development. It will provide more engagement with other stakeholders and the opportunity to work with partners to develop collaborative approaches to soil protection and the provision of improved guidance for sustainable use and management of soils.

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THE IMPACT OF AGRI-ENVIRONMENT SCHEMES ON SCOTLAND'S BIODIVERSITY

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Summary: 230 scheme farms (Countryside Premium (CPS), Rural Stewardship (RSS) or Organic Aid (OAS)) were surveyed each with a paired, nearby non-scheme farm in order to compare biodiversity between farm types and (for RSS) to compare change in biodiversity over time. The CPS farms supported more wildlife (individuals and species) than the non-scheme farms. The RSS farms were also richer in wildlife than the non-scheme farms during both surveys. Biodiversity increased on the RSS and non-scheme farms between surveys, but the rate and nature of this increase was not significantly different. There were no differences in biodiversity between the small sample of OAS and non-scheme farms.

INTRODUCTION

The recent loss of biodiversity on UK farmland, particularly during the last quarter of the 20th century, has been well documented (e.g. birds: Vickery *et al.* 2004; invertebrates: Benton *et al.* 2002; plants: Sutcliffe & Kay 2000) and it is widely accepted that changes in agricultural practices prior to and during this period have caused this decline in biodiversity.

Agri-environment schemes (AES) are currently the main vehicle through which farmers can receive compensatory payments to introduce management changes that would have environmental benefits. The Countryside Premium Scheme (CPS) was open to farmers in Scotland between 1997 and 2000 when it was superseded by the Rural Stewardship Scheme (RSS), which itself closed to new applicants in 2007. Both aimed to conserve valuable habitats and other features on the farm (e.g. archaeological sites, landscape features) and also to increase biodiversity by introducing novel habitats and management practices (Anon 2000, 2006). The Organic Aid Scheme (OAS) was open to applicants between 1994 and 2006 with its prime aim being to encourage and support those wishing to convert to or maintain organic farming practices, but with the proviso that biodiversity was conserved where possible and not destroyed (Anon 2005). This paper reports some of the farmland bird results from a five year study of the impact of these three schemes on biodiversity in Scotland (Parish *et al.* 2009)

MATERIALS AND METHODS

This study was conducted across Scotland between 2004 and 2008. In all, 88 RSS farms were surveyed, 105 CPS and 37 OAS farms, together with 230 similar, nearby (< 10 km) paired non-scheme farms. The CPS farms and some of the OAS farms (the ones already established in the scheme) were surveyed in one year only. The RSS farms and the remainder of the OAS farms were surveyed first when they had just joined their schemes but before management plans had been implemented. Then they were resurveyed three years later to allow an estimate in the change in biodiversity over time and how the scheme and non-scheme farms compared. Surveys of vegetation, invertebrates and birds were carried out, but this paper focuses on the bird data. Full details of the bird survey methodologies and analytical approaches are provided in Parish *et al.* (2009).

Analyses

The maximum count for each bird species across the two counts per year was used in the analyses. As well as looking at the total number of individuals/species recorded, the bird data were divided into groups based on conservation status (e.g. species having a Biodiversity Action Plan and/or those Red and Amber listed species designated as being of 'greatest' and 'moderate' conservation concern in the UK) or shared ecological traits (i.e. 'Crows', 'Pigeons', 'Tits', 'Finches', 'Hirundines', 'Woodland warblers', 'Farmland warblers', 'Waders', 'Ground feeders' and 'Gamebirds'). The latter classification allowed exploration of potential mechanisms behind possible major effects, e.g. if only seed-eating birds showed a response then it might be that the schemes were providing something only the seed-eaters could exploit. Details of the species included in each functional group are provided in Parish *et al.* (2009).

Analysis of CPS and single-visit OAS farms

For the CPS and single-visit OAS analyses, univariate tests were used. Paired t-tests were carried out on the log-transformed data (x+1). Binomial tests (distribution-free) were also performed, but the results agreed with the t-tests and are not mentioned further. The Shannon diversity index was also calculated and compared between scheme and non-scheme farms.

Analysis of RSS and re-surveyed OAS farms

For the basic analyses (simply comparing the bird data between the scheme and non-scheme farms and the change over time) of RSS and OAS farms which were re-surveyed after three years, an Analysis of Variance (ANOVA) was performed on log-transformed data (x+1). Here, the ANOVA models included a Pair effect (identifying each pair of farms in the sample), Time effect (visit 1 or visit 2), Scheme effect (scheme or control) and the Scheme by Time interaction. The interaction measures whether the change in biodiversity over time is different between the scheme and non-scheme farms, and is the effect of most interest in this study. The pair effect reduces the residual variance by accounting for differences between the different pairs. The log transformation is appropriate because any effects would be expected to be multiplicative rather than additive, and because it reduces the influence of the occasional very high count. The assumptions of the ANOVA are met because although the log data may not themselves be normally distributed, the residuals after fitting the main effects are nearly so.

RESULTS

CPS

Fifty two percent of the 20 comparisons revealed significant differences in bird numbers between the CPS and non-scheme farms, each showing that CPS farms were more biodiverse than the non-scheme farms. For the total count 30% more birds, of 15% more species, were found on the CPS farms compared to the non-scheme farms (Table 1). This difference was reflected in the numbers of red and amber-listed birds, and amber-listed species, and amongst a diverse sub-group of functional categories (tits, finches, woodland and farm warblers, and ground feeders), which includes insectivorous and granivorous species.

RSS

The ANOVA results show that both Time and Scheme had a major effect on the bird data with 75% and 90% of comparisons significant respectively, including the total number of individuals, species and Shannon Index (Table 1). This reflects the fact that in nearly all cases bird numbers (or numbers of species) increased between surveys on both the RSS and non-scheme farms, and that the number of individuals or species was higher on the RSS farms than the non-scheme farms (90% of comparisons in year-1; 100% of comparisons in year-4). The total number of species increased between surveys by 16% on RSS farms and the number of individuals by 29%, whereas for the non-scheme farms these figures were 12% and 16% respectively. The number of species was 10 to 16% higher on the RSS farms, and the number of individuals was 25-41% higher. However, only one interaction term was significant (suggesting a difference in the rate of change over time between the RSS and non-scheme farms) – that for waders. This could have been a chance result given the large number of comparisons. The number of waders decreased over time on the RSS farms whilst increasing on the non-scheme farms. Therefore, there was no conclusive evidence of the RSS influencing bird numbers after the farmers joined the scheme, either in terms of the rate of change in bird numbers (or number of species), or in the nature of the changes over time – all sub-groups responded similarly.

Table 1. Total bird counts for all three schemes. CPS = Countryside Premium Scheme; RSS = Rural Stewardship Scheme; OAS = Organic Aid Scheme. OAS single = OAS farms only visited during one year. C1 = count from year 1; C2 = count from year 2. C2-C1 = C2 minus C1. S = Scheme farms; NS = Non-scheme farms. N = number of pairs of farms.

Scheme	N	No. species		No. individuals		Shannon Index	
		S	NS	S	NS	S	NS
CPS	105	23.2±0.7	20.2±0.7	140.0±10.0	107.6±6.5	2.5±0.0	2.4±0.0
RSS C1	88	19.1±0.7	17.3±0.7	107.7±8.3	85.9±7.2	2.3±0.0	2.2±0.1
RSS C2	83	22.0±0.7	19.0±0.8	139.2±10.3	98.6±6.8	2.5±0.0	2.4±0.1
RSS C2-C1	80	3.0±0.7	2.1±0.6	31.1±12.1	13.7±7.9	0.1±0.0	0.2±0.1
OAS single	22	23.6±1.8	20.8±1.5	127.8±14.8	116.7±13.9	2.5±0.1	2.4±0.1
OAS C1	15	19.9±1.4	17.9±1.7	82.7±8.2	82.1±10.5	2.6±0.1	2.3±0.1
OAS C2	16	22.9±1.6	19.3±2.0	119.9±18.0	113.2±17.5	2.6±0.1	2.3±0.1
OAS C2-C1	15	2.7±1.0	0.3±1.6	24.3±11.8	25.5±11.5	0.0±0.1	0.0±0.1

OAS farms visited once

Total bird/species number did not differ between OAS and non-scheme farms (Table 1). In fact there was only one significant difference among the 20 comparisons between OAS and non-scheme farms: there were 23% more species of amber-listed birds on the OAS farms than non-scheme farms (Table 1). Sample sizes here were smaller than for the CPS and RSS analyses, but the other (non-significant) comparisons in the OAS analyses do not suggest a consistent effect of the scheme relative to the non-scheme farms as the number of species or individuals present is not necessarily larger on the OAS farms.

OAS farms visited twice

There were only four (20%) significant Scheme effects here. The total number of bird species and the overall bird diversity (as estimated by the Shannon Index) were higher on organic farms than the non-scheme pairs (Table 1). Tits and finches were also more abundant on OAS farms. The only significant Time effect (5% of 20 comparisons) was for woodland warblers which increased over time on both the OAS and non-scheme farms.

DISCUSSION

The CPS and RSS farms supported more birds, of more species, than their non-scheme pairs and for the RSS farms this was the case before their management plans were implemented as well as three years later. Bird numbers increased between the two survey years on RSS farms and their non-scheme pairs, but there was no difference in the rate or nature of that change over time. There was little evidence of increased bird numbers on OAS farms relative to their non-scheme pairs and for those monitored twice after a three-year interval there was little evidence of any increases over time (nor for the non-scheme farms here). The number of pairs of farms was lower for the OAS analyses than for the other schemes, but it was not insubstantial.

The difference in abundance or number of species of birds between the CPS and RSS farms and their non-scheme partners was repeated in a diverse selection of functional groups, with the scheme farms supporting more insectivorous, granivorous and omnivorous species, and species with varied nesting requirements. This in turn suggests that the mechanisms behind the difference (not directly investigated here) were likely to be relatively complex involving more than one demographic and ecological route.

The high bird numbers on the CPS and RSS farms almost certainly arose because of the selection procedure for these schemes, which favoured farms that already had potentially valuable wildlife habitats or species present. The OAS farms were selected primarily on other characteristics and so were not unlike their non-scheme pairs in terms of bird numbers at the outset of the study. In a review of Agri-environment schemes (AESs) across Europe, Kleijn and Sutherland (2003) found that only 54% of species or groups studied (not just birds) demonstrated a greater abundance or species richness on the scheme farms than the non-scheme farms, even though there were severe weaknesses in the design of many studies that meant greater levels of biodiversity were likely to be found on AES farms than the non-scheme comparisons (very few studies collected baseline data before farm management changed and then monitored changes over time). More recently, a study of Ireland's AES

concluded that little benefit was to be had from the scheme and that landscape features were more pertinent in influencing biodiversity (Feehan *et al.* 2005).

In contrast to the RSS and CPS sites, no substantive differences in biodiversity between OAS and non-scheme sites were found, either in terms of differences between OAS and non-scheme farms at any one point in time, or in terms of changes over time. Other studies have reported mixed results for the impact of organics on biodiversity, with the majority suggesting they have a positive impact (e.g. Fuller *et al.* 2005, Holzschuh *et al.* 2008), but some showing mixed effects (Bengtsson *et al.* 2005). Others have again shown that any effect may vary among taxa (Fuller *et al.* 2005) and from place to place (Rundlöf & Smith 2006). Organic farming is distinct from both the CPS and RSS in that the conservation of biodiversity is not one of its prime aims. Perhaps differences between OAS and non-scheme farms were not found here because of relatively small sample sizes compared to the other scheme comparisons. This is a possibility, but compared to many of the other studies on organic farming quoted above the sample sizes are larger. The finding suggests that over much of Scotland, organic farming does not produce the benefits for biodiversity that AESs do and that, on its own, organic farming cannot be relied upon to restore the post-intensification losses of farmland biodiversity. That is not to say that organic farming should no longer be promoted and supported as there are many other aims to this system of agriculture which fall outside the remit of this study, but it does mean that perhaps additional elements need to be added to the requirements of organic farming in Scotland to maximize the multi-functional benefits of the system.

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BIOMASS OPPORTUNITIES – ADDRESSING AGRICULTURAL AND ENVIRONMENTAL REQUIREMENTS

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Summary: The UK aims to obtain 15% of all energy from renewables by 2020, requiring a significant increase in renewables contribution to each of the three energy sectors; electricity, heat and transport. Large areas of bioenergy crops are predicted to be required. This paper is based on a study commissioned by the National Non Food Crop Centre (www.nnfcc.co.uk) to consider the environmental and agricultural implications of changing cropping patterns to increase supplies of biomass for energy generation. The key focus was on biomass crops and conventional crop co-products (straw) for fuel and energy end-uses. Factors necessary to increase biomass production from agriculture, whilst minimising environmental impacts, were outlined. The overall conclusion was that increasing biomass production can potentially provide a range of positive environmental outcomes but a number of agricultural, economic and non-economic issues will need to be addressed before this can be achieved fully.

INTRODUCTION

The UK aims to obtain 15% of all energy from renewables by 2020, requiring a significant increase in renewables contribution to each of the three energy sectors; electricity, heat and transport. Bioenergy in the form of biomass or biofuel crop products can contribute to each of these sectors. Large areas of bioenergy crops are predicted to be required; the UK Biomass Strategy concluded that 350,000 ha of perennial energy crops were needed by 2020, in addition to an estimated 740,000 ha for transport fuel from biofuel, whilst the European Environment Agency anticipated that 1.1 m ha could be made available by 2020. These predictions are set against current biomass crop production, comprising of willow from short rotation coppice (SRC) and Miscanthus, of 15,500 ha, indicating that a sharp increase in area of production is needed to meet demand.

This paper is based on a study commissioned by the National Non Food Crop Centre (Booth *et al.,* 2009) to consider the environmental and agricultural implications of changing cropping patterns to increase supplies of biomass for energy generation. The key focus was on biomass crops and conventional crop co-products (straw) for fuel and energy end-uses.

Factors necessary to increase biomass production from agriculture, whilst minimising environmental impacts were outlined.

BIOMASS CROP PRODUCTION; CURRENT STATUS AND FUTURE PROSPECTS

The current and potential future biomass crop feedstock supply has been determined in a previous study (Kilpatrick *et al.*, 2008). Potentially available biomass could be available in the form of straw from set-aside land brought into production, energy crop production from set-aside land brought into production, energy crops on arable land, conversion of temporary grassland to energy crops and conversion of permanent grassland and rough grazing to short rotation forestry with consequences for land use change as shown in Table 1.

Table 1. Scenario for land use change with increasing biomass crop production

Current land use	New land use	Hectares	Oven Dried Tonnes (million)	% Land use change
Bare fallow	Miscanthus	296 000	3.6	7% arable
Arable	Miscanthus	211 000	1.8	5% arable
Temporary grass	Miscanthus	67 000	}	5.5% temporary
			} 1.6	grass
Temporary grass	Short Rotation	67 000	}	5.5% temporary
	Coppice (SRC)		}	grass
Long term grass/	Short Rotation	1 800 000	7.5	16% temporary
rough grazing	Forestry (SRF)			grass/ rough grazing

IMPACTS FOR THE ENVIRONMENT

Implications of increasing the cultivation of biomass crops for the wider environment

Carbon dynamics of the soil/crop system are key indicators of the environmental implications of land use change. This was assessed using computer modelling techniques (ECOSSE, SUNDIAL). Detailed management information, taking into account all factors affecting input and output, were considered in order to simulate changes in the soil/crop system on a site basis. Implications of these site changes were determined at a national scale with reference to weather and soil data.

Effect of land use change of converting arable land to Miscanthus and long term grassland to SRC were determined, following the scenarios outlined in Table 1. Converting arable soils to Miscanthus results in an exclusive increase in soil organic carbon stocks. By comparison, converting grassland to SRC result in a loss of soil carbon due to soil disturbance and initial reduction in plant input as trees are not fully grown. It was estimated to require 7 decades for the carbon lost from disturbing soil under long term grass to be accumulated under SRC.

Effects on Biodiversity

In terms of biodiversity, biomass energy crops are associated with generally higher ground flora and bird species richness than for arable crops. SRC is harvested every 2 – 4 years and Short Rotation Forestry (SRF) will be harvested after 8 – 20 years and this extended harvesting cycle will maintain biodiversity compared to annual crops.

As Miscanthus is not native to the UK it may support less biodiversity than SRC and as it matures its biodiversity value may decline due to increased crop density. The species used for SRF will determine lightness of its canopy, a major influence for biodiversity. The relatively long lived nature of SRF before harvesting can mean that it introduces a different and potentially more stable habitat into the agricultural landscape. Provided that large-scale monocultures and habitats with greater biodiversity value are avoided, then the establishment of SRC, Miscanthus or SRF on a farm would generally be expected to add to the mix of already existing habitats and thereby enhance the bird and invertebrate biodiversity on that farm.

Effects on Crop Inputs

Biomass energy crops contrast to conventional arable crops in that they are perennial, clearly not requiring soil cultivation every year. This has an immediate effect to reduce fuel inputs compared to annual crops. In addition, biomass energy crops require less fertiliser and agrochemical inputs, giving benefits for greenhouse gas emissions. The manufacture of nitrogen fertiliser is particularly demanding on energy and the lower N requirement from biomass crops makes a significant contribution to improving the environmental profile of these crops.

Effect of biomass cropping on carbon accounting on-farm

Carbon footprinting, a measure of the impact of an activity on greenhouse gas emissions from a product over its lifetime, was carried out to consider the impact of introducing biomass crops into farm systems.

The effect of introducing Miscanthus or SRC into an intensive arable farm to replace 12% total arable had a positive effect in reducing emissions. Reductions in emissions were due to reductions in fuel and fertiliser, mainly in nitrous oxide emissions caused by fertiliser. The effect of introducing SRC or SRF into an upland/hill farm to replace 12% improved grassland use and consequently reducing number of cattle resulted in a very small reduction in GHG emissions due to reduction in fuel and fertiliser use. A significant reduction was due to reducing cattle number and therefore minimising methane emissions.

IMPACTS FOR AGRICULTURE

Increasing Cultivation of Biomass Crops

Analysis of the current returns from biomass crops, at currently expected yields on a farm basis indicate they do not compete with many arable cropping situations in the UK. To achieve an increase in plantings of perennial energy crops on arable or improved grass land it is estimated that an increase in biomass price from the current £45/odt ex-farm to £60 would

be needed. For the large scale planting required, a further increase in biomass price, in the order of £70/odt would be needed. Government support for energy crops has declined recently due to reductions in direct support (planting grants, Energy Aid payment) and the expected removal of set aside. While biomass prices have increased in recent years these have not been sufficient to compensate for reduced support and to keep up with gains in grain and oilseed prices.

In the more intensive livestock areas, short term lets currently offer better returns than energy crops with much less risk and greater future flexibility. However UK grazing livestock numbers continue to decline due to poor profitability and a lack of skilled labour and this will give an opportunity for energy crops to become more competitive in livestock areas over time.

Current biomass contracts offer price security at index linked price levels for the next 10 years which represents a longer period of price security than is available in the grain market. For this reason the crop may suit businesses seeking long term price security and a minimum of risk. Perennial energy crops are also less exposed to fluctuations in input costs. Developing ways that farmers can participate in future market movements could encourage greater interest in biomass production perhaps through contracts linked to the energy market or grain futures.

With increased support likely for renewable heat in the future, growing energy crops could offer the opportunity for farmers to develop a heat supply business retailing energy direct to local homes and businesses. The potential value of biomass for these markets could be significantly higher than current contract levels but much depends on being able to develop a cost effective and efficient supply chain and on the introduction of incentives to support heat generation from biomass.

The present work confirmed that non financial barriers also inhibit uptake of biomass crops by farmers and need to be addressed to encourage uptake. The long term commitment required for perennial energy crops, uncertainty over yields and contracts, lack of familiarity with the crops and a reluctance to move away from traditional cropping patterns are significant disincentives to farmers. Independent sources of data, particularly on realistic yields, costs and returns are essential to build farmer confidence in these new enterprises.

Impact of using Straw as a Renewable Feedstock

A considerable amount of straw is produced in the UK each year and it is estimated that there is potential for 16 large scale power stations utilising straw for electricity/heat generation. Straw is currently in demand as a livestock bedding and these would be based in areas which do not have a high demand for straw for this purpose. Future developments in second generation biofuels may also increase demand for straw although technology required is some years away from commercial viability.

Much straw across the UK is currently incorporated in the soil following harvest and provides a nutrient source for the following crops. The nutrient value of straw is a major factor in determining the price required to encourage farmers to sell straw off the farm and price swings in the value of fertiliser could readily lead to minimum required straw values of anywhere between £30/t and £60/t based on changes in the value of fertiliser. Any strategy to secure straw for biomass would have to include a suitable pricing mechanism in order to

accommodate swings in fertiliser and other costs to adequately compensate farmers and ensure supplies of straw were maintained.

Opportunities for Reducing Energy Inputs in Crop Production

Use of legumes and organic manures has significant potential to reduce energy inputs for crop production. The use of legumes has a limited role in perennial biomass crop production but cultivation in the season prior to planting could provide nitrogen for the establishment phase, or use of a nitrogen fixing tree species, such as alder, in short rotation forestry may provide potential. Where straw is used for biomass production undersowing a legume with a cereal from which straw is to be used for biomass purposes may also be an option.

The use of some organic manures, such as sewage sludge, is limited for crops destined for food use, but this constraint may be avoided if the crops are to be used for non-food purposes.

CONCLUSIONS

Increasing biomass production can provide a range of positive environmental outcomes:

- The environmental implications in terms of effect on soil organic carbon of increasing biomass production, certainly on arable land are positive.

- Substituting annual arable crops for the perennial biomass crops SRC, SRF and Miscanthus leads to an improvement the carbon footprint on-farm.

- The introduction of biomass crops into farming systems is likely to provide benefits for biodiversity.

There are however several agricultural issues which require consideration if take-up of biomass is going to increase to meet demand.

- Uptake is constrained due to competition from other, conventional agricultural enterprises.

- Straw availability for large biomass plants is limited to the main cropping area in the East of England. Use as animal bedding or feed provides direct competition to biomass in areas where livestock production is important.

- Earning potential from biomass crops currently compares unfavourably to conventional crop and livestock enterprises. Farmer take up will be limited until further incentives are introduced e.g. an option to reward carbon savings from cultivation of these crops could be considered.

Increasing biomass production from agriculture requires economic and non-economic issues to be addressed.

- Current perennial biomass crops require considerable commitment. Production of perennial biomass crops, with associated long term commitment to land use, represents a major change for arable, or indeed livestock farmers, and converting

farmers to cultivation of these crops is challenging. The challenge will be even greater where land is occupied by tenant farmers

- Farmers are generally unfamiliar with the agronomy requirements of biomass crops such as SRC and Miscanthus. Yield results currently available are largely limited to trial sites with good agronomic potential and performance on a range of soil types and situations is not well known. These factors mean that there is little confidence in cultivation of the crops.

- Introduction of crops with lower establishment costs such as reed canary grass and switchgrass may encourage farmer uptake. Even though these crops have a slightly lower yield than SRC or Miscanthus options they may be worthy of consideration if a larger area of cultivation is achieved.

- The environmental implications of land use change from long term grassland are negative, in the short to medium term, with a release of soil carbon. It could be advantageous to retain long term grass in these situations and find an alternative use. Conversion using anaerobic digestion could be an option (e.g. Germany).

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FARMING FOR FOOD AND BIOLOGICAL DIVERSITY: POLICY CHALLENGES AND NEW APPROACHES

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Summary: Biological diversity is known to be an essential component of ecological functioning, health and stability. Farming specifically aims to reduce biodiversity by focusing on the husbandry of a very limited range of plants and animals. Reduction in biodiversity on farmland continues and is closely aligned to perceptions of farm efficiency and ambitions of yield. The dangers of further drastic genetic erosion have been recognised, and biodiversity action plans and agri-environment schemes are examples of measures put in place as a result. The achievements of these and other measures have so far been limited. It is now time to assess whether biodiversity conservation and enhancement can become an explicit integrated output of farming practice rather than continue as an ancillary output. This new approach will demand changes in mindset, research and policies but offers significant new opportunities for farmers.

INTRODUCTION

Food security is now an issue of global concern and along with climate change, threatens to sideline biodiversity in the hierarchy of policy issues. Achievement of food security however depends on more than secure access to staple foodstuffs. Diverse diets are needed to help meet the challenges of malnutrition and obesity, and biodiversity has a contribution to make to the breeding of new varieties and development of effective farming systems. For example, the Secretariat of the Convention on Biological Diversity (2008) notes that different varieties of rice vary in their protein content from 5 to 14 per cent whilst in different banana varieties, the provitamin A content ranges up to 8,500 fold from one variety to another. Agricultural ecosystems that maintain a wide genetic resource base can therefore provide a diversity of foods that can increase food security and improve nutrition at the same time as maintaining ecological stability and health.

Arguments for conserving biodiversity within ecosystems are usually summarised under one or more of four headings: life-support, production and its regulation, moral/ethical/philosophical, and aesthetic/spiritual/cultural. Scherer-Lorenzen (2005) articulates the role of biodiversity within ecosystem analysis as:

> 'Both theoretical and experimental work has shown that within a habitat, changing diversity has profound effects on biomass production, nutrient retention, and other ecosystem characteristics such as stability. In most experiments, a positive relationship between plant diversity and productivity has been found, while the level of unconsumed resources was inversely related to diversity.'

It is logical to conceptualise agricultural systems as managed ecosystems and it is also logical to consider agro-biodiversity capable of providing the same range of benefits and services as biodiversity in wild or semi-wild ecosystems. Agricultural biodiversity is defined by the UK Agricultural Biodiversity Coalition (http://www.ukabc.org/) as:

> 'Agro-biodiversity comprises the diversity of genetic resources (species, varieties, breeds) cultivated for food, fodder, fibre, fuel and pharmaceuticals. It also includes the diversity of non-harvested species that support production (soil micro-organisms, predators, pollinators), and those in the wider environment that support agro-ecosystems as well as the diversity of the agro-ecosystems.'

FARMLAND BIODIVERSITY: THE CURRENT SITUATION

Farmland biodiversity in Scotland is generally acknowledged to have declined significantly over the last fifty years, two important examples being birds and crop landraces.

As a consequence of changes in farming practice, between 1970 and 1990, 12 species of farmland bird, such as the barn owl (*Tyto alba*), contracted in range by more than 10 per cent. Similarly between 1994 and 1999, the abundance of 3 out of 13 widespread farmland species, the skylark (*Alauda arvensis*), lapwing (*Vanellus vanellus*) and kestrel (*Falco tinninculus*), showed a statistically significant decrease (Scottish Biodiversity Forum, 2003).

Landraces are among the most threatened component of UK plant biodiversity as the traditional farming systems which maintain them are threatened. The dependence on continued local regeneration makes them particularly vulnerable. An indication of the state of agro-biodiversity is given by survey work undertaken for the compilation of the UK National Inventory of Plant Genetic Resources (Sholten *et al*., 2006). This study identified just five landraces which were still being grown and used in agriculture in Scotland: Bere Barley (*Hordeum vulgare),* Small Oat (*Avena strigosa*), Rye (*Secale cereale*), Shetland Cabbage (*Brassica oleracea*) and 'Scots' Timothy (*Phleum pretense)*.

CURRENT FARMLAND BIODIVERSITY POLICY IN SCOTLAND

The UK was one of 150 countries to sign up to the Convention on Biological Diversity (1993) which commits the parties to achieve 'a significant reduction of the current rate of biodiversity loss at the global, regional and national level as a contribution to poverty alleviation and to the benefit of all life on Earth' by 2010.

As a part of this commitment, the UK Biodiversity Action Plan (UKBAP) was published in 1994 to develop strategies to protect biological diversity. In Scotland, this is implemented via the Scottish Biodiversity Strategy (SEERAD, 2004) and overseen by the Scottish Biodiversity Forum. The Forward Strategy for Agriculture (SEERAD, 2001) and Custodians of Change (SEERAD, 2002) made significant contributions to the policy debate.

The establishment of Sites of Special Scientific Interest, which gained additional protection from the Wildlife and Countryside Act (1981), was followed by a new approach. Environmentally Sensitive Areas were created as a mechanism to alter the management of agricultural land in order to enhance sensitivity to the natural heritage character of specific areas. Set Aside, the Farm Woodland Premium, the Native Pinewood, the Countryside Premium, the Rural Stewardship and the Organic Aid Schemes all made further contributions. Currently, Rural Priorities and Land Managers Options include manifestations of this agri-environment approach.

The Scottish Government also has a commitment to conserve its plant genetic resources through its international treaty obligations, and to this end the Scottish Landrace Protection Scheme was launched in 2006 to provide a safety net for the continued use of landraces by storing seed produced by each grower each year. Sholten *et al.* (2006) consider there is little evidence the mainstream UK conservation agencies have considered crop wild relatives or wild harvested species in formulating their conservation priorities and there seems to be widespread ignorance of the real socio-economic value of them.

The effectiveness of agri-biodiversity policies

Agri-environment schemes have been subject to some scrutiny but conclusions as to their effectiveness have sometimes been mixed. Kleijn & Sutherland (2003) reviewed 62 studies and found a variable set of results. In general, however, agri-environment measures were associated with increases in species richness, which outweighed overall any reverse outcomes. Subsequent studies have indicated a more positive series of outcomes. There is good evidence that the much-increased coverage, and the kinds of management options now being used within the UK agri-environment schemes, will deliver significant benefits in terms of biodiversity, particularly in respect of the vegetation and birdlife of arable habitats, species-rich grasslands, hedgerows, moorland and lowland heaths, and some types of wetlands. While there is less evidence of benefits for mammals and invertebrate species, there are cases of measured benefits in respect of, for example, butterflies, bumblebees, sawflies and plant bugs, and some mammal species, especially in arable habitats. In general the higher level schemes are likely to deliver significantly more benefits per hectare of land than the entry-level schemes, but the extent of the latter is clearly much more significant at the landscape scale (Boatman *et al*., 2008). Wilson *et al.* (2009) notes that the population decline of farmland birds in Britain has not yet been reversed into national population recoveries and contrasts this with the successful delivery at a population level of targeted recovery initiatives built upon higher-level agri-environment schemes for range-restricted species.

Seed banks of native flora on agricultural land are now depleted due to intensive agricultural practices and the return of arable flora is unlikely to occur naturally (Ford, 1997). The creation or re-establishment of habitats such as wildflower meadows from seed carries a potential risk of the genetic structure of native species being affected by the use of non-native provenances, either by displacing plants of local origin or through hybridisation (Welch *et al*., 2001).

FUTURE CHALLENGES

There is now a strong case for arguing that farmers must in future deliver biodiversity conservation and enhancement as an explicit objective of their activity. The word explicit is important because up until now biodiversity has been seen as either a by-product or an added-extra that can be tacked on to the main business of maximising food production. The achievement of such a significant change in attitude and ambition will require that certain intellectual, political and economic challenges be faced.

The most fundamental of these is recognition and acknowledgement by both policy makers and farmers that biodiversity conservation and enhancement must be achieved at the same time as outputs rewarded through the conventional marketplace. Agro-biodiversity must also be recognised as an integral element of biodiversity strategies and as an enabling feature of the design and management of managed ecosystems. Land use systems that make best use of, and work most efficiently with enhanced diversity must be the focus of agricultural research and development. A systems approach would recognise multiple outputs and involve a very wide range of internal processes such as energy and nutrient fluxes, and management inputs. Intensive farming will probably have to become biologically and endogenous-energy intensive, rather than input and exogenous-energy intensive.

This necessitates a significant increase in the research emphasis towards the integration of agricultural production and the delivery of environmental services. In the opinion of Wilson *et al.* (2009), it is necessary to create greater ecological heterogeneity in agricultural systems by making space (and time) for the life cycles of non-crop organisms at all spatial scales. They suggest that there may also be utilitarian and cultural benefits from more rather than less integration. The agri-ecological approach long advocated by Altieri (1987) is a model that attempts to achieve this integration:

> 'The ultimate goal of agro-ecological design is to integrate components so that overall biological efficiency is improved, biodiversity is preserved, and the agro-ecosystem productivity and its self-regulating capacity is maintained. The goal is to design an agro-ecosystem that mimics the structure and function of local natural ecosystems; that is, a system with high species diversity and a biologically active soil, one that promotes natural pest control, nutrient recycling and high soil cover to prevent resource losses.'

This theoretical approach has much in common with organic farming principles and with the ecological integrated paradigm that constitutes the favoured alternative to the productionist paradigm as defined by Lang & Heasman (2004). The concept of High Nature Value farming (HNV), which has increasing profile in Scottish land use policy, is also concerned with maintaining high levels of diversity within the farmed environment.

There is a need for a deeper understanding of the relationships between organisms that are considered components of wild systems, and those that are considered components of agricultural systems and those that are both. The possibilities for integrating the proposed heterogeneity within productive systems need to be explored further.

New approaches to delivery

Post-war Britain demanded food security as its primary agricultural priority and farmers duly obliged, supported by the EU Common Agricultural Policy. New systems of agriculture which will contribute to both food security and environmental security will demand new delivery mechanisms. Whilst existing regulatory mechanisms such as cross compliance, and market mechanisms via certified produce will continue to play their part, there is a need for new markets and new approaches.

The marketplace has in the past failed to recognise and reward the value of environmental services provided by well-managed systems. Making the transition to sustainable development (http://www.iisd.org/sd/) means learning to invest more of the proceeds of economic development to maintain the environmental conditions for its continuation. In order to create a new market, the value of biodiversity has to be recognised and evaluated. This valuation process, whilst contentious, is an essential component of any attempt to try to integrate environmental services into a market economy. This also has implications for the direction and funding of the CAP.

The perceived dichotomy between farming and environmental ambitions must be disentangled. For farmers there are opportunities if the deeply ingrained culture of productionism can be balanced with revitalised cultures of environmentalism and aestheticism and these can be aligned with the responsibility of land ownership. The goals of ecological health and sustainable food production can be shown to be complimentary rather than contradictory.

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SCOTLAND'S ENVIRONMENTAL AND RURAL SERVICES (SEARS)

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Summary: SEARS arose as a result of a challenge set by the Environment Minister Michael Russell to produce a single environment and rural delivery service in June 2007. The nine bodies within the Rural Affairs and Environment portfolio of Scottish Government were set a very demanding schedule to produce a proposal for consideration by the Minister in September that year. The proposal was presented and accepted by the Minister in the presence of the Chairs and Chief Executives (or equivalents) of the nine organisations. The acceptance signalled the go ahead for the design and delivery of Phase 1 of SEARS. Two years on the pace of SEARS remains unrelenting. It has become a successful model whose achievements are cited in Ministerial speeches. Phase 1 was largely focused on culture change and reducing visits and inspections and Phase 2 was more aimed at reducing bureaucracy and enhancing bio security. At the time of writing Phase 3, continuing the reducing bureaucracy theme, is being designed for delivery during 2010 and 2011.

BACKGROUND AND CONTEXT

Scotland's Environmental and Rural Services (SEARS) brings together nine delivery-focused bodies within the Rural Affairs and Environment portfolio to provide "a single environment and rural delivery service… (operating) within the current statutory framework" (Russell, 2007) leading to more joined-up services. It was formed at the instigation of the Environment Minister Michael Russell in the summer of 2007 and is part of the Scottish Government's Simplification Programme, aimed at re-aligning public services to achieve more effective delivery.

Three principles of public service delivery are central to SEARS, namely:

- User focus – putting the person and not the institution first;
- Effectiveness – focusing on real improvements in services delivered for the people of Scotland;
- Value for money – making sure that each and every public pound is spent wisely.

SEARS is a partnership project involving the design and implementation of improved services for **rural land managers**. In particular, it aims to improve customer experiences by:

- Co-ordinating inspections and visits;
- Removing duplication;
- Providing flexible access to service through any door;
- Facilitating customer access to multiple and co-ordinated services;
- Sharing and using information more effectively, reducing multiple data requests;
- Empowering individual staff to provide a wider range of services.

This project, known as the SEARS Frontline Delivery Project, is chaired by Campbell Gemmell, Chief Executive, SEPA. It is an integral part of a wider programme including projects delivering on Co-locations, Communications and Education and Direct Land Management which is chaired by Peter Russell (Scottish Government Rural Director) and overseen by a Strategic Reference Group, Chaired by the Minister for the Environment. Scottish Government provides the Project Manager and support services including access to a communications specialist.

The partners are:

- Scottish Government Rural Payments and Inspections Directorate (RPID)
- Loch Lomond and the Trossachs national Park Authority (LLTNPA)
- Cairngorms National Park Authority (CNPA)
- Scottish Natural Heritage (SNH)
- Deer Commission Scotland (DCS)
- Scottish Environment Protection Agency (SEPA)
- Forestry Commission Scotland (FCS)
- Animal Health Agency (AHA)
- Crofters Commission Scotland (CCS)

The design and preparation for delivery of the first Phase of SEARS took place between 2007 and 2008 and was launched at the Royal Highland Show in June 2008. Phase 2 was initiated in the autumn of 2008 and a third phase is under development running through to 2011. This paper focuses mainly of SEPA's involvement in SEARS.

Partnership Process

SEARS is a partnership approach, not an organisation *per se*. A Memorandum of Agreement (MOA) (Scottish Government, 2008a) setting out the framework for the operation of this partnership and minimising the risks of complex cross-charging arrangements, was agreed by the Programme Board in March 2008. All the partners are Scottish public bodies responsible to the Scottish Parliament through Scottish Ministers, so the principle that costs and benefits should be assessed at the level of the public purse as a whole reinforced the sense of common purpose. Similarly a data sharing agreement has been drawn up which sets in place the foundations to enable better data sharing across the SEARS organisation in agreement with the Information Commissioner for Scotland.

Evidence Base and Stakeholder Engagement

Research (Scottish Government, 2008b) was commissioned early in 2008 to establish land managers experiences of dealing with the multiple environmental and rural agencies. The findings from interviews with a cross section of more that 1500 land managers revealed they had little difficulty identifying the appropriate organisation to deal with on specific issues. However, they would welcome further integration of the services provided as long as they also had access to specific skills and experience as and when required. Most only dealt with one or two of the partners on an annual basis. The most valued aspect of the service was local access and expert support. A high percentage supported the idea of inspections being conducted by a single officer on behalf of a number of the partners. There were variations in responses from different sectors, customer groups and variations from the same customer group in different areas of Scotland. A key finding was that the burden of paperwork

(averaged at 1.83 hours/week) was not substantially greater than that faced by other commercial businesses. However this was seen as the biggest single problem with a strong demand for both simplification and an overall reduction in this administrative burden. The results of this survey are used as a benchmark for satisfaction levels with SEARS' partners.

The importance of securing stakeholder support for SEARS was recognised early on and in the summer of 2007 when the original SEARS proposal was at the design stage valuable input was sought from key stakeholder groups, for example, National Farmers Union (Scotland), Scottish Rural Property and Business Association, and Confederation of Forest Industries. Biennial engagement with these groups and the Scottish Crofting Foundation, the Association of Deer Management Groups, the Scottish Tenant Farmers Association and the Scottish Countryside Alliance has continued to demonstrate strong support for SEARS.

PHASE 1

A total of 18 opportunities were identified for delivery in Phase 1. It was seen as important to get building blocks in place early on to help facilitate the future development of SEARS. Key opportunities for SEPA are listed below:

- The SEPA communications centre was to handle calls on a dedicated 0845 number which operates 24/7 transferring the burden of complexity from the customer directly onto the SEARS organisations
- A SEARS web portal was created on the SEPA IT system to provide access to data, information, guidance and contacts for customers. Customers can also use the portal to update personal details which can then be made available to all partners if required.
- To embed the necessary cultural change in staff 78 training events were arranged involving a total of 1400 frontline staff across the family of organisations and in offices throughout the country
- Rationalisation of inspection activity relating to the Groundwater Regulations, Controlled Activity Regulations (CAR) (Scottish Government, 2005) Engineering and Diffuse Pollution General Binding Rules (Scottish Government, 2008c) and the Silage, Slurry and Agricultural Fuel Oil (Scotland) Regulations 2003 (Scottish Government, 2003). SEPA played a major role in designing and delivering guidance and training to RPID and SNH and in future FCS frontline field staff to enable them to carry out inspections on behalf of SEPA during planned visits for other purposes and provide SEPA with the results.
- Reduction in SEPA's groundwater subsistence charge to mainly sheep farmers (by c. £150k) due to the streamlined SEARS approach to groundwater licence work.
- New bracken control leaflet produced and application process streamlined and now available on-line.

These opportunities were delivered via 5 work streams namely IT, Data Sharing, Inspections, Training, and Communications. Overseeing delivery was the Delivery Design work stream which provided a "buddy" for each work stream. The buddy acted as a conduit for information and an important sounding board for the work stream membership.

A key success measure of the highlighted opportunities is the empowering of staff to carry out duties on behalf of another SEARS' partner. The staff involved now have a much greater

knowledge of a wider range of environmental issues and play an enhanced role in the delivery of customer and environmental benefits. During Phase 1 SEPA authorised and trained 453 RPID staff to carry out SEPA duties on its behalf and to report the findings to SEPA. Quality assurance arrangements are in place providing reassurance these inspections are carried out with the appropriate rigour.

Feedback from rural land managers and stakeholders has been extremely positive and the target of around 2000 saved separate visits and inspections was achieved six months ahead of schedule. Support has been reflected in an almost 100% attendance by invited stakeholders at SEARS engagement events.

An unexpected consequence of SEARS is that trust and credibility has been raised within stakeholder organisations membership. For example, generally RPID would be able to address small non compliances with the SEPA legislation with the land manager at the time of the visit without having to refer the matter to SEPA. Also where the few cases referred to SEPA have been followed up the engagement has been of an amicable nature since the subject has been previously discussed with the RPID inspector. Staff involved have also benefited considerably by gaining a wider knowledge and understanding of partner roles and responsibilities.

There were areas within Phase 1, however, where SEARS failed to deliver, either because circumstance changed or the opportunity was not cost effective or difficult to achieve by the land managers.

PHASE 2

Immediately following on from the launch of Phase 1, at the Royal Highland Show in 2008, a lessons learned exercise was carried out which helped inform the model for the design and delivery of Phase 2 with a greater use of task and finish groups. It was clear that effort had to be focused on cementing the benefits from Phase 1 ensuring business as usual across the family. Alongside this a methodology for capturing the metrics, rolling out refresher and awareness training for new employees and existing staff into the SEARS approach was required. With the benefit of research and stakeholder input there was a growing confidence that Phase 2 would build on the customer benefit achieved in Phase 1. There was a desire amongst stakeholders to widen SEARS to include appropriate elements of work undertaken by the Local Authorities and the Food Standards Agency.

The main focus of Phase 2, informed by the research findings and stakeholder input was on opportunities for reducing bureaucracy and enhancing bio security. Key opportunities are listed below:

- SEARS portal review including numbers of forms in use. Compilation of number of forms in use with a view to reducing and simplification and making available via SEARS portal
- Production of "consultation principles" for use across the family to streamline the process and reduce delays
- Controlled Activity Regulations (CAR) and Pollution Prevention and Control (PPC) applications within or near designated sites. Reaching agreement with SNH on

licence level activities to avoid need for consultation enabling decisions to be reached within a shorter timeframe

- Organic waste to land exemptions. Agreeing a position on the 50ha limit for registering exemptions and the development of a one farm: one form approach
- Bio security. Agreeing a SEARS Bio Security Protocol (Scottish Government, 2009a) for use by all partners when visiting land to reduce the risk of spreading animal, fish or plant disease. There has been widespread interest in the protocol from other agencies in Scotland and throughout the UK
- International catering waste going to landfill sites. SEPA and the Animal Health Agency reaching agreement that SEPA carry out the Animal Health aspects of landfill inspections when on routine planned visits to these sites
- Development of a template to help the Intensive Agriculture PPC permitted sites draw up disease outbreak contingency plans. This was an industry led opportunity, which will help facilitate permit compliance.

Further customer research (Scottish Government, 2009b) and stakeholder engagement provided reassurance the opportunities would help meet customer expectations. Phase 2 was launched by the Environment Minister Roseanna Cunningham at the Royal Highland Show in June. The Show saw the launch of the first SEARS Annual Review (Scottish Government, 2009c) which also contains information relating to the wider programme.

Development of guidance, training and "awareness raising" is being rolled out with the opportunities embedded as business as usual across the family.

PHASE 3

A "lessons learned" review of Phase 2 has been carried out. Ideas for Phase 3 are being considered, focusing on further opportunities to reduce bureaucracy and widen SEARS. From an original 27 opportunities in the mix this has been narrowed down to the following principal areas of focus:

- Review, update and extend the use of the bio security protocol
- Modify the disease outbreak contingency plan for wider general use [non PPC]
- Simplify and strengthen the process for handling CAR engineering registrations within designated sites
- Modify CAR abstraction for irrigation regime to enable it to be used for CAP cross compliance assessment
- Examine the potential roll for Quality Assurance schemes in risk based inspections
- Review and update of portal
- Consideration of SEARS proofing policy development

Phase 3 will be supported by further customer research and stakeholder engagement. Piloting of the Standard Cost Model (BRE, 2005) to assess administrative burden of SEARS's opportunities is underway at the time of writing. SEARS partners have given presentations to the Scottish Rural Property and Business Association (SRPBA) Regional Committees during the summer of 2009. One to ones with individual land managers is also underway involving a study of "A Year in the Life" profiles to attempt to tease out the critical bureaucratic burdens

faced by a range of land managers. Delivery will be via a mixture of work streams and task and finish groups reporting to the delivery design team.

CONCLUSIONS

Whilst the partners have different drivers and regulatory requirements SEARS has shown the benefit of working together to streamline processes, create efficiency and help sustain the rural economy. Staff have benefited from learning to deliver a wide range of services and gained knowledge and insight of the wider roll of partners and how these can compliment each other. Rural land managers have benefited from streamlined service delivery, reduced numbers of separate inspections and visits, quicker decisions, and modest financial savings. Successful stakeholder engagement has been crucial to the success of the process.

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SAMPLING FOR THE NEW ZEALAND FLATWORM

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Summary: The New Zealand flatworm *Arthurdendyus triangulatus* is an alien terrestrial planarian which is a predator of our native earthworms in British Isles and the Faroes. The detrimental impact on earthworm numbers has potential to reduce wildlife biodiversity, e.g. moles, and cause economic losses to farmers. The unique attributes of the New Zealand flatworm has meant that so far, no sampling protocol has been devised which will allow an estimate of a population to be made. Sampling in the west of Scotland over a 17 month period produced data which were analysed to determine the characteristics of such an infestation. The results showed that the New Zealand flatworm had an over-dispersed distribution and that variability increased with sampling mean. For practical reasons it was suggested that the minimum sampling size to determine populations should be at least 15. A log transformation was also shown to normalise the data.

INTRODUCTION

The New Zealand flatworm (*Arthurdendyus triangulatus*) (Dendy, 1894) is an alien terrestrial planarian which is a predator of native earthworms in the British Isles and the Faroe Islands. It has been implicated in the eradication of moles from areas in the west of Scotland (Boag 2000) and has the potential to have a detrimental economic impact on Scottish agriculture (Boag and Neilson, 2006). Sampling procedures have been devised to detect and quantify other introduced economically important alien soil inhabiting animals e.g. potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) (Been and Shoemaker 1996) which are relatively immobile in the soil. However no sampling protocols have been devised for any animals with similar attributes to the New Zealand flatworm which is a mobile, nocturnally active, organism which is thought to live and feed for the majority of the time on the soil surface. The purpose of this paper is to investigate the relationship between the sample variance and samples size associated with the New Zealand flatworm and given guidance on the number of samples required to achieve a desired level of confidence in estimating the population.

MATERIAL AND METHODS

The study site was in the south end of a field at Dalinlongart Farm Sandbank, Dunoon, Argyll, Scotland (Ordnance Survey Grid reference NS145820) which had previously been used to study the spatial distribution and relationship between *A. triangulatus* and earthworms (Boag *et al.,* 1999) and considered typical of grassland field in that part of Scotland. This field was in an area between Dunoon and Loch Eck where over fifty adjacent

fields were known to be infested with the New Zealand flatworm and therefore considered representative of infested agricultural land in western Scotland (Boag, 2000). The technique used to collect the data was to put out "traps" which were strong polythene bags filled with approximately 6 kg gravel and placed on bare soil. These "traps" acted as refuges which were generally cool, dark and damp and under which the flatworms hid during the day. The surface area of the traps in contact with the soil was approximately 0.25 m^2. Forty traps were initially placed in an 8 by 5 rectangular grid on 18 July 1999 but on 4 November 1999 this number was increased to 64 traps in an 8 by 8 grid. The traps were placed 2m apart and were inspected at least monthly by lifting them and examining both the soil surface and the underside of the polythene bags. The experiment was concluded on 16 October 2000 after a period of 17 months during which there were 25 sampling occasions. The monitoring of this site was extended for over a year to give data which encompassed all seasons as populations are known to fluctuate throughout the year. The data collected gave an easily accessible index of flatworm numbers but probably only represented about 30-40% of the total population (Blackshaw, 1990, and unpublished data from the same field). The complete data set consisted of six samples of size n = 40 and nineteen samples of size n = 64. These were referred to as data sets 1 to 25.

The objective was to estimate the variance of the flatworm counts at each sampling date with a view to ultimately make recommendations about sample size. However it could not be assumed that the counts are approximately normally distributed and therefore could not appeal to the usual theory about the properties of the sample variance s^2 as an estimator of the population variance σ^2.

Two approaches were adopted to study the properties of variance estimators for flatworm counts, namely bootstrapping and moment estimators.

Letting $\boldsymbol{x} = (x_1 \ldots x_n)$, n = 40 or 64, denote a complete set of flatworm counts for a single sampling occasion, the corresponding sample variance s^2, was computed as

$$s^2 = \sum_{i=1}^{n} (x_i - \bar{x})^2 / (n-1) \qquad (1.1)$$

A bootstrap sample $\boldsymbol{x}^* = (x_1^* \ldots x_n^*)$ was obtained by sampling n times with replacement from the original data points $\boldsymbol{x} = (x_1 \ldots x_n)$. This was repeated one thousand times for each of the 25 data sets. Corresponding to each bootstrap sample was a bootstrap replication of s^2, denoted by s^{2*} which was obtained by calculating the variance of each bootstrap sample

$$s^{2*} = \sum_{i=1}^{n} (x_i^* - \bar{x}^*)^2 / (n-1). \qquad (1.2)$$

Finally, the mean and variance of the s_j^{2*}, $j = 1 \ldots 1000$ were derived to give an indication of the accuracy and precision of s^2 as an estimator of the population variance σ^2. To estimate these statistics for smaller sample sizes, the original data, $\boldsymbol{x}$, was used to obtain sub-samples of size m, where $m = 2 \ldots n-1$. For example, for m=5, a sub-sample was obtained by randomly sampling 5 times with replacement from the original data points x_i. As before, this was repeated one thousand times and again s^{2*} calculated as in 1.2 above except with m replacing n. The mean and variance of the one thousand s^{2*} were calculated exactly as before to discover how the accuracy and precision of s^2 changed with sample size m. To obtain further information about the behaviour of s^2 the 2.5- and 97.5 percentile statistics of the s^{2*} were derived.

The second approach is based on the properties of moment estimators. It has be shown (Kendall and Stuart, 1948) that the mean and variance of the usual variance estimator s^2 can be expressed as

$$E(s^2) = \sigma^2 \qquad (1.3)$$

and

$$\text{var}(s^2) = \frac{\mu_4}{n} - \frac{(n-3)}{n(n-1)}\sigma^4 \qquad (1.4)$$

where μ and σ^2 are the (unknown) population mean and variance. Replacing μ and σ^2 in (1.4) by their sample estimates $\bar{x}$ and s^2 provides large sample approximations to the sampling mean and variance of s^2. These approximations, like the bootstrap estimates, are independent of any underlying distributions. For a simulated sample size less than n, m is substituted in (1.4) to give the estimates of the sampling variance.

Logarithmic transformations of the mean and variance of the 25 data sets were taken and the relationship calculated to give the index of aggregation (Taylor, 1961). This provides a normalising transformation that equalises the variance.

RESULTS

Flatworm counts were generally low in all 25 data sets but representative of data obtained from other sites (Boag et al., 2005). The minimum count was zero and the maximum count thirteen. The distribution of counts in all data sets had a tail to the right (positively skewed). Figure 1 plots the 25 data set means against their corresponding variances and shows that the sample variances increase as the sample means increase and that the increase is approximately linear.

Results from these example data sets show the range between the 2.5 and 97.5 percentiles are very wide at sample sizes less than 10. The distance of the 97.5 percentile from the average $\mathbf{s}^{2*}$ is greater than the distance of the 2.5 percentile indicating that the distribution of sample variances is strongly positively skewed at smaller sample sizes. This effect lessens as the sample size increases until the upper and lower 2.5 percentiles become approximately symmetrical about the mean. The sample size at which the upper percentile is less than twice the population variance estimate varied different for all data sets.

Figures 2a and 2b are plots of the sample size against the mean bootstrap s_j^{2*} and the 2.5 and 97.5 percentiles of s_j^{2*}, for the data sets of the 8th and 12th dates respectively.

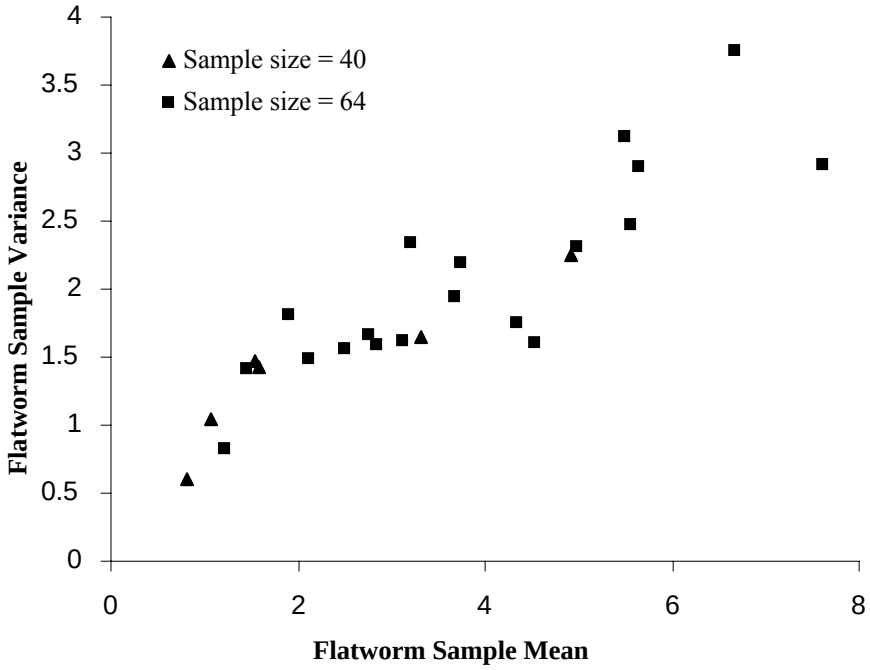

Figure 1. Flatworm sample variance plotted against sample mean indexed by sample number

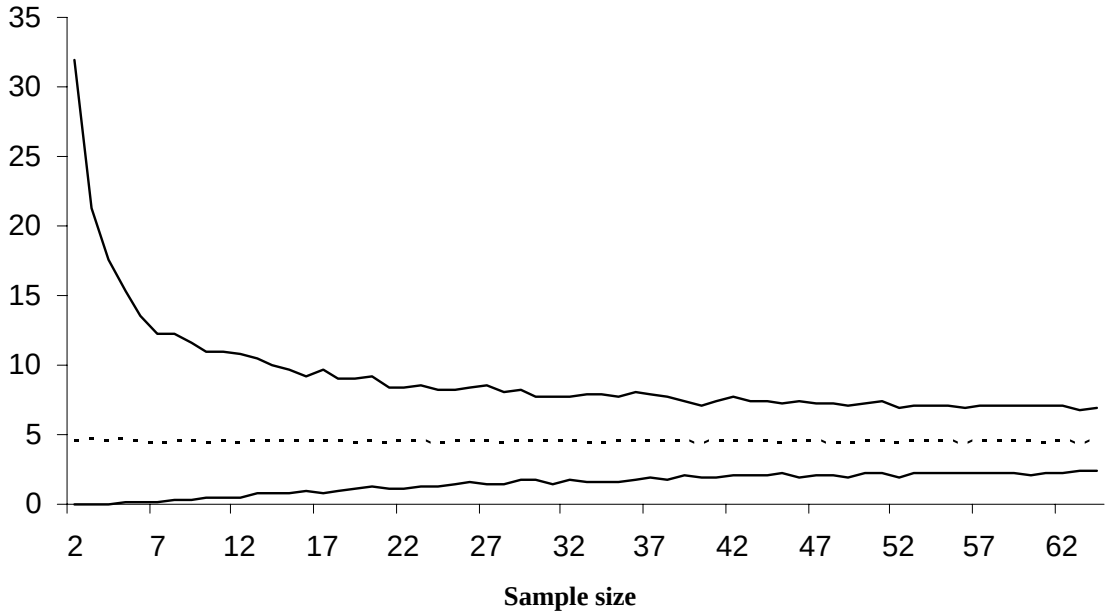

Figure 2. 2.5 and 97.5 percentiles and mean of 1000 bootstrap estimates of s^{2*} for data set 8

Results from these example data sets show the range between the 2.5 and 97.5 percentiles are very wide at sample sizes less than 10. The distance of the 97.5 percentile from the average $\mathbf{s}^{2*}$ is greater than the distance of the 2.5 percentile indicating that the distribution of sample variances is strongly positively skewed at smaller sample sizes. This effect lessens as the

sample size increases until the upper and lower 2.5 percentiles become approximately symmetrical about the mean. The sample size at which the upper percentile is less than twice the population variance estimate varied different for all data sets. For example, in data set 8, it can be seen that the variability of the variances at smaller sample sizes is greater than that of data set 12. Therefore the 97.5 percentile curve is steeper initially than that of data set 12 and takes longer to fall to below twice the estimated variance.

Figure 3 indicates that using the transformation $\log_e(\text{variance}) = 0.315 + 1.344 \times \log_e(\text{mean})$ normalises the relationship between the mean and the variance

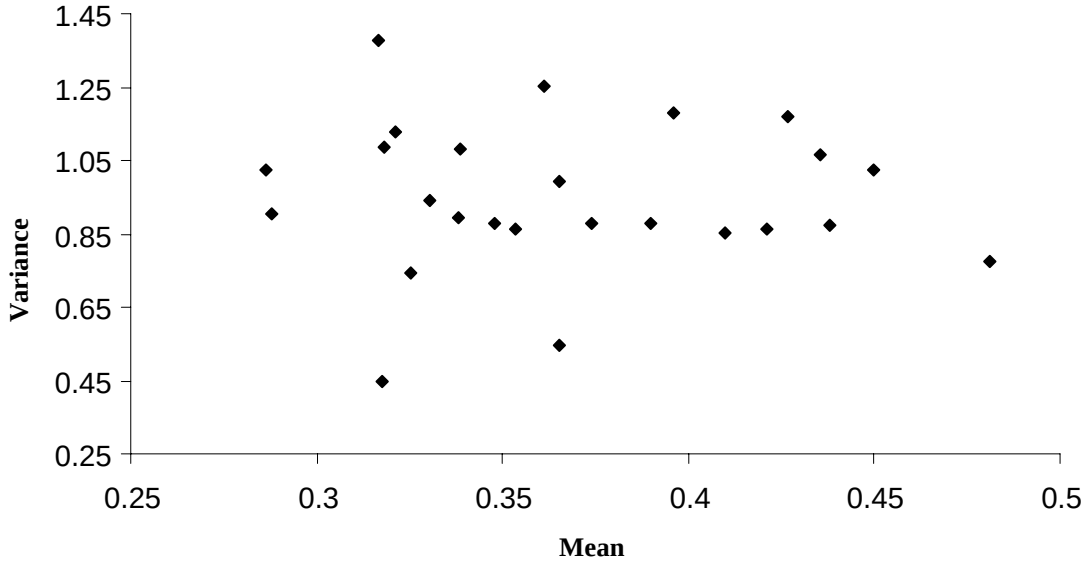

Figure 3. Variance versus mean for the transformed flatworm worm counts

DISCUSSION

The New Zealand flatworm, A. *triangulatus*, is a relatively recent representative of a number of successful alien species which have become established in countries throughout the world. Taylor's Power Law Index of Aggregation has been used to determine the number of samples required to estimate accurately the population size of a wide range of organisms. It has the advantage over some other frequently used indices of aggregation e.g. the negative binomial, since it can be used to produce a normalising equation devised for specific individual species (Taylor et al., 1979). Logarithmic transformations of the mean and variance of the 25 data sets were taken and the relationship calculated as $\log_e(\text{variance}) = 0.315 + 1.344 \times \log_e(\text{mean})$. This gives an index of aggregation (Taylor, 1961) of 1.344 which suggests that the distribution of the flatworms is not random ($P<0.05$). Transforming the flatworm counts using $Z = x^{1-1/2b}$ where b = 1.344, the Taylor's Power Law Index of Aggregation provides a normalising transformation that equalises the variance.

The minimum number of samples required for the 97.5 bootstrap percentiles of s^2 to fall below twice the estimated σ^2 varied considerably so no definitive all encompassing recommendation can be proposed as to the number of samples which need to be taken to estimate New Zealand flatworm populations. However a subjective minimum sample size estimated visually from Figure 2 would indicate that a sample size of 15 could be a practical

compromise between the requirements for statistical analysis and the time taken to sample for flatworms under field conditions.

The analysis of the data in this paper, which was collected over a 17 month period, would suggest that using 15 traps would be an acceptable compromise for the detection of flatworms under polythene traps and meet both the above criteria. Setting out more traps would take considerably more effort for relatively little more information.

ACKNOWLEDGEMENTS

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THE SCOTTISH LANDRACE PROTECTION SCHEME

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Summary: Seed of landraces are not usually sold commercially and their survival is entirely dependent on farm-saved seed from growers. The Scottish Landrace Protection Scheme was set up to conserve seed of Scottish landraces in *ex situ* collections and, to provide growers with a safety deposit system for seed they donate. In the event of seed harvest failure, seed, which is already adapted to the donor's growing environment, can be returned to the original donor.

INTRODUCTION

Landraces are probably the most threatened component of UK cultivated plant biodiversity as they are maintained by farmers, not conservationists, within both traditional and modern farming systems. In Scotland, maintainers are an aging population, many of whom are crofters in the Northern and Western Isles. In some areas the number of growers is declining as upcoming generations work part-time in other jobs or are using modern cultivars.

Wright *et al*. (2002) outlined historical evidence for the use of a number of traditional cereal varieties in Scotland. The diversity and potential of Bere Barley and other cereal landraces were reported and several areas of research for traditional Scottish cereal crops were recommended. However, without a government policy on the conservation of plant landraces, few of these recommendations have been considered since. Few landraces survived to the present day, but those still being used have depended on their continued harvest *in situ* and their conservation in *ex situ* seed collections.

UK NATIONAL INVENTORY

Survey work undertaken for the compilation of the UK National Inventory of Plant Genetic Resources (Scholten *et al.* 2004), commissioned by Defra, identified five landraces [Bere Barley (*Hordeum vulgare*), Small Oat (*Avena strigosa*), Rye (*Secale cereale*), Shetland Cabbage (*Brassica oleracea*) and 'Scots' Timothy (*Phleum pratense*)] which were still being grown and used in agriculture in Scotland. Apart from 'Scots' Timothy, for which seed production is in the Stirling and north Tayside area, Scottish landraces are mostly grown in the Northern and Western Isles. A recently updated summary of UK landraces has been described (Scholten *et al.* 2009).

SEED COLLECTION

Prior to the UK National Inventory, relatively few samples of landrace seed had been collected and conserved in *ex situ* collections. In the 1980s Shetland cabbage seed was collected and deposited at the vegetable genebank in Wellesbourne. Accessions were screened for clubroot (*Plasmodiophora brassicae*) resistance and plants were selected for having specific race resistance (Crute & Pink 1989). In the 1930s seed of small oat (*Avena strigosa*) was collected by the Welsh Plant Breeding Institute to breed improved disease resistance.

Although genebanks made a determined effort to obtain seed of traditional varieties from breeders and horticulturalists in the 1980s (Dr. Astley pers. comm.), few attempts were made to systematically collect varieties in the UK, other than a European-funded *Brassica* survey (van der Meer *et al.* 1984).

The systematic collection of seed of Scottish landraces followed the work of the UK National Inventory. As the use of landraces in agriculture is rare, and the populations used were very variable and dynamic, there was a need to collect seed from all available growing populations from different harvest years to protect their diversity in *ex situ* collections.

In 2004 Southworth collected seed in the Northern and Western Isles to undertake a genetic diversity analysis of bere barley (Southworth 2007). In 2006 seed of bere barley, small oat and Shetland cabbage was collected in Shetland (Lever 2006) and in 2008 and 2009 seed of bere barley, small oat and Hebridean rye was collected, both as pure crops and as crop mixtures, from the Western Isles (Scholten *et al.* 2008). Seed from these collecting missions is stored at SASA at -22^{o}C (see Table 1).

Table 1. Scottish Landraces and Traditional Varieties: accessions in SASA *ex situ* seed collections as at 30.11.2009.

Crop	Species	Accessions	Landrace/Traditional Variety
Bere Barley	*Hordeum vulgare*	52*	Landrace populations
Small Oat	*Avena strigosa*	32	Landrace populations
Common Oat	*Avena sativa*	8	Traditional varieties††
Wheat	*Triticum aestivum*	1	'Red Standard'
Hebridean Rye	*Secale cereale*	4	Landrace populations
Shetland Cabbage	*Brassica oleracea*	27	Landrace populations
Timothy	*Phleum pratense*	1	'Scots'†

**32 small samples were collected for a PhD project*
† registered on the UK National List
†† Further information on Scottish Landraces can be found at www.scottishlandraces.org.uk which is maintained by Science and Advice for Scottish Agriculture, part of Scottish Government.

THE SCOTTISH LANDRACE PROTECTION SCHEME

The Scottish Landrace Protection Scheme **(**SLPS) was launched by SASA in August 2006 (Green *et al.* 2009) to provide a safety net for the continued use of landraces by storing seed produced by each grower each year. In the event of harvest failure, a grower can request some of the seed already deposited and stored at SASA. With the consent of the donor, some seed can be made available for general distribution for research, breeding and education.

The aim of the SLPS is to encourage growers to use seed they produce in their own locality and remove the need to use seed which is not adapted to their local area. This prevents the loss of seed selected for local use.

Each stored sample is notionally divided with the aim of conserving a sufficient quantity of seed for emergency regeneration, monitoring (germination and vigour of seed in store), re-supplying the donor (the quantity being dependent on the size and quality of the original sample), morphological and molecular characterisation and general distribution for *bona fide* research, breeding, education or further evaluation.

On receipt at SASA, each collected or donated seed sample is registered, examined for seed health and tested for germination. The growers are informed of the results and consent is sought for general distribution of seed. Seed is then cleaned, dried at 15% r.h. and stored at -22^{o}C. A sub-sample is removed for safety duplication elsewhere and is also stored at -22^{o}C.

Seed of barley, small oat and rye landraces are often grown together as a mixture; seed received is sampled to undertake seed quality tests and to calculate the proportion of each component. The sample is stored as a mixture.

To meet the above aims, a minimum viable seed quantity is required for participation in the scheme and for making seed available for general distribution. Seed requests can be made to *Genetic.Resources@sasa.gsi.gov.uk*

SCOTTISH LANDRACES

Bere barley

Bere is grown in Orkney and Shetland as a pure crop, and in the Western Isles both as a pure crop and as a mixture with other cereal landraces (small oat and Hebridean rye). As landrace populations are variable and dynamic, they quickly adapt to their local growing environment and after some time, seed grown in one area may not be suited to other localities. This was the case following seed harvest failure in the Western Isles; bere sent from Orkney did not grow well in the Western Isles (South Uist). Support for this observation was confirmed by Southworth (2007) who raised awareness of the considerable diversity in bere barley populations, both between the different island groups (Orkney, Shetland and the Western Isles) and within each island group. Southworth provided evidence that bere accessions harvested from different island groups were distinguishable, which suggests that populations should be sampled from the entire range of distribution in order to conserve this landrace. The extent of this diversity was probably not previously understood and raises issues of how to conserve such diversity and encourage local sustainable use.

Shetland cabbage

Shetland cabbage has been mainly used as winter feed for cattle, but has also been used as a vegetable. Seed quantities harvested each year are small; but seed sown is often mixed with a seed harvested from a previous year. Growers are widely distributed around Shetland, but their numbers have rapidly declined in recent years.

Seed, collected from 17 growers from different parts of Shetland, is now conserved at SASA. Of the 27 accessions stored, 24 qualified for participation in the Scottish Landrace Protection Scheme and 23 of these have been given consent for general distribution by donors.

Characterisation of 19 accessions, collected on the Shetland mainland and the islands of Foula, Yell and Whalsay in 2006 (Lever 2006, Scholten *et al* 2008), started at SASA in 2007. Visual assessment and preliminary analysis of field-grown material shows wide morphological variation within and between accessions for traits such as foliage colour, head formation, head density and powdery mildew resistance. Molecular characterization was started in late 2008, but further work is needed to summarise the results.

The variation in Shetland cabbage does not appear to be linked with any particular region in Shetland, but preliminary analysis of all data suggest the variation within populations is extraordinary compared to traditional cross-pollinated *Brassica* varieties.

Small oat and rye

In the Northern Isles small oat and bere barley are grown as ‘pure’ crops, but the number of growers is small and the area has declined. In the Western Isles, cereal landraces (small oat, bere barley and Hebridean rye) are mostly grown as mixtures on the west coast on the sandy machair soils. Each grower creates their own seed mixture, the proportion of the components being selected so that the mixture is best suited to the local soil and exposure to local weather conditions. The area of landraces grown in the Western Isles is over 300 ha (Scholten 2009) and the number of growers is stable; however, most seed sown in mixtures originates from a small number of pure crops.

Timothy

As a landrace, ‘Scots’ Timothy has long been established in the Scottish Seed Certification system, which has meant that there is a certain assurance that quality seed can be maintained and sold. However, the area grown and the number of growers have declined in recent years, and the supply of the source seed has narrowed to a small number of populations.

SEED MARKETING LEGISLATION

The recent EU Directive on the marketing of seed of agricultural Conservation Varieties (Anon. 2008) was introduced to encourage the marketing of seed of landraces and traditional varieties. Although most of the usual requirements for marketing of seed are retained, reduced standards can be applied for varietal purity; there is also no requirement for official input, although variety testing, seed testing and seed sampling must be undertaken to international standards. The only official input required is the checking of varietal purity and varietal identity by growing varieties in control plots. Registration can be achieved without a

full DUS test, provided a representative variety description, based on technical questionnaire characters in CPVO technical protocols or UPOV technical guidelines, is submitted.

CONCLUSIONS

With poor weather conditions at harvest and the lack of good on-farm drying and storage conditions, it is difficult to maintain seed quality of Scottish landraces over years. The decline in the number of growers, particularly in Shetland cabbage and Timothy, means that these landraces are threatened.

Although the SLPS provides a useful reservoir of seed which is adapted to local environments, the amount of seed submitted by donors is small; the amount of seed that can be returned to growers in the event of harvest failure is therefore limited. For cereal crops, the amount stored under the SLPS would not be sufficient to grow a crop, but would be enough to re-establish the landrace in its local environment.

The SLPS supports *in situ* regeneration by identifying active local growers from seed donations, building *ex situ* safety duplication so that growers can have some of their own seed returned to them in the event of harvest failure, providing information to growers about germination and disease for the next crop, and by advising on the best methods of seed harvest and local short term seed storage.

The future of Scottish landraces depends on their continued regeneration over years. If seed harvest fails, the landrace will be lost, unless a representative seed sample is stored in *ex situ* collections. Further seed samples of Scottish landraces need to be collected to conserve and characterise and study them, so that we can understand how to develop an appropriate long term strategy to conserve and sustain this diversity.

The breeding of highly uniform modern cultivars excludes much potentially useful genetic diversity. This diversity, which exists in surviving landrace populations, may have potential for the development of cultivars which are able to cope with low nutrient soils, climate change and sustainable, low input farming. The breeding of future cultivars may depend on utilising this genetic resource within landraces and traditional varieties (Green 2008).

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PLANET SCOTLAND SOFTWARE FOR NUTRIENT MANAGEMENT PLANNING AND NVZ COMPLIANCE ON FARMS IN SCOTLAND

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Summary: PLANET Scotland (Planning Land Application of Nutrients for Efficiency and the environmenT) has been developed by SAC and ADAS (funded by the Scottish Government) as a software tool designed for routine use by Scottish farmers or advisers to plan and manage nutrient use on individual fields in a quick, easy, user-friendly way. It is designed to encourage careful planning and recording of nutrient applications to crops and grass and to help farmers comply with the legal and scheme requirements, notably the NVZ Action Programme rules. PLANET is publicly owned and is available free of charge to farmers and their advisers. Users need to set up a few relevant details of the farm and each field. The PLANET website and Helpline will be updated for use in Scotland as well as England and Wales, and retains the domain name www.planet4farmers.co.uk.

INTRODUCTION

Key policy objectives of the Scottish Government are to reduce nutrient pollution of water and air while fostering a competitive farming industry. It is estimated that about 74% of nitrate in Scottish waters originates from agricultural land (SEPA, 2006). A mix of advisory measures, regulatory measures and incentives are in place or being developed to help farmers adapt where necessary so that production methods are economically and environmentally sustainable. The revised NVZ Action Programme (AP) rules (Scottish Government, 2008) are designed to reduce diffuse nitrate pollution. Farmers with land inside a designated NVZ must comply with these rules. Future implementation of the requirements of the Water Framework Directive is likely to place further emphasis on nutrient management planning.

DEVELOPMENT OF PLANET SCOTLAND

PLANET Scotland (Planning Land Application of Nutrients for Efficiency and the environmenT) has been developed by SAC and ADAS (funded by Scottish Government) as a software tool designed for routine use by Scottish farmers or advisers to plan and manage nutrient use on individual fields in a quick, easy and user-friendly way that is also technically correct and meets compliance requirements. It is designed as a 'tool' for practitioners, to help and encourage careful planning and recording of nutrient applications to crops and grass and to help farmers comply with the legal and scheme requirements that are in place, notably the NVZ AP rules. PLANET is publicly owned and is available free of charge to farmers and their advisers. PLANET Scotland is developed in the .NET software language and will be

supported to run on Windows XP and Vista. The software will be made available, under a license agreement, for use by commercial software developers. Technically, the field-level fertiliser recommendations generated by PLANET Scotland mimic those that would be generated by using SAC technical notes including those published during 2009 (SAC TN621, SAC TN622, SAC TN623, SAC TN625). PLANET can be used to carry out the necessary calculations and produce a report showing compliance with the NVZ Nmax limits (Scottish Government 2008).

PLANET Scotland users need to set up a few relevant details of the farm and each field (e.g. farm name, average annual rainfall, field name, soil type). To obtain recommendations for each crop, information is needed for past cropping (at least one previous year), soil analysis, use of organic manures, fertilisers and lime. Some minimum information must be entered to obtain a recommendation (e.g. crop type, soil type, organic manure type), but default information is used for other attributes if specific information is not available. Users are encouraged to obtain specific information to improve the accuracy of recommendations. Once recommendations have been generated, users can devise their own nutrient application plan for each field by rate and timing of application of each nutrient. The details of the nutrient plan may be edited and then confirmed as an accurate record of what actually happened. The field record for one harvest year then becomes the basis for generating recommendations for the next harvest year.

The PLANET project was originally developed by ADAS for England and Wales with funding from Defra. It was recognized from the outset that there was a need for a close working relationship with the agricultural software industry. It was clear that farmers with existing commercial farm recording software do not want to duplicate entry of field-level information into a separate software system. Also, since field records for the majority of the national arable area are held on commercial software systems, effective integration of PLANET provides rapid market penetration. To date, the PLANET recommendations DLL (Dynamic Link Library) has been integrated into 5 commercial software systems. Each company is required by their license to ensure that their database can hold and present all of the data-types required by the DLL. Each company designs its own Graphical User Interface (GUI) for data entry and output.

PLANET Scotland also contains calculation modules that use approved methods for the calculation, recording and reporting of the following measures in the revised NVZ AP, including (Scottish Government 2008);

- An *Organic Manures Inventory and Storage Requirements* module which calculates monthly quantities and the nutrient content of farm manures, and the minimum slurry storage requirement as required for compliance with this NVZ AP rule.
- An *Organic Manure Storage Capacity* module which calculates the storage capacity of existing slurry and solid manure stores based on store dimensions.
- A *Livestock manure N farm limit* module which calculates the whole-farm manure N capacity for derogated or un-derogated farms, and the current N loading as required for compliance with this NVZ AP rule
- An *Nmax* function that calculates the farm-average maximum N rate (Nmax) for individual crop types, and compliance with this Nmax rate, as required by this NVZ AP rule.
- A *Farmgate Nutrient Balance* that calculates the balance of nitrogen, phosphate and potash coming onto the farm (e.g. in feeds, fertilisers, organic manures) against these nutrients exported off the farm (e.g. in farm produce, organic manures).

WEBSITE AND HELPLINE

Following release, it is important to provide support so that farmers and advisers can quickly and easily start to use PLANET, both correctly and with confidence. Inadequate support will significantly undermine the potential benefits that will come from wide use of PLANET in the industry.

In England, since the launch of PLANET v1.0 in January 2005, Defra has funded the PLANET website (www.planet4farmers.co.uk) developed by ADAS, and for ADAS to operate the PLANET Helpline. The website and Helpline will be updated and modified in order to service PLANET v3.0 users in England and Wales, and PLANET Scotland. PLANET emails will be sent to alert registered users to PLANET developments, new versions, events, training workshops and other nutrient management information (e.g. NVZ related matters).

1. PLANET website and user registration

The PLANET website is being updated to meet the following specifications:

- Retain the domain name www.planet4farmers.co.uk for use in England, Wales and Scotland.
- Modernise the general look and feel of the existing site to make it more attractive and user-friendly.
- The Home page will have an option to direct users to one of 3 areas - PLANET for England and Wales, PLANET for Scotland, or ENCASH. ENCASH is used to calculate the N produced by pigs and poultry based on specific dietary input, which may be used instead of standard values of manure N production in NVZs. Users will then provide tailored information as part of the process for registering to obtain a copy of PLANET Scotland (CD or download), download documentation, web links, etc.
- In each area, there will be sub-areas for:
    - 'About PLANET' or 'About ENCASH' information
    - 'News' items will be loadable to be shown between set start and end dates.
    - 'Downloads' (split into 'Software' and 'Documents') that can be uploaded/deleted/edited by registered site Administrator(s).
    - 'Register to receive a copy of PLANET'.
- On registration, a minimal amount of information about a new user will be requested and stored in the PLANET database. Details contained in the current database will be migrated to the new database.
- Provide web links.

2. PLANET Helpline

The current PLANET Helpline will be updated to meet the following specifications:

- Access to the Helpline will be via the PLANET website, email or telephone (08456, local call cost).
- Helpline reception will be managed by ADAS (as currently). Reception staff are trained to deal with simple queries, administer dispatch of CDs, booking in delegates to meetings, etc.
- Calls will have a target 'same day' response time:
    - Software queries (to ADAS)

- PLANET or ENCASH operation queries (England and Wales) (to ADAS Nutrient Management specialist on duty rota)
- PLANET Scotland operation queries (Scotland) (to SAC Nutrient Management specialist on duty rota)

3. Training farmers and advisers to use PLANET Scotland

The provision of effective and easily accessible training is essential so that farmers and advisers can confidently and correctly use all the tools and the full functionality of PLANET. To date in England, support to PLANET users has been through a Helpline and face-to-face, hands-on, training workshops. However, with the increasing number of PLANET users (currently over 10,000 registered), it was necessary to explore alternative, widely available and cost-effective ways of providing training to both new and existing users. Face–to-face workshops are very effective for those who are able to attend, but have proved to be inaccessible to many interested users due to problems of location, date, available places, etc.

Development of a series of web-based training tutorials is under consideration as the internet allows software training and demonstrations to be available to users of the software in their own home or office, and for accessing in their own time. Such training could be provided as a structured programme of training tutorials; each pre-recorded section of the tutorial would demonstrate the steps required for completing a PLANET task, or series of tasks, with an audio commentary talking the trainee through the sequence, and annotations highlighting key aspects. Each tutorial could be paused, fast forwarded or rewound. The training tutorials would be hosted on a website which the PLANET user could visit to download and view in their own time.

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FARM SCALE DIFFUSE POLLUTION MONITORING: UNDERSTANDING THE ISSUES AT THE FARM SCALE

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SUMMARY: Diffuse pollution arising from agricultural sources is receiving increased attention from regulatory agencies in Scotland. Through its Environmental Focus Farm project (EFF), the Scottish Agricultural College (SAC) has in partnership with the Macaulay Land Use Institute been working with the Scottish farming community to understand their impact on Scotland's water quality and to reduce diffuse pollution from agricultural sources. Two EFFs have been established, one in Ayrshire and a second in Angus. Both are in priority water catchments identified by SEPA and Scottish Government. As part of this work SAC has been monitoring the water quality on the EFF in Ayrshire to understand its magnitude and to assess the impact of implementing diffuse pollution mitigation methods. This paper reviews the results from 2007 to 2009 monitoring of Faecal Indication Organisms (FIO) and phosphorus from a burn that runs through the Ayrshire EFF. Proposed diffuse pollution measures have yet to be implicated and are scheduled for completion in 2010. The preliminary results for FIO monitoring confirm that higher levels of FIO occur in the burn following intense rainfall. They also show that FIO and phosphorus loading of the burn is occurring upstream from the EFF and isolating its contribution will be difficult. In absolute terms the levels of FIO and phosphorus in the burn are of concern when compared to proposed regulatory targets.

INTRODUCTION

The Scottish Government along with SEPA have recently announced and implemented a range of strategies and legislation aimed at ensuring Scotland is compliant with the timeframe of the European Water Framework Directive (WFD) which sets specific targets by which member countries must characterise and protect their water resources. Compliance with the existing legislation such as the Bathing Water Directive has also been a long standing issue. Both directives have clearly stated the risk that diffuse pollution from agricultural sources can have on Scotland's water quality. Diffuse pollution monitoring and mitigation from rural land uses is an objective of Scotland's WFD strategy and is a focal point for the activities of the Scottish Government and SEPA through range of initiatives including listing it as a priority under Scotland Rural Development Programme (SRDP) and through the recent enactment of the Water Environment (Diffuse Pollution) (Scotland) regulation in 2008.

SAC in partnership with the Macaulay Land Use Institute have established EFF in two diffuse pollution priority watersheds. The objectives of the projects are to:

1. identify and test practical and affordable ways in which farmers and other stakeholders can address diffuse pollution issues;

2. demonstrate and evaluate the effectiveness of measures to mitigate diffuse pollution by appropriate monitoring;

3. achieve effective mitigation of diffuse pollution in catchments representative of typical land uses, through implementation of appropriate measures;

4. investigate the potential of alternative policy scenarios for achieving Good Ecological Status in surface waters and groundwater resource protection within a sustainable socio-economic framework.

A central part of the project has been the water quality monitoring for key diffuse pollutants on the SAC EFF Dairy Farm in Ayrshire in conjunction with farm diaries showing day to day activities. Understanding and reducing the diffuse pollution loading of manures and slurries is regional priority in Ayrshire due the impact that farming in the Ayrshire can have on Bathing Water Quality on the Ayrshire Coast (Lipp *et al.*, 2001: Vinten *et al.*, 2008). The monitoring results are helping to inform local farmers about the impact of their activities on water quality and to try and assess the impact of implementing diffuse pollution mitigation measures. A range of parameters have been monitored but this poster will discuss results for Faecal Indicator Organism (FIO) and phosphorus monitoring.

BACKGROUND

In 2006 SAC established an EFF on Low Holehouse farm near Mauchline in Ayrshire. Low Holehouse is a 120 head; slurry-based dairy operation within the Cessnock watershed. The farm extends to 57 ha and including rented and separately owned land within the catchment the land base for the operation stretches to approximately ~165 ha. Low Holehouse farm is bisected by the Killoch Burn, a tributary of Cessnock Water. The Killoch drains 372 ha and there are eight livestock enterprises located within the sub-catchment, six of which are dairies located upstream from Low Holehouse which is near the outflow of the Killoch to Cessnock water.

In 2007 five monitoring stations were established on Low Holehouse farm, three along the Killoch, and two located on a farm ditch. ISCO 6712 automatic samplers (ISCO-Teledayne, Nebraska, USA) were installed at each sampling point and equipped with a rain gauge and area velocity meter. During the bathing season (1 June – 15 September) the samplers where programmed to take one sample per hour once rainfall intensity exceeded 1.5 mm in a 15 min period. The first samples where taken to represent low flow concentration and was augmented with opportunistic samples through the summer. All samples were collected and submitted for analysis with 24 hours of collection. The analysis for total coliforms (TC),

faecal coliforms (FC) and Enterococci (EN) was done using methods defined by the Scottish Environmental Protection Agencies (SEPA) as part of their Bathing Water Program (Fowler, G., SEPA *per comm*.).

For Total Phosphorus and Orthophosphates the above monitoring was augmented by bi-monthly sampling throughout the year and the analysis was conducted by SEPA using methods defined for the water classification systems as part of their monitoring obligation under the WFD (Walker, S., SEPA *Per comm*.). Following a comprehensive diffuse pollution audit of Low Holehouse by SAC staff, a range of diffuse mitigation measures were proposed. Due to the high capital cost of the proposed measures the implementation phase of the project has been delayed to early 2010 from its original 2009 target. The monitoring is therefore still in a pre-implementation phase.

FIO Monitoring

The monitoring for FIO has been going for three bathing seasons (2007-2009). For purposes of this paper only the results from two monitoring stations (Stations A and B) are discussed. Station A is where the Killoch Burn flows onto Low Holehouse farm and Station B is where it exits the property. These stations were established to monitor and isolate the degree of diffuse pollution loading occurring from the farm as a whole. Results for low and high water flow monitoring of FIO over all three seasons are summarised in Table 1.

Table 1. Geometric mean of FIO (cfu/100ml) result for high and low flow. TC: total coliforms; FC: faecal coliforms; EN: enterococci.

	Low Flow		High Flow	
	Station A (n = 18)	Station B (n = 18)	Station A (n = 51)	Station B (n = 51)
TC	2158.8	1878.4	17488.5	17460.1
EC	872.3	779.5	8356.6	7686.4
EN	153.6	583.1	107.5	2153.8
	(n = 15)	(n = 13)	(n = 15)	(n = 15)

These preliminary results confirm the established relationship between increased flow resulting from intense rainfall events and FIO concentrations (SEPA, 2002). The high flow results also show that a concentration of FIO in the Killoch as it flows onto Low Holehouse (Station A) are similar and in some cases higher then when it leaves the farm (Station B). Work is ongoing to relate these results to volume of flow and allow for estimate of total loading at both locations which would confirm these findings. The exception is EN which has consistently higher contractions at Station B. A portion of the total load of FIO entering the Killoch occurs upstream for Low Holehouse and isolating the farm as a source of FIO is difficult. Table 2 and 3 show the summary of the annual results for both stations during high and low flow periods.

Table 2. Annual geometric mean of FIO (cfu/100ml) result for high and low flow for Station A. TC: total coliforms; FC: faecal coliforms; EN: enterococci.

	2007		2008		2009	
	High Flow	Low Flow	High Flow	Low Flow	High Flow	Low Flow
TC	13211.1	725.9	23415.0	6032.2	19464.0	1704.7
EC	6104.8	274.2	9262.4	1878.1	13341.9	1047.9
EN	NA	NA	2804.7	1101.1	770.5	100.0

Table 3. Annual geometric mean of FIO (cfu/100 ml) result for high and low flow for Station B. TC: total coliforms; FC: faecal coliforms; EN: enterococci.

	2007		2008		2009	
	High Flow	Low Flow	High Flow	Low Flow	High Flow	Low Flow
TC	9306.8	820.2	28442.6	8854.4	14106.9	985.5
EC	3868.8	270.5	12284.4	2291.3	8092.6	958.7
EN	265.5	168.8	7523.4	1253.6	615.4	327.5

The annual results also show that that loading of FIO is occurring upstream of Low Holehouse particularly for high flow periods. The higher FIO results for 2008 are consistent with larger number of intensive rainfall event that where captured as compared to 2007 and 2009. The assessment of individual event combined with the seasonal averages is showing that discreet loading from Low Holehouse is occurring at low flow which is consistent with some of the concerns raised during the diffuse pollution audit. No comparison of FIO results has yet been made to other studies or to companion a study being conducted by SEPA in a neighbouring catchment.

Phosphorus Monitoring

Total phosphorus (P) and Orthophosphate (OP) results are available from July 2007 through to June 2009. Table 4 shows summary results for both P and OP for the summer (May-September) and winter periods for both Stations. Due to analytical cost results for Total P are limited.

For both P and OP the winter results have been heavy influenced by two individual samples taken in April and October 2008 during winter flooding. An additional bias applies in the summer results due increased sampling following rainfall events. The results show an increased loading of phosphorus to the burn in the summer months. Additional work is ongoing to assess low and high flow concentration and to relate the result to volume flow and

suspended solid analysis. Results from individual sampling events during high rainfall periods are also showing a relationship between volume of flow and P concentrations. As with the FIO results a diffuse loading of P is occurring upstream this may obscure the contribution from Low Holehouse.

DISCUSSION

The monitoring is ongoing and by the spring of 2010 it is expected that diffuse pollution mitigation measures will be fully implemented on Low Holehouse. These preliminary results for FIO, P and OP concentrations in the Killoch show that a diffuse pollution loading is occurring upstream of Low Holehouse and isolating its input will be difficult. Work is ongoing to relate these results to volume of flow and to assess increases that can be attributed to drainage from Low Holehouse. In absolute terms many of the individual results for FIO concentrations are of concern when compared to the tighter standard being introduced under the EU Directive 2006/7/EC which is coming into force in Scotland. The OP and Total P results are also systematically high when compared to standards under proposal from the UK Technical Advisory Group on the Water Framework Directive.

Table 4. Summary of OP and Total P for the winter and summer Period between 2007 and 2009.

	Summer (May to September)					
	Orthophosphate (mg/l)			Total P (mg/l)		
	Average	Max	Min	Average	Max	Min
Station A	0.21 (n = 65)	0.61	0.01	0.22 (n = 29)	0.51	0.08
Station B	0.23 (n = 60)	0.95	0.04	0.25 (n = 30)	0.79	0.05
	Winter (October – April)					
Station A	0.18 (n = 29)	1.2	0.01	0.26 (n = 29)	1.61	0.033
Station B	0.16 (n = 31)	0.69	0.06	0.22 (n = 31)	1.02	0.07

Through SAC's EFF program this monitoring has and will continue to help farmers in Ayrshire understand and directly link their activities to water quality issues in Scotland.

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INTEGRATED MANAGEMENT OPTIONS FOR AGRICULTURAL CLIMATE CHANGE MITIGATION (IMPACCT)

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IMPACCT is a European Commission research project that seeks to develop a software tool to help European agriculture reduce its climate change impacts. The tool will be designed to facilitate farmers and growers take action to reduce their greenhouse gas emissions and improve carbon sequestration by modifying farming practices. It will also support policy makers in the development and improvement of climate change mitigation policies.

Although the focus of IMPACCT is on climate change, it is important not to forget all the other goods and services that agriculture needs to provide. Sustainable agriculture is about finding a balance between environmental, economic and social objectives. Achieving one objective, i.e. climate change mitigation, should not be pursued at the expense of other objectives. Agriculture needs to be economically viable, produce enough food, fibre and oils to equitably meet the needs of an increasing global population, and ensure that any other detrimental environmental impacts are minimised to acceptable levels. Therefore, the project will take a whole farm integrated approach seeking to identify any benefits and/or burdens on the environment, farm economics or society more generally that changing farming practices to mitigate climate change might have.

The model development process is supported by a comprehensive literature and data review and a number of farm case studies/consultation exercises that will be undertaken in several EC Member States. This process will help define the requirements of the model, based on the needs of end users, provide concrete examples of mitigation actions and provide a picture of what is already happening across the EU.

The project consortium is being led by the Agriculture and Environment Research Unit at the University of Hertfordshire, England. The project partners are Institut National de la Recherché Agronomique, France; Centro Ricerche Produzioni Animali, Italy; Wroclaw University of Environmental and Life Sciences, Poland; University of Ljubljana, Slovenia; FH Land Management, Scotland; Szent Istvan University, Hungary and Ingenieurbüro Feldwisch, Germany. Further details of the project and partners are available on the project website: http://www.herts.ac.uk/aeru/impacct/

MOVING TOWARDS SUSTAINABILITY – WHAT ARE THE POTENTIALS FOR INDIAN NEEM TREE (*AZADIRACHTA INDICA*) EXTRACTS TO BE USED IN THE INTEGRATED MANAGEMENT OF THE LARGE PINE WEEVIL (*HYLOBIUS ABIETIS)*?

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The Large Pine Weevil (*Hylobius abietis*) is the most significant insect pest affecting the commercial reforestation process of temperate coniferous forests in Northern Europe. At present the synthetic insecticide alpha-cypermethrin is registered for use as a plant protection product, however, with the stringent changes to EU regulations governing insecticides, synthetic pyrethroids for use in forestry are progressively becoming non-viable. The findings of laboratory and field bio-assays using extracts from the seeds of the Indian Neem Tree (*Azadirachta indica*) are presented, showing that the feeding of *H. abietis* is reduced, thereby increasing the conifer seedlings chance of survival.

The seeds of *A. indica* contain a wide range of triterpenoid compounds which have a number of effects on insects including repellency, antifeedant and growth-regulating properties. The findings of this study indicate that there is potential to manufacture a product from a sustainable resource which is comparable in efficacy to alpha-cypermethrin. Shifting the dependence of *H. abietis* management from a synthetic neurotoxin to a product derived from a natural resource, which is innocuous to mammals and beneficial insects, contains many advantages towards the well-being of human and environmental health. With the likely adoption next year of the new EU Regulation on Plant Protection Products with its themes of low-risk to people and the environment, utilisation of *A. indica* extracts as a plant protection product has great future potential.

A BREEDERS PERSPECTIVE ON DISEASE RESISTANCE BREEDING

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Summary: In order to meet the demands for wheat, yields must increase by 50% by the year 2030 (Marathee and Gomez-MacPherson, 2001). Population growth, demand for biofuels and a radical change in dietary requirements in the developing world are driving the need. To meet these demands the ability to maximise grain yield *potential* will be critical. This will depend on the development of strategies that will harmonise the use of genetic resistance to pathogens and the use of appropriate fungicides.

Whilst not wishing to understate the value of other wheat diseases, priority diseases are *Septoria tritici* (leaf), eyespot (root) and *fusarium spp* (ear). Each disease will demand a different genetic strategy to maximise the utilisation of genetic resources as well as synergistic use of fungicides to optimise control.

INTRODUCTION

Wheat breeders are faced with a large number of breeding objectives. Although grain yield *'per se*' carries the highest value, it can be argued that there are considerable constraints on breeding for higher yields – end use market requirements, agronomic performance and breeding for disease resistance '*per se*'. This last point is particularly relevant as over the last thirty years a range of disease resistant winter wheat varieties have been added to the UK Recommended Lists. However none have found favour in the market place by virtue of their lower yield potential when treated with a full prophylactic fungicide programme. As a result breeders have lost enthusiasm for breeding for disease resistance, resulting in less than optimal genetic control of a range of damaging diseases.

The 'Green Revolution' which fed the world following the introduction of semi dwarf wheats in the 1970s has often focused on the development of this new genetic material which possessed higher yield *per se*. This is not unjust but the real benefits of this new germplasm was the opportunity to change farming practices – the utilisation of higher rates of nitrogen and the wide-scale use, particularly in the developed world, of highly efficient fungicides. The UK was the beneficiary of this new approach to growing wheat as a range of high yielding cultivars were developed which would respond to the higher inputs being applied. However this approach resulted in a selection system which favoured varieties with moderate or even poor disease profiles but which nevertheless responded to higher inputs of fungicides producing high yields in farm situations.

DISEASE RESISTANCE – PRIORITIES

Wheat breeding in the UK cannot act in a vacuum whereby it denies the opportunities that the wide range of agrochemicals available present. There should be no conflict between the two approaches – breeding for high yield potential and breeding for high levels of response to fungicides – albeit at much reduced active ingredient rates in the future. In reality, yields of wheat in the UK grown without fungicides would decrease by 30% overall and in years of severe disease breakdown by significantly more than this.

Breeders must ascribe priorities and allocate precious breeding resources accordingly. In terms of disease resistance breeding the wheat plant can be divided into three components

1. The stem/ leaf tissues
2. The root system
3. The ear

It can be argued that for each of these components there are higher priority diseases – based upon potential damage and the opportunities that breeders identify to address the problem.

1. The stem/ leaf tissues

Much attention is drawn to the developing wheat plant above ground as it is easy to relate to, being visible throughout the growing cycle and easy to assess for disease susceptibility. Although attacked by a range of diseases through its life cycle, genetic and fungicide control of most diseases is relatively simple. Yellow rust (*Puccinia striiformis*) will be particularly problematic in the next few years as there is a reliance on a narrow genetic base. There has been a history over the last 50 years of 'boom and bust' cycles as breeders have deployed major genes for resistance which have been overcome by race changes in the pathogen. With unknown background resistance many varieties have become severely infected resulting in dramatic changes in the variety profiles over relatively short periods. However, whilst this disease is a very emotive disease – being particularly visible – control measures can be put in place to mitigate any substantive losses.

The most damaging foliar disease of wheat is septoria tritici blotch (STB) caused by *Mycosphaerella graminicola* (anamorph *Septoria tritici*) which has proven to be difficult to breed for and with reducing efficacy of key fungicide products difficult to control in the field. In addition, unlike the other foliar diseases, it is prevalent in every field in every year – an endemic disease. In addition, yield losses ascribed to *Septoria tritici* are often underestimated and grain quality deterioration as a result of attack is often overlooked.

Prior to the 1970s, the predominant Septoria disease was stagonospora nodorum blotch (formerly septoria nodorum) caused by *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum)*. The introduction and widespread growing of semi dwarf varieties, such as Longbow, Riband and Consort, sustained high levels of inoculum, thereby maintaining the threat until the present day. A substantial reduction in the level of atmospheric pollution by SO_2 may also have influenced this major change (Arraiano *et al*., 2009). *Septoria tritici* must therefore be considered by wheat breeders to be the most important target for disease resistance breeding.

The most effective and sustainable means of keeping damage from STB to a minimum has to be a combination of both chemistry and varietal resistance. The contribution required from each component of this integrated approach will vary from situation to situation. The weakening of genetic resistance to STB has been reported and the emergence of widespread insensitivity to strobilurin fungicides has been of major consequence. Concerns relating to the gradual erosion in efficacy of azole fungicides have been exacerbated by recent reports of the possible discovery of new azole insensitive pathotypes in France and Ireland.

Breeding for high resistance to STB together with acceptable yield potential is not easy. The genetics of resistance are complex and there frequently appears to be a cost, in terms of yield potential, of having superior resistance. Thirteen major genes for resistance have been identified and assigned gene symbols. These specific resistance genes differ in their reaction to known isolates of STB. In addition, work done at the John Innes Centre (JIC), indicates that a wide range of partial STB resistances are present among UK wheat varieties. The IMPRESSIV (Improved Resistance to *Septoria Tritici* in Superior Varieties) collaborative project involving the JIC and a consortium of five wheat breeding companies and funded by DEFRA and the Home-Grown Cereals Authority through Sustainable Arable LINK is currently working to provide breeders with increased knowledge of STB. This new knowledge should help improve the supply of *Septoria* resistant varieties with high yield.

After testing 226 new and old varieties (Arraiano *et al*., 2009), it was noted that several of the lines showing the greatest resistance to STB, including Exsept, Boxer and Flame, were bred by the Nickerson wheat breeding programme. This paper concludes that there may be useful partial resistance genes within the Nickerson germplasm. Such resistant varieties were produced by historical and subsequent crossing and identified by the establishment of highly effective STB screening nurseries. Our experience shows that the crossing of two or three varieties, all with moderate resistance, can produce progeny with significantly greater resistance. This suggests that there are indeed different resistance factors which must be additive in effect. It is certainly more difficult to breed for high resistance combined with high yield potential but the problem is not insurmountable. The final results coming from the IMPRESSIV project and 'in-house' experiments should identify molecular markers which are associated with the most favourable partial resistance QTL (quantitative trait loci) i.e. those associated with the greatest reduction in STB severity and without a negative effect on yield. Future crossing and selection can, therefore, be even more targeted towards a continued improvement in STB resistance.

The relatively recent introduction of varieties derived, in part, from wild emmer wheat (*Triticum dicoccoides*) has provided a broadening of the UK germplasm base. Such varieties exhibit novel traits, some desirable and some less so. Robigus and Timber have improved levels of STB resistance. Even more recently, Stigg has shown to be exceptionally resistant to STB. However, it is known that breakdown of resistance based on major genes does occur as witnessed by the demise of the variety 'Gene' in Oregon over a relatively short five year timescale (Chartrain *et al.*, 2004)

Breeders can quite rightly claim to have steadily pushed STB resistance levels upwards using traditional pathology techniques. This is exemplified by the continued increases in disease ratings for this disease found within the UK CEL Recommended list.

The rapid increase in the prevalence of *Septoria tritici* in the UK can give an insight into potential problems if climate change continues and new diseases (for the UK) such as tan spot (*Pyrenophora tritici-repentis*) become prevalent.

2. The root system

Roots are often neglected by pathologists as they are regarded as being difficult to work with as a subject because they are subject to a range of diseases. However, the incidence of eyespot *(Pseudocercosporella herpotrichoides*) in the UK is particularly important as this is an endemic disease and one which decreases both grain yield and quality. Whilst usually associated with growing a second wheat after wheat crop this disease is prevalent even within first wheats. This is a consequence of shorter rotations and the earlier sowing dates now being used. In essence, early drilling will result in first wheats expressing many of the root disease symptoms normally associated with 'second wheats'.

The primary source of resistance for eyespot is that derived from the French bred winter wheat variety Cappelle Desprez – designated Pch2. This resistance was deployed throughout UK germplasm very effectively during the mid to late twentieth century. Recognising the narrow genetic base being used within the UK, attempts to broaden the range of material being used in crossing programmes were carried out in the 1970s. This process continues to this day. However, the majority of material used within these programmes carried little or no resistance to eyespot. The genetics of resistance are known to be complex and without robust field selection it is soon lost – hence the gradual degradation in resistance today. There is a real need for a laboratory based marker to increase selection efficiency to maintain this resistance within the UK germplasm base.

In the 1970s a more 'powerful' resistance was deployed within UK material derived from the wild oat grass *Aegilops ventricosa* and designated Pch1. This resistance provided a higher level of resistance and was introduced commercially via the variety Rendezvous in 1987. However, associated with this resistance was a 'yield drag' whereby yields were less competitive (2-4%) than their non resistant counterparts. Some 30 years later this association has still not been broken and a range of varieties carrying this resistance (Lynx, Hyperion, etc.) have been ignored by growers as they took advantage of higher yielding but less resistant varieties.

There is now, however, strong evidence that deployment of these varieties would have reduced overall eyespot levels within wheat crops with consequential benefits for succeeding wheat crops. The development of earlier drilling of winter wheat and reduced cultivations will increase further the build up of this disease.

The most important 'economic' root disease is 'Take all' (*Gaeumannomyces graminis* var. *tritici)* but as yet there is no evidence of 'resistance' in wheat. However there are indications of higher levels of tolerance in some varieties which could be exploited. The importance of this disease has been recognized by the investment being made by the Wheat Genetics Improvement Network (WGIN) sponsored by Defra. However, in the long term it is probable that resistance will have to be transferred from other related species either through conventional or genetic modification (GM).

3. The ear

Fusarium spp are the most significant ear diseases with significant yield reductions and grain quality degradation serious consequences. Fusarium ear blight or Fusarium head blight (FHB) is caused by a number of *Fusarium* species, the main ones being *Fusarium graminearum* and *Fusarium culmorum*. *Microdochium majus* and *Microdochium nivale* also cause FHB. All species produce similar symptoms and many of the species produce mycotoxins which contaminate the grain and, above certain levels, can affect the health of human and animal consumers.

FHB is a sporadic disease in the UK. Wet weather, particularly at the time of flowering, is favourable to the development of the disease. Wheat following maize as the previous crop is subjected to a greatly increased FHB risk as the fungi causing the disease overwinter on the maize residues. Relatively little maize is grown in the UK which reduces the incidence of FHB and associated mycotoxin problems compared to many other countries. The advent of global warming and the potential for increased areas of grain maize in the UK will increase the threat of this damaging disease.

No varieties are immune to FHB and there is little variation in resistance between the majority of UK varieties. A few exhibit resistance levels outside the range normally observed and the best of these are made use of in current breeding programmes. More potent sources of resistance have been identified in Chinese germplasm. The most notable of these is the variety 'Sumai 3'. A few QTL (quantitative trait loci) have been identified as being responsible for controlling the resistance of this variety and breeders worldwide are working to exploit these.

Increasing legislation regarding mycotoxin levels in blighted grain and the potential impact from climate change has already increased the priority of FHB resistance as a breeding objective. This trend will continue and new sources of resistance and the molecular tools to track resistance will be sought.

FUTURE PROSPECTS

As the demand for wheat increases and environmental issues increase in importance, control of disease within wheat will become increasingly important. Already world wheat supplies have been threatened by the evolution of a new stem rust (*Puccinia graminis*) race (Ugg 99). In addition, new virulent races of yellow rust have been reported in Australia and southern Europe (Milus *et al*., 2009). Resistance to azole chemistry continues to increase (Defra-funded Sustainable Arable Link project (LK0976)).

The control of key diseases within the UK is also under threat from the 'New rules on pesticide residues to strengthen food safety in the European Union' (ec.europa.eu/food/plant/protection), whereby a number of established plant protection products will be withdrawn from the market over the next few years. The timescale for new products to come forward from the agro-chemical industries is so long that there could be deficiencies in plant protection products for key diseases. Products which control *Septoria tritici* (primarily azole chemistry) are particularly vulnerable.

With climate change will come new threats to the UK, with the incidence of tan spot (*Pyrenophora tritici-repentis*) – now a very minor disease in the UK – forecast to increase significantly. A major disease problem in continental Europe, this disease is likely to become readily adapted to a 'warmer' UK in the future. In addition stem rust (*Puccinia graminis*), a major threat within southern Europe, can be expected to become a threat should summer temperatures rise as predicted.

The UK is well placed to counter these threats as UK wheat breeding activities are primarily within European programmes with consequential prior exposure to these threats. In addition, the range of technologies available to the breeding community has increased over time with molecular marker assisted selection systems in place for the current threats. Resistant material is available within germplasm resources both worldwide and domestically and this can be made available in relatively short timeframes via enhanced delivery streams using either single seed descent or double haploids. In addition, the UK has one of the most competitive wheat breeding environments in the world, with good levels of investment both from the private and public sectors. Future success will depend largely on the mechanisms put into place which will identify and exploit the synergies between these two sectors. The development of structures such as the Wheat Genetics Improvement Network, the Crop Improvement Club (CIC) and the Technology Strategy Board (TSB) will be important if the UK is to meet the demands of maximising wheat production and mitigating yield losses through disease.

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GENE MAPPING OF DISEASE RESISTANCE PHENOTYPES IN BARLEY

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Summary: We report the analysis of 16 field disease nursery and nine detached leaf scores of Rhynchosporium conducted on 150 random inbred lines from the barley cross Derkado x B83-12/21/5. By using a new SNP map and a QTL x Environment mapping procedure, we could directly compare the genomic locations of resistance effects detected by both types of test. The most significant resistance effects were associated with the absence of dwarfing alleles at the *sdw1* and *ari-e*.GP loci but, as these were also detected by the detached leaf tests, these appear to represent genuine partial resistance effects. Two other partial resistance effects were located in the region of the *Ml(La)* and *mlo* mildew resistance loci, one with a major flowering time locus and the final locus was not associated with any known major gene. None of the effects were consistent across all environments and the causes of their instability need to be established.

INTRODUCTION

Recent EU legislation severely limits the prospects for continued use of fungicides to control foliar pathogens of crops. This means that improved plant resistance to foliar pathogens is required for the future sustainability of crops. From harvest 2008, spring barley cvs Optic and Oxbridge accounted for 75% of the Scottish maltster purchases but neither has very strong whole-plant resistance to Rhynchosporium. Whilst there are a number of sources of whole-plant resistance to Rhynchosporium, the most effective are based upon single major genes for which there is matching virulence in the pathogen population (Zhan *et al*., 2006). Whilst a newly introduced cultivar can have an effective resistance, its effectiveness declines as it increase in popularity. For instance, cv. Doyen was rated as an '8' when first recommended in 2004 and is currently rated a '7' (www.hgca.com). Continuous cropping of a resistant cultivar can also lead to a rapid decline in the effectiveness of a major-gene resistance to Rhynchosporium. Cv. Livet was used as a spreader in a Net Blotch nursery at SCRI as it had an effective major-gene resistance to Rhynchosporium but was susceptible to Net Blotch yet substantial amounts of Rhynchosporium developed upon it by only the second year of the nursery, indicating strong selection for a strain of the pathogen that could overcome the resistance (WTB Thomas and AC Newton, unpublished data). This suggests that the deployment of major gene resistances to Rhynchosporium will have limited impact upon the long-term sustainability of spring barley cropping in Scotland.

By contrast, whole-plant resistance to Rhynchosporium appears to be much more effective in winter barley cultivars with one, cv. Suzuka, achieving the maximum rating of '9' on the 2009 UK Recommended List and 10 others scoring '8' and none scoring less than '6'

(www.hgca.com). Genomic surveys show that the winter and spring barley gene-pools are clearly distinct (Rostoks *et al.,* 2006), indicating little inter-breeding between the two. However, it is highly likely that the resistance of winter barley cultivars is due to some of the same major-genes that have been deployed less successfully in a spring background.

Previously, we have described the mapping of resistance factors to Rhynchosporium in a spring barley cross in which there were no major-gene sources of resistance. These 'partial' resistance effects are presumed to be race non-specific and therefore potentially durable. These resistance factors were detected from scores of resistance obtained from natural infection in special Rhynchosporium nurseries and detached leaf tests using a range of isolates (Newton *et al.,* 2004). The major problem with the analysis employed was that it was not possible to combine the two different sets of data obtained from the disease nurseries and detached leaf tests. This means that it is not possible to detect any consistent resistance effects. Furthermore, the previous analysis only detects QTL main effects, i.e. those that are consistent across all the disease assessments. Resistance effects are highly subject to variations in the test environment, which can be dependent on weather, pathogen race properties, or a combination of both. Recent developments in QTL mapping now permit the separation of QTL main effects from true QTL x Environment interaction effects whilst accounting for the observed relationship of means and variances across the different environments (Boer *et al.,* 2007). The net effect is to improve the power of the analysis.

The development of a high throughput SNP genotyping platform for barley (Close *et al.,* 2009) has greatly improved the ability to analyse barley genotypes with molecular markers. The first Barley Oligo Pooled Array (BOPA1) enables genotyping of barley with molecular markers representing 1536 different barley genes distributed throughout the genome. Because the markers are located in known barley genes, we can exploit the syntenic relationship between barley and rice, for which a full genomic sequence is available, to explore lists of potential candidate genes underlying genomic regions affecting the control of important characters. We have incorporated some new data and utilised the new mapping methodology with the BOPA1 map for the Derkado x B83-12/21/5 population to identify genomic regions for partial resistance to Rhynchosporium.

MATERIALS & METHODS

The Derkado x B83-12/21/5 population consisted of 150 doubled haploid lines derived by anther culture from the F1 of the cross. Derkado had the *sdw1* and *mlo* dwarfing and mildew resistance genes respectively and B83-12/21/5 had the *ari-e.*GP dwarfing gene so the population also segregated for these three major genes. For this study, we created a genetic map from the above major-genes and just over 500 polymorphic SNP markers detected by surveying the population with BOPA1. This resulted in seven linkage groups corresponding to and representing good coverage of all seven barley chromosomes.

Previously reported data represented means from disease nurseries grown in 1994 (one scoring date), 1995, 1996, 1997 and 1999 (all with two scoring dates). For the current study, we treated each scoring date within each year as a separate 'environment' and augmented these with new data obtained from disease nurseries grown in 2005 (two scoring dates) and 2006 (five scoring dates). Thus we had a total of 16 environments for the disease nurseries.

We also scored the population for resistance to 9 individual isolates of Rhynchosporium in detached leaf tests (Newton *et al.,* 2001). All data was recorded on a 1-9 scale with 1 = complete absence of any infection. Relationships between the 25 different scores were explored by Principal Component Analysis, with the results displayed as a bi-plot, using the software SC-Biplot (Smit Consult, The Netherlands). In a bi-plot, the angle formed by the projections of environmental scores from the origin represents the correlation between them. Angles close to 0º represent high positive correlations and angles close to90º represent un-correlated environments.

Data from the 25 separate scores of Rhynchosporium infection on the population were combined with the genotypic data and the genetic maps to detect genomic regions where QTL main effects and QTL x Environment interactions were located. We used the same methodology as that employed by Boer *et al.,* 2007 to detect QTLs for yield in maize which is now implemented in Genstat 12 (Payne *et al.,* 2009). The test statistic was –Log10(P) and genomic regions where it exceeded the 5% genome wide error rate threshold of 3.785 were declared as QTLs.

RESULTS & DISCUSSION

Figure 1 is a bi-plot summarising the Rhynchosporium infection data for the Derkado x B83-12/21/5 mapping population over the 25 individual environments in which we gathered data. It can be seen that the majority of the projections of the vectors for the detached leaf tests are perpendicular to those for the field nurseries, indicating no correlation between the two types of test. Scores on the first principal component reflect the overall resistance of the individual genotypes with high and low scores representing susceptibility and resistance respectively. It is noteworthy that the scores for Rh06_1 are on the other side of the origin for the remaining scores gathered in 2006. This indicates a strong negative correlation between the Rh06_1 and the other scores, especially those for Rh06_2 and Rh06_3. The spread of the lines either side of this axis indicates that there is considerable Genotype x Environment interaction within the data set, thus validating our strategy of detecting QTL x Environment interactions.

QTL x Environment analysis of the data detected at least one QTL in all environments apart from DLRh1 and Rh1DN06. The analysis revealed two extremely significant regions associated with Rhynchosporium resistance (Figure 2). These were, however, the locations of the two major dwarfing genes and, as the effects are consistent with taller plants being more resistant, suggest that these effects largely represent escape mechanisms. The detached leaf scores are, however, completely independent of height and Figure 2 shows that the regions of the dwarfing genes show significant associations with some of the detached leaf scores, indicating that there are some genuine resistance effects located in these regions. The resistance effects of the dwarfing gene regions in the detached leaf tests were, however, nowhere near as consistent as those detected in the disease nurseries and both showed clear evidence of cross-over interactions. The next most significant effect was located on the long arm of barley chromosome 2H and this was, in fact, the strongest effect detected for the detached leaf tests, being significant in six of the 9 tests. Apart from a barley significant decreasing effect from Derkado for Rh1DN94, all the resistance effects at this locus were derived from B83-12/21/5. We are not aware of any major resistance genes segregating in this region, although it is likely that the Ml(La) mildew resistance is located in the same

region (Giese *et al.,* 1993) and it is possible that B83-12/21/5 carries this mildew resistance gene as well as the Mla13 resistance.

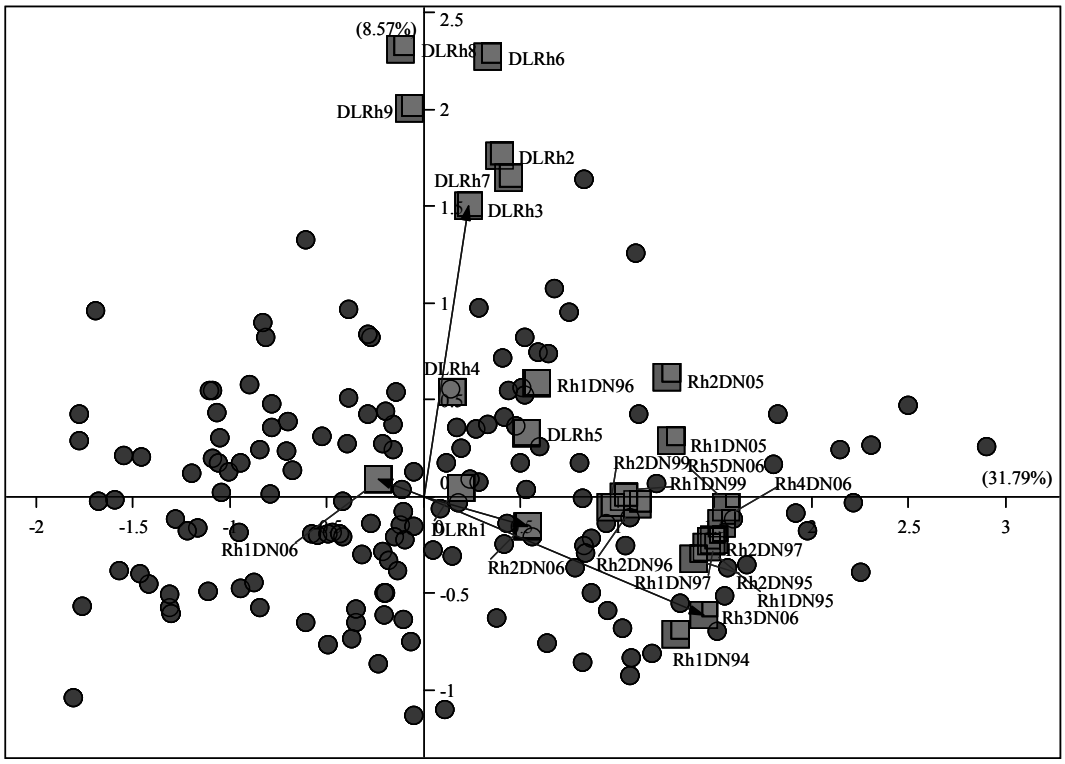

Figure 1. Bi-plot showing Rhynchosporium infection data effects for detached leaf and field data for Derkado x B83-12/21/5 mapping population over the 25 individual environments. Squares and circles represent environments and genotypes respectively. Straight lines represent the projections of selected environments from the origin.

Three other significant effects were detected on each of barley chromosomes 4H, 6H and 7H. The effect on 4H is located in the region of the *mlo* mildew resistance locus but there was clear evidence of a cross-over interaction for this locus with the resistant allele being derived from the non-*mlo* parent (B83-12/21/5) in three of the nine environments where there was a significant effect of this region. The association of the wild-type *mlo* allele with resistance was found in both environments in the 2005 disease nursery and also in one of the detached leaf tests (DLRh5). In contrast, the only associations of the *mlo* resistant allele with field resistance were detected in the last three environments of the 2006 nursery. The reasons for these quite contrasting results may reflect environmental differences between 2005 and 2006 but it is also noticeable that we did not detect any evidence of associations of *mlo* with Rhynchosporium in the nine environments that we tested the population between 1994 and 1999.

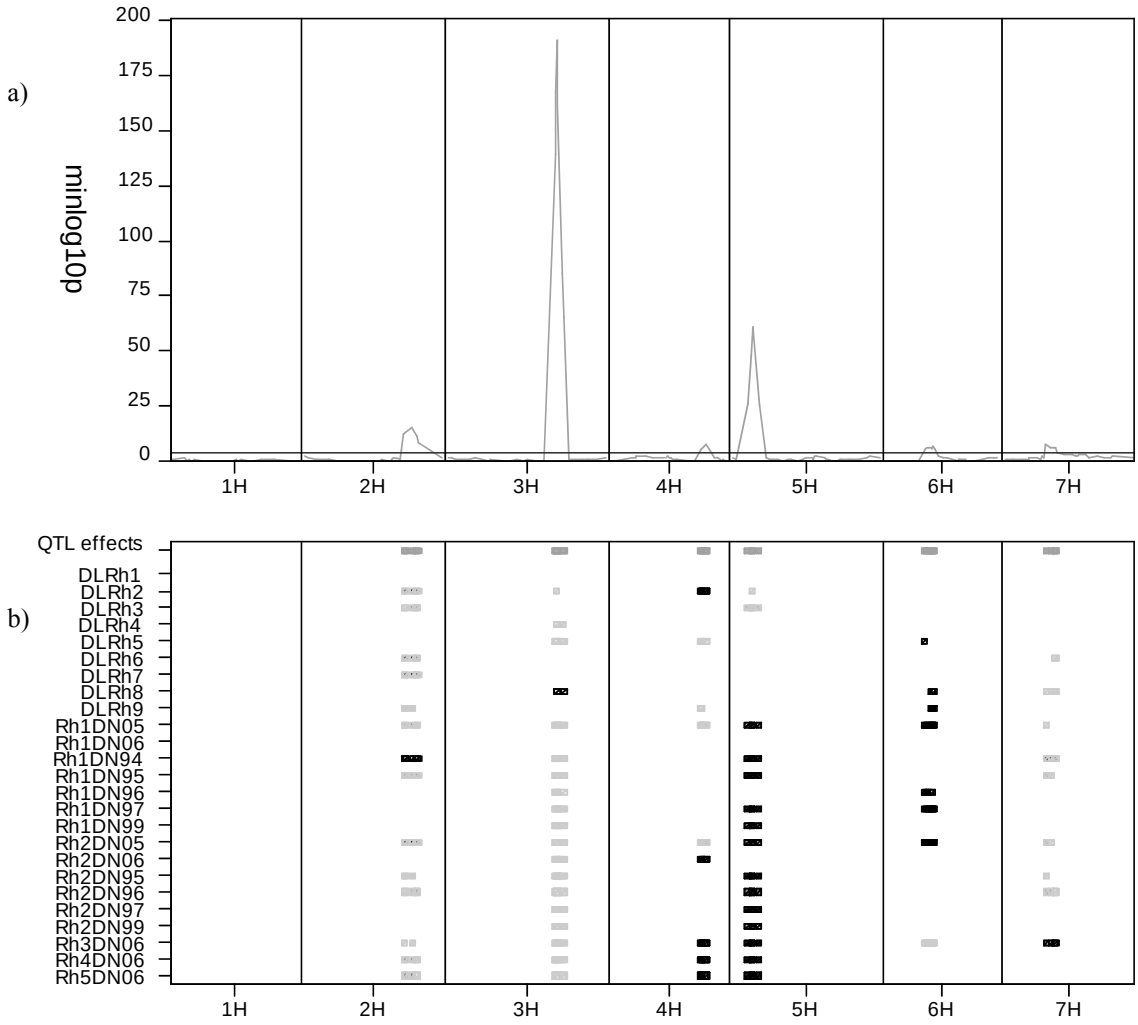

Figure 2. a.) Plot of test statistic for QTL x Environment analysis (undulating line) compared to test statistic (horizontal line).
b.) Genomic location of significant QTL effects detected in each of 25 environments. Black and grey lines indicate resistant alleles from B83-12/21/5 and Derkado respectively.

The effects detected on chromosomes 6H and 7H were quite consistent with the resistant allele being derived from Derkado and B83-12/21/5 respectively, apart from the third environment in 2006. The *Rrs13* major-gene resistance is located on the short arm of chromosome 6H (Zhan *et al.,* 2008) but is located closer to the telomere of the short arm than the effect that we have detected. Whilst we have detected some QTLs for other characters in this region, we have not detected any height or maturity effects, suggesting that this is a genuine partial resistance effect. Some supporting evidence for this is provided by the fact that Derkado alleles provide resistance effects in all three detached leaf tests that were significant. The effect on 7H is located in the same region as QTLs for height and heading date (WTB Thomas, unpublished data) with B83-12/21/5 alleles associated with later and taller plants, suggesting escape rather than true resistance. As with the *sdw1* dwarfing gene, we also detected significant associations of the region with two of the detached leaf tests, indicating that height escape cannot be the cause of the resistance effect. As the resistance effects are both in the same direction, maturity escape cannot yet be excluded as the cause of the apparent resistance.

In conclusion, we have used new genetic information and methodologies to identify a number of putative partial resistance factors to Rhynchosporium. By including measurements from both detached leaf studies and field measurement in the same analysis, we have been able to eliminate height effects as the cause of some of the resistance effects that we have detected but further study is required to identify the causal mechanisms behind these effects.

ACKNOWLEDGEMENTS

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THE EPIDEMIOLOGICAL IMPORTANCE OF ASYMPTOMATIC INFECTION OF WINTER BARLEY BY *RHYNCHOSPORIUM SECALIS* AND ITS CONSEQUENCES FOR CROP PROTECTION AND BREEDING

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Summary: Leaf scald (caused by *Rhynchosporium secalis*) is one of the most economically important diseases of barley. Data were collected from three seasons of trials using two susceptible winter barley cultivars (Sumo and Saffron) and two resistant cultivars (Flagon and Manitou). Levels of *R. secalis* DNA were quantified from several growth stages (GS) throughout the growing season and visual symptoms assessed. Results demonstrate that the pathogen was able to colonise and sporulate extensively on apparently healthy leaves, and spread to grain without symptoms being seen in the crop. This raises questions about how the fungus interacts with the plant, what triggers lesions to develop and the role of asymptomatic infection in pathogen spread, plant defence and crop yield.

INTRODUCTION

Leaf scald (caused by *Rhynchosporium secalis*) is one of the most economically important diseases of barley. Yield losses of 10 to 40% are not uncommon (Shipton *et al*., 1974) and crop failures have been reported under severe epidemics. Control of *R. secalis* by the use of resistant cultivars relying on major *R*-genes has proved unsustainable (Shipton *et al*., 1974; Zhan *et al*., 2008) and additional disease control is achieved by using partial resistance traits and fungicide applications.

Leaf scald is a polycyclic disease, normally involving several pathogen generations during the growing season, and secondary disease spread by splash-dispersed conidia (Fitt *et al*., 1989; Zhan *et al*., 2008). Primary inoculum is thought to be via splash-dispersed conidia or mycelium from infected plants or crop debris. Infection of barley seed by *R. secalis* has also been shown to be important in the dissemination of the pathogen (Lee *et al*., 2001). While no sexual stage has been discovered, most populations of *R. secalis* are genetically diverse (Abang *et al*., 2006), Furthermore, considerable genetic variation for neutral markers has been identified in *R. secalis* populations (Salamati *et al*., 2000) and the measurements of similar frequencies of both mating types (Foster and Fitt, 2003) in populations sampled at different locations (Linde *et al*., 2003) are consistent with the presence of a teleomorph.

The fungus is able to grow under the leaf cuticle and produce new conidia without the development of visual symptoms (Jorgensen *et al*., 1993; Zhan *et al*., 2008). Infection by *R. secalis* in UK winter barley generally produces few visual symptoms before January/February, although *R. secalis* has been detected by PCR during early season (Fountaine, 2005). Due to the polycyclic nature of the disease, several pathogen generations may occur before symptom development, during which time *R. secalis* may interact with both major-R gene and partial resistance in barley cultivars (Zhan *et al*., 2008). Symptom

development has been demonstrated to be a plant host reaction in response to secreted fungal proteins, such as NIP1, in barley cultivars carrying the matching gene *Rrs1* (Steiner-Lange *et al*., 2003; Slot *et al*., 2007). To improve guidelines for growers and breeders, it is important to understand the impact of the asymptomatic phase of *R. secalis* on yield. For example, such understanding would help to establish if early fungicide applications targeting asymptomatic infection can improve yield.

Both winter and spring barley cultivars are annually assessed for leaf scald resistance in a series of experimental trials at different sites (HGCA-sponsored Recommended List (RL) trials). Fountaine *et al*. (2007) demonstrated that there was a poor correlation between RL resistance ratings and severity of disease assessed by visual symptoms or quantitative PCR (qPCR) data.

The aim of this study was to monitor the development of *R. secalis* in winter barley crops using both visual assessments and qPCR assays on four winter barley cultivars during 2007-2010. This would investigate the role of asymptomatic infection in *R. secalis* epidemics at different growth stages during the season.

MATERIAL AND METHODS

Trial sites

Winter barley trials were established at Rothamsted Research over three growing seasons 2006-2009. In each trial, there were six randomised blocks, each block included plots of two resistant (Manitou [9] and Flagon [8]) and two susceptible (Sumo [5] and Saffron [6]) cultivars [figures in brackets represent the cultivar RL rating]. Plots were untreated. Visual assessment of disease symptoms and qPCR analysis of leaf and grain samples was done at growth stages (GS) 13, 22, 26, 39 and 75 in 2007; in 2008 the GS 13 sample was not taken and in 2009 the GS 13 and 22 samples were not taken.

DNA extraction from leaf and grain material

DNA was extracted from leaf samples (10 leaves per plot) or grain samples (5 g) in accordance with the protocol of Fraaije *et al*. (1999) with the modification outlined by Fountaine *et al*. (2007). Grain samples were ground in a coffee grinder (CG100 model, Kenwood, UK). DNA was quantified using a nano-drop spectrophotometer (ND-1000, Labtech International Ltd, Sussex, UK) and the DNA solution diluted to working stocks of 20 ng DNA μl^{-1}. A total of 50 ng DNA was used for subsequent qPCR reactions. The qPCR reactions were performed as described by Fountaine *et al*. (2007).

Green leaf assays

Single leaf samples were taken from winter barley cultivar Sumo at GS 39 from the untreated plot in 2009. Samples consisted of green leaves without any symptoms and leaves that had single *R. secalis* lesions present. Leaf samples were separated into two lots: one lot was used for DNA extraction and the other for staining for microscopy. DNA was extracted from individual leaves as stated above but the pre-extraction phase was modified by extracting DNA from leaves using liquid nitrogen and grinding in a pestle and mortar. DNA was quantified and aliquoted. Levels of *R. secalis* DNA in each leaf were quantified by qPCR. For microscopy, leaves were cleared by immersion overnight in 100% ethanol. Leaves separated for staining were then immersed in trypan blue stain for 1 hour then de-stained overnight. Leaves were observed under x20o magnification.

RESULTS

Rhynchosporium secalis DNA was detected in leaf samples early (GS 13) in 2007 (Fig. 1). Levels increased significantly by GS 26 although levels fluctuated between seasons (Fig. 1) and for the different cultivars (Fig. 2). Visual symptoms were not seen early in the season and were low at GS 26 even though levels of DNA were high. Highest levels of *R. secalis* DNA were detected in Sumo, whereas lowest levels were measured in Manitou (Fig. 2)

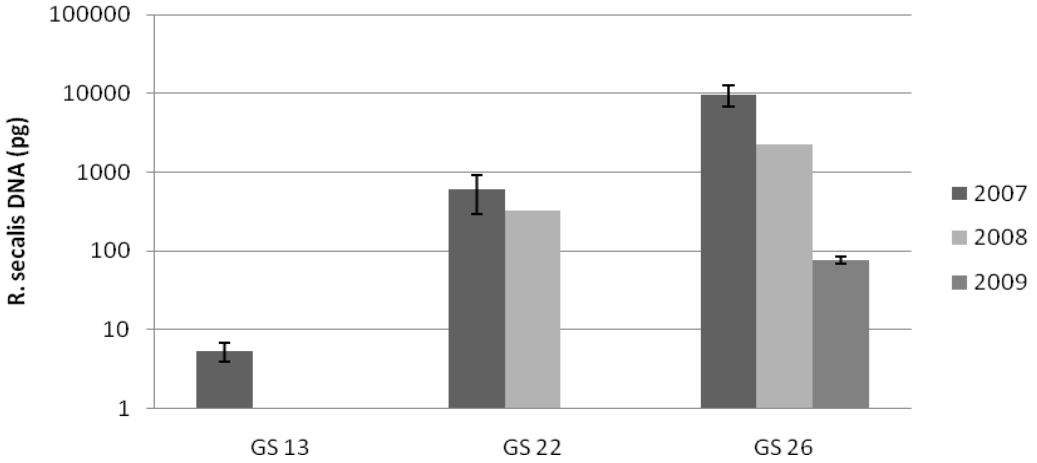

Figure 1. Plot of *R. secalis* DNA (pg) in 50 ng leaf samples taken from untreated winter barley (cultivar Sumo) at different growth stages over three growing seasons (2007-2009) at Rothamsted Research

In 2009, visual symptoms of the pathogen were seen on cultivar Sumo at GS 39 (Table 1). *Rhynchosporium secalis* DNA was detected in all grain samples at harvest (Table 1) even though visual symptoms of disease had not been seen during the season for most cultivars. Levels of DNA fluctuated according to cultivar and season. Levels were greatest in 2008 when there was high rainfall in July and August that spread spores from leaves onto the grain.

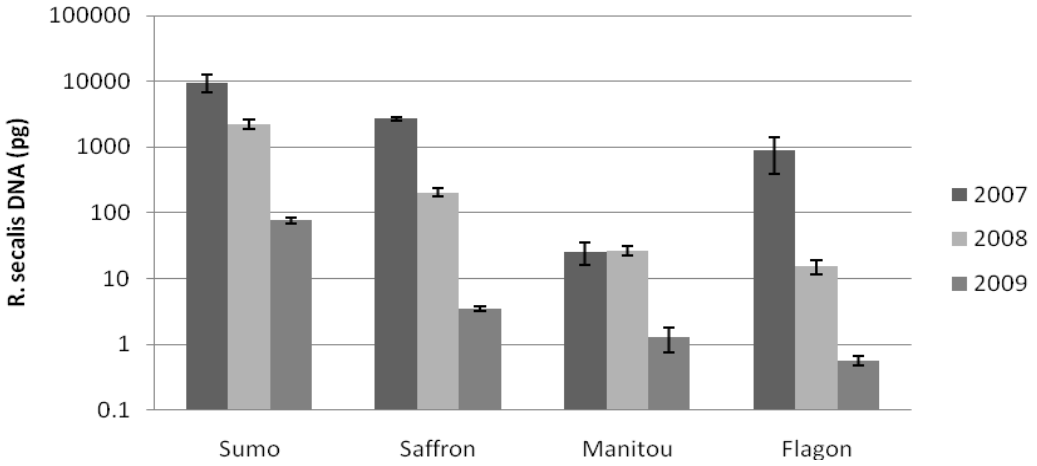

Figure 2. Plot of *R. secalis* DNA (pg) in 50 ng leaf samples at GS 26 from untreated winter barley cultivars over three growing seasons (2007-2009) at Rothamsted Research

Levels of *R. secalis* DNA were high in most of the leaves showing visual symptoms of disease (Fig. 3). Most of the asymptomatic leaves had very low levels of *R. secalis* DNA indicating that colonisation had not occurred. A few symptomless leaves had high levels of *R. secalis* DNA, comparable to levels found on leaves with symptoms. Microscopic observation of leaves with lesions showed high levels of sporulating *R. secalis*, often limited to the area within the lesion. *Rhynchosporium secalis* was not detected on the majority of asymptomatic leaves but a few did show high levels of *R. secalis* sporulation on the leaf without the appearance of lesion development.

Table 1. Percentage leaf area affected by *R. secalis* at GS 39 (leaf 2) and level of *R. secalis* DNA on grain samples taken at harvest over three growing seasons (2007-2009) on untreated winter barley (Cultivars S = Sumo; Sa = Saffron; M = Manitou; F = Flagon) at Rothamsted Research

	2007				2008				2009			
	S	Sa	M	F	S	Sa	M	F	S	Sa	M	F
% leaf area affected (leaf 2, GS 39)	0	0	0	0	1.4	0	0	0	6.3	2	0	0
Level of *R. secalis* DNA (pg) in 50 ng grain DNA at harvest	6.5	9	0.3	1.1	14	13	5.2	1.1	8.8	0.8	0.9	0.4

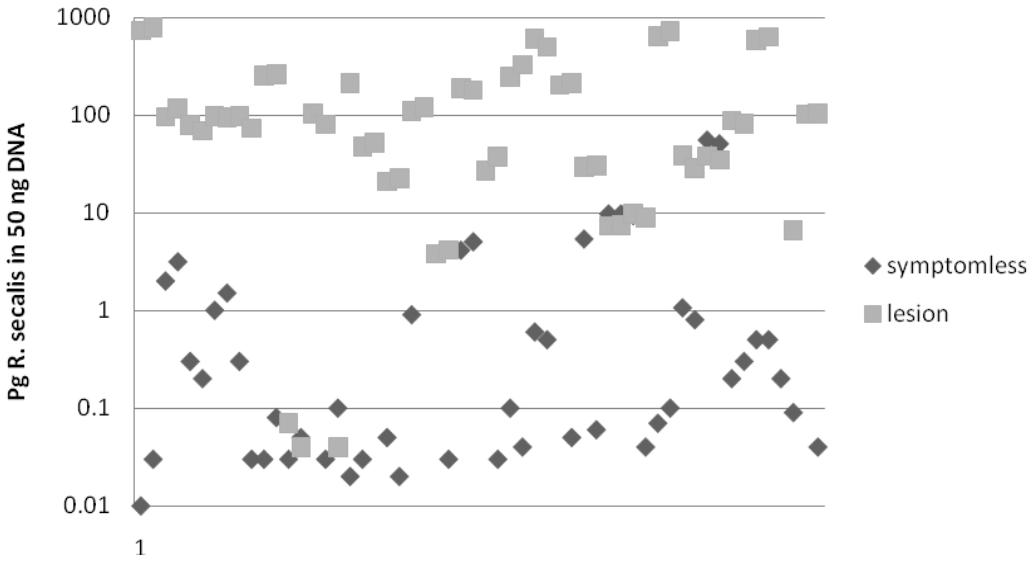

Figure 3. Plot of *R. secalis* DNA (pg) in individual leaves of winter barley (cultivar Sumo) either asymptomatic or with leaf scald lesions

DISCUSSION

This study has demonstrated that further research is necessary to understand how *R. secalis* interacts with the host plant. Results have demonstrated that *R. secalis* is able to colonize the host plant extensively without causing visual symptoms of disease, thus providing a potential inoculum source for later epidemics and is supported by other published data (Davis and Fitt, 1993; Fountaine *et al*., 2007; Zhan *et al*., 2008).

Colonisation early in the growing season goes undetected and increases the risk of later epidemics. Environmental conditions greatly influence the spread of the pathogen up the plant later in the season (reviewed by Zhan *et al*., 2008). In 2008, greater rainfall was recorded at Rothamsted later in the season than in 2007, and the results indicate that this lead to a greater level of *R. secalis* contaminating the grain in 2008 than in 2007, even though at the beginning of the season the DNA levels suggest that risk was greatest in 2007. Dry spring periods may allow the plant to escape the disease even though early season risk may be high.

The results also indicate that grain can become infected even if symptoms have not been observed in the crop. Throughout the three growing seasons there was little visual detection of *R. secalis* symptoms, but in all three seasons grain became infected. International trade in seed has been implicated in the spread of the pathogen (Zaffarano *et al*., 2006). Without detection of airborne spores (Atkins *et al*., 2008) and in the absence of crop debris and volunteers, seed borne infection may be the most important source of primary infection. Results indicate that even if resistant cultivars are used seed can still become infected. Further research is necessary to determine if a protectant spray late in the season may be necessary to produce clean seed.

In areas where risk of *R. secalis* epidemics is high, it may be necessary to apply an early season application of fungicide to decrease the risk of subsequent epidemics. Why the fungus produces lesions needs further investigation, as do the trigger factors that determine lesion development. From the results, it is apparent that the fungus can grow and reproduce on green leaves without the need to develop lesions. Therefore, it is not clear why lesions are formed; is it an advantage to the plant or to the pathogen? It is also unclear if a high level of asymptomatic colonisation early in the season affects the plant and subsequent yield?

The use of qPCR to accurately estimate *R. secalis* DNA may provide a more reliable assessment of cultivar resistance than visual assessment of disease symptoms, particularly when assessments during the asymptomatic colonisation of the host plant can be performed. There is a need to construct a predictive model for *R. secalis* epidemics that takes into account environmental, pathogen population, cultivar and agronomic factors, and to determine inoculum sources for *R. secalis* so that more sustainable control strategies can be designed.

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USE OF QPCR TECHNIQUES TO PREDICT LIGHT LEAF SPOT RESISTANCE OF OILSEED RAPE VAREITIES

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Summary: The project aimed to test whether measuring components of disease resistance in winter oilseed rape will improve selection of resistant cultivars, and to produce new methods to rate cultivar resistance. Visual assessment of light leaf spot (LLS) after incubation of newly expanded leaves was demonstrated to provide a better discrimination in cultivar resistance than either visual assessment of plots or of close inspection of plants in the field. Amounts of DNA of *P. brassicae* in leaves or main shoots (meristems) related well to subsequent severity of LLS symptoms at some dates. Ability to discriminate between susceptible and resistant cvs was limited from early samples taken in December/January, particularly in Scotland, probably because there had not been sufficient thermal time to allow the pathogen to grow and spread on plants through secondary infection. Sampling in late winter/spring appears to provide the best material for qPCR studies to discriminate cultivars for resistance to *P. brassicae*.

INTRODUCTION

The components of resistance to diseases in winter oilseed rape cultivars (CORDISOR) project aimed to test whether measuring components of resistance to disease, especially symptomless growth, could improve selection of resistant cultivars, and to produce new methods (using quantitative PCR) to rate resistance in winter oilseed rape cultivars. CORDISOR focused on resistance to the two main diseases of winter oilseed rape in the UK, stem canker (caused by *Leptosphaeria maculans*) and light leaf spot (LLS) (caused by *Pyrenopeziza brassicae*). Disease resistance ratings are currently calculated from the visual assessment of disease and do not explain the relative importance of resistance expressed in different tissues (leaf, stem, pods) at different times or stages of disease development – information which is valuable for breeding new cultivars with enhanced disease resistance and reduced reliance on fungicides for disease control. This paper concentrates on work with light leaf spot, since this is the major disease of northern Britain.

The approach used in the project was to evaluate 20 different oilseed rape cultivars for their growth and amounts of disease at a range of field sites in England and Scotland. Cultivars were chosen to represent a wide range of resistances and susceptibilities to canker and light leaf spot, using predominantly cvs from the HGCA recommended list at the start of the project, and were: Apex, Aragon (replaced by ES Astrid for the latter 2 years of the project),

Bristol, Canberra, Castille, Courage, Disco, Elan, Escort, Expert, Fortis, Hearty, Lioness, NK Bravour, NK Victory, Ontario, Recital, Royal, Shannon and Winner. The visible disease symptoms recorded in different ways were compared with measurements of amount of DNA of the causal pathogens using quantitative PCR on selected plant tissues sampled at key growth stages.

MATERIALS AND METHODS

Visual plant and disease data were recorded on the set of cvs at 12 different sites (RL trials and breeders' nurseries in Scotland and England, and trials at Rothamsted) in each of three seasons (harvest years 2005, 2006 and 2007). At Rothamsted, over the first two seasons of the project, one cv (NK Bravour) was grown in additional plots next to the main experiment and was sampled for quantification of pathogen DNA in plant tissues more frequently than the main experiment. The same set of cold-stored seed was used each year for field experiments. Disease and plant assessments were made according to an established protocol (Table 1) at specific times. Additionally, growth stage was recorded and presence of other diseases on leaves, stems or pods (e.g. downy mildew, powdery mildew, Alternaria, botrytis, white leaf spot, etc.) noted.

Table 1. Summary of CORDISOR plant and disease assessments (10 plants/plot unless stated)

LLS1	Light leaf spot (incubate plants in plastic bags in cool (10°C) room for 5 days then score N^{o} leaves affected and % total leaf area affected on individual plants, plus 1-9 plot score at time of sampling according to Recommended List protocol)
LLS2	Apical meristem infection (whole plants wrapped in blue-roll in a plastic bag and dispatched for PCR)
LLS3	LLS symptoms on leaves of individual plants (without incubation score N^{o} leaves affected and % total leaf area affected), plus 1-9 plot score at time of sampling according to RL protocol.
LLS4	(subsequently deleted as part of LLS1 and LLS3)
LLS5	LLS stems (score % area affected on main stem)
LLS6	LLS pods (score % pods affected and % total area of pods affected)

Data were collated at Rothamsted, and analysed by ANOVA using a statistical software package (Genstat) for each site x sample date. Cultivar means for each variable recorded were copied into Excel databases, for each season. These databases were used for further analysis of relationships between different variables and especially the relationships between severity of visual symptoms (e.g. canker or light leaf spot) and the amount of pathogen DNA (*L. maculans* or *P. brassicae*) in the same samples or samples from the same plots taken at earlier time-point.

The pathogen DNA data was produced from plant samples taken at selected times from field experiments and processed directly at Rothamsted or NIAB, or posted from other sites (SAC sites and breeders' nurseries in Scotland and England) to Rothamsted. In some cases, posted samples were assessed for visible disease symptoms in addition to processing of selected plant tissues for pathogen DNA measurement. At Rothamsted, all selected tissue samples (upper or lower petioles, meristems or recently expanded leaves) were frozen, freeze-dried,

and powdered and DNA was extracted using a commercial kit (DNAmite, Microzone, Haywards Heath) (see West *et al*., 2008 for details). Different quantitative PCR (qPCR) protocols were used according to equipment available at each site (Rothamsted and NIAB).

Although various methods with different primers were initially investigated at Rothamsted, both NIAB and Rothamsted sites used SYBR green systems to quantify *L. maculans* in plant tissues and a similar method was used at Rothamsted to assay *P. brassicae*. This method measures fluorescence of a dye that binds to double-stranded DNA. Non-target DNA (e.g. from the plant), that is not replicated in the PCR reaction produces a background low level of fluorescence but this is below a set detection threshold. Therefore, only the DNA of the target (plant pathogen in this case) is measured, the amount produced after a set number of replication cycles depending on the initial amount of target DNA in the sample.

RESULTS

Disease development and measurement

Light leaf spot severity varied greatly between sites and was greatest in March and April. Cultivars cvs Hearty, Shannon and Recital were most susceptible, while cv. Elan alone had less LLS than all other cultivars.

Incubation of ten newly expanded leaves per plot in plastic bags in a cool room for 4-5 days allowed good visualisation of LLS symptoms to discriminate differences in cultivar resistance to *Pyrenopeziza brassicae*. Substantially more light leaf spot symptoms were visible after a 4-5 day incubation than 2 day incubation or no incubation, especially in early to mid-winter. Generally there were large differences in LLS severity on leaves between the 20 cvs. tested at each site with greatest severity on cv. Hearty and least on cv. Elan.

There was no clear relationship between light leaf spot (LLS, *Pyrenopeziza brassicae*) severity on leaves in late winter and LLS severity on pods in June.

There was a good relationship ($R^2 = 0.55$ in 2005) between LLS severity on pods (1-9 scale in June-July, means of four sites) and the HGCA LLS resistance score for different cultivars.

There was very little or sporadic development of alternaria leaf and pod spot, powdery mildew, downy mildew and sclerotinia stem rot (SSR) with SSR more severe than normal in 2007 (but with no significant differences between cvs).

Quantification of pathogen DNA in plant by qPCR

There were significant differences in amounts of pathogen DNA for both *L. maculans* and *P. brassicae* between sites that had high or low severity of visible disease and between untreated and fungicide-treated plots. Amounts of *P. brassicae* DNA were well related to subsequent severity of LLS symptoms at some sites but not at others (particularly not if samples were taken early in the season in Scotland).

There was always much more *P. brassicae* DNA in samples of lamellae of newly expanded leaves than in the main shoot tip (meristem).

Goodness of fit of regressions of *P. brassicae* DNA against LLS severity were not related to the total amount of pathogen DNA.

DISCUSSION

The project showed for the first time that *Pyrenopeziza brassicae* infection of the main shoot tip (meristem) of OSR plants in winter was a common and widespread occurrence. Amounts of DNA of *P. brassicae* in leaves or main shoots (meristems) related well to subsequent severity of light leaf spot (LLS) symptoms at some dates (particularly when visible symptoms were severe in late winter/early spring, when visual assessments are normally made for Recommended List evaluation). It is probable that the early samples taken in December and January, particularly in Scotland, had not had sufficient thermal time to allow the pathogen to grow and spread on plants (increasing biomass) through secondary infection, which limited the ability to discriminate between resistant and susceptible cvs. Sampling in late winter/spring appears to be the best timing to provide the material for qPCR studies to discriminate cultivars for resistance to *P. brassicae*.

It is suggested that the lack of clear relationship between LLS severity on the leaves in winter and the severity of symptoms on the pods in June may indicate that either the second release of ascospores in spring is influential and/or that a different resistance mechanism occurs on pods and/or that avoidance of meristem infection is critical. PCR data confirmed that at some sites meristems in untreated plots became infected before winter; this could result in stunting of plants and ensure that subsequent growth stages would be infected. Similarly, fungicide applied in autumn decreased LLS severity on pods the following June for most cultivars.

The lack of influence of total amount of pathogen DNA on relationship between *P. brassicae* DNA and LLS severity indicates that the ability to predict LLS severity is not reduced by quantity of pathogen DNA being limited.

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CONTROL OF FOLIAR DISEASES OF SPRING BARLEY USING RESISTANCE ELICITORS

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Summary: A cocktail of the resistance elicitors: *cis*-jasmone, β-aminobutyric acid (BABA) and acibenzolar-S-methyl (ASM) was examined for its ability to induce resistance in barley to foliar pathogens in field studies over two seasons. The efficacy of the elicitor cocktail on its own was poor and variable, depending on both barley variety and season. Greater levels of disease control were obtained using a combination of elicitor and fungicide. However, although a mixture of elicitor and fungicide applied together gave reasonable levels of disease control in 2007, most consistent disease control was obtained by treating plants with the elicitor combination at GS24, followed by reduced rate fungicide at GS31 and GS39. Despite the moderate levels of disease control, most elicitor and elicitor + fungicide treatments resulted in increased grain yields.

INTRODUCTION

Application of various agents to plants can lead to the induction of resistance to subsequent pathogen attack, both locally and systemically (Walters *et al.,* 2007; Reglinski & Walters, 2009). Such induced resistance can be split into systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is characterised by a restriction of pathogen growth and a suppression of disease symptom development compared to non-induced plants infected with the same pathogen. Its onset is associated with an accumulation of salicylic acid (SA) at sites of infection and systemically, and with the coordinated activation of a specific set of genes encoding PR proteins. Treatment of plants with SA or one of its functional analogues e.g. acibenzolar-S-methyl (ASM; marketed in Europe as Bion®), induces SAR and activates the same set of PR genes. ISR develops as a result of colonisation of plant roots by plant growth promoting rhizobacteria (PGPR) and has been shown to function independently of SA and activation of PR genes, requiring instead jasmonic acid (JA) and ethylene (ET) (Pieterse & Van Loon, 2007).

Because induced resistance offers the prospect of broad spectrum disease control using the plant's own resistance mechanisms, there has been great interest in the development of agents which can mimic natural inducers of resistance (Lyon, 2007). These include elicitor molecules released during the early stages of the plant-pathogen interaction and the signalling pathways used to trigger defences locally and systemically. Examples include ASM, which has been shown to elicit SAR in a wide range of plant-pathogen interactions (Leadbeater & Staub, 2007), the non-protein amino acid β-aminobutyric acid (BABA), and the oxylipin, *cis*-jasmone (CJ) (Walters *et al.,* 2007).

The efficacy of induced resistance under field conditions is variable, representing a major obstacle to its use in practical crop protection. Induced resistance is a complex plant response to pathogen attack and as such, will be modified by many factors including genotype. However, insufficient attention has been paid to investigating the mechanisms underlying variable efficacy and approaches that might be adopted to incorporate elicitors into crop protection practice. SAC, together with colleagues at SCRI, have been examining the prospects for the control of barley diseases using resistance elicitors. Here we report the results of field experiments over several years, undertaken as part of this programme.

MATERIALS AND METHODS

The following elicitors were used in the experiments described in this paper: *cis*-jasmone (INDOFINE Chemical Company, Inc, Hillsborough, NJ, USA); ASM (Syngenta); β-aminobutyric acid (BABA, Sigma Chemical Company, UK). Elicitors were dissolved in distilled water and made up to the appropriate concentration (1 mM for ASM and BABA; 625 mg/l for *cis*-jasmone). For all elicitors except ASM, 0.01 % Tween 20 was added to act as a surfactant.

Field experiments were conducted in 2007 and 2008 at two sites, Lanark and Perth. Two spring barley varieties (Cellar and Optic) were sown in a randomised block design at a seed rate of 360 seeds/m^2 and an individual plot size of 10 m x 2 m. Plots received standard fertiliser and herbicide regimes and seven treatment programmes were compared (Table 1). Spray dates for fungicides were based on plant growth stage as described by Zadoks *et al.* (1974). Fungicides were applied with a knapsack sprayer using an equivalent spray volume of 200 l ha^{-1}. Disease symptoms were assessed at spray dates and at 14 day intervals after the final spray. Plots were harvested at the end of the trial and yields expressed as tonnes/hectare at 85 % dry matter content.

RESULTS

In 2007, the elicitor cocktail applied on its own at GS24 reduced powdery mildew infection in both Cellar and Optic (significantly so in Cellar), but had no effect on *Rhynchosporium secalis* or *Ramularia collo-cygni* (Table 1). In contrast, application of the elicitor cocktail at GS24, followed by fungicide at GS31 and GS39, reduced infection by all three pathogens in both cultivars. Thus, in Cellar, the elicitor cocktail plus full rate fungicide reduced mildew by 63%, *R. secalis* by 70%, and *R. collo-cygni* by 50%, while the elicitor followed by half rate fungicide reduced disease levels by 50%, 100% and 50% respectively, for mildew, *R. secalis* and *R. collo-cygni* (Table 1). In Optic, the elicitor cocktail provided levels of disease control at the same levels to that obtained using the standard fungicide regime.

Table 1. Effects of an elicitor cocktail and combinations of elicitor + fungicide on disease control and grain yield in the spring barley cultivars Cellar and Optic in 2007.

Treatments	Mildew (%)	Rhynchosporium (%)	Ramularia (%)	Grain yield (%)
Cellar 2007				
Untreated	4.0	1.0	6.0	6.40
Elicitor cocktail (E)	2.0	1.0	6.0	6.40
E + fungicide (full rate)	1.5	0.3	3.0	6.80
E + fungicide (half rate)	2.0	0	3.0	6.55
Fungicide	1.5	1.0	3.0	6.80
LSD (P = 0.05)	1.27	1.10	2.30	0.38
Optic 2007				
Untreated	5.0	1.0	8.0	6.00
Elicitor cocktail (E)	3.0	1.5	8.0	5.90
E + fungicide (full rate)	3.0	0	4.0	6.30
E + fungicide (half rate)	2.0	0.3	4.0	6.30
Fungicide	2.0	0.5	3.5	6.30
LSD (P = 0.05)	2.27	1.60	3.1	0.45

Table 2. Effects of an elicitor cocktail and combinations of elicitor + fungicide on disease control and grain yield in the spring barley cultivars Cellar and Optic in 2008.

Treatments	Mildew (%)	Rhynchosporium (%)	Ramularia (%)	Grain yield (%)
Cellar 2008				
Untreated	-	10.0	8.0	5.96
Elicitor cocktail (E)	-	6.0	8.0	5.76
E + fungicide (full rate)	-	1.0	4.0	6.68
E + fungicide (half rate)	-	0.6	3.0	6.55
Fungicide	-	0.3	3.5	6.80
LSD (P = 0.05)	-	5.42	4.51	0.75
Optic 2008				
Untreated	14.0	3.0	6.0	5.31
Elicitor cocktail (E)	9.0	4.0	6.0	5.51
E + fungicide (full rate)	3.0	0.6	4.0	5.85
E + fungicide (half rate)	4.0	0.6	4.0	6.01
Fungicide	6.0	0.6	4.0	6.78
LSD (P = 0.05)	3.92	4.56	3.40	0.53

Greater levels of disease control were observed in 2008, with the elicitor + full rate fungicide treatments, for example, reducing mildew by 79%, *R. secalis* by 80%, and *R. collo-cygni* by 50% in the cultivar Optic (Table 2). In Cellar, the elicitor cocktail reduced mildew by 94%. As in the 2007 season, the elicitor cocktail applied on its own at GS 24 provided less substantial levels of disease control. With the exception of mildew, where best control was achieved using the elicitor + fungicide treatment, the standard fungicide treatment provided similar levels of disease control as the elicitor + fungicide treatments (Table 2).

Compared to the untreated control, most treatments increased grain yield in both seasons, although a small decrease in yield was observed in the elicitor treatment on its own on Cellar in 2008 (Tables 1 and 2).

DISCUSSION

These data demonstrate that a cocktail of resistance elicitors, used in combination with full rate or reduced rate fungicide, can provide levels of disease control equivalent to, and in some cases better than, standard fungicide treatments in spring barley. In contrast, the elicitor cocktail on its own yielded poorer levels of disease control. The elicitor treatment used a combination of ASM, BABA and *cis*-jasmone. Using combinations of elicitors which trigger different resistance pathways has been suggested as a means of maximising the efficacy of induced resistance (Pieterse & Van Loon, 2007). These authors suggest that combining SAR and ISR might provide a useful approach to achieving durable disease control under field conditions. Recent work has shown that the elicitor cocktail used in the present work induces greatly increased expression of the SAR marker gene *PR1a,* suggesting that the activation of SAR (Sablou, Walsh & Walters, unpublished results).

It is thought that the intense activation of host defences during induced resistance might be associated with costs (Walters & Heil, 2007). In other words, the diversion of host resources away from growth and development towards defence might lead to reduced plant growth and yield. However, the data presented in this paper show quite clearly that use of the elicitor cocktail, especially in combination with fungicide, increased yields. Interestingly, use of the elicitor cocktail on its own on Cellar in 2008 was associated with a small reduction in yield, although the mechanism underlying this effect is unknown. Direct induction of resistance, where defences are triggered following elicitor application, has been shown to incur costs in terms of plant growth and reproduction (Heil *et al.,* 2000; Van Hulten *et al.,* 2006), while priming for induced resistance, where defences are triggered only following pathogen attack, has been shown to provide benefits to the plant under disease pressure (Van Hulten *et al.,* 2006; Walters *et al.,* 2009). Although ASM can prime plants, it is also associated with direct induction of resistance (Latunde-Dada & Lucas, 2001; Van Hulten *et al.,* 2006). Depending on the concentration used, BABA can either induce defences directly or prime plants for enhanced resistance (Van Hulten *et al.,* 2006). In the present work, since plants were treated with a combination of ASM, BABA and *cis*-jasmone, it seems possible that treated plants would have exhibited a mixture of direct induction of resistance and priming, and indeed, recent research suggests that this is the case (Paterson & Walters, unpublished results). In any event, it is clear from these field studies that using elicitor + fungicide treatments can lead to increased yields.

Although the efficacy of induced resistance can be variable, being dependent on genotype and environment, perhaps by using combinations of elicitors and reduced rates of fungicides, together with appropriate choice of variety, more consistent and higher levels of disease control can be achieved. These possibilities are under continued investigation at SAC.

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POTENTIAL OF SEED TREATMENT TO CONTROL *RAMULARIA COLLO-CYGNI* IN BARLEY

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Summary: *Ramularia collo-cygni* has become an increasing problem for barley growers throughout Europe in recent years and the pathogen continues to spread in cereal producing areas. Recent studies have indicated that the pathogen has a seed-borne stage and that conventional chemical seed treatments have only limited activity against the pathogen. This paper describes experimental results using a number of alternative treatments from field trials in 2009. The movement of the pathogen into new growth was measured using quantitative PCR techniques and disease severity was measured by visual assessments. The potential for the application of these treatments to control Ramularia Leaf Spot in barley production is discussed.

INTRODUCTION

Ramularia collo-cygni is the major biotic component of the leaf spotting complex which attacks barley crops in the late growing season (Walters *et al.,* 2007). Symptoms are most commonly observed on foliage after flowering in the crop. Initial signs of infection are small brown to blackish spots, 1-2mm long. The spots develop a chlorotic halo and eventually neighbouring lesions may coalesce to form a larger necrotic region. The subsequent loss of green leaf area leads to deleterious effects on yield quantity and quality (Walters *et al*., 2007). The pathogen rose to prominence in barley production areas in the 1990's as part of the leaf spotting complex. Ramularia leaf spot (RLS) has been recorded in many European countries and the disease is still being identified in new countries in Central Europe (Walters *et al.,* 2007; Manninger & Muranyi, 2009; Gubiš *et al*., 2009).

The fungus has been found to be present in seed (Havis *et al.,* 2006; Salamati & Retian, 2006 Frei *et al.,* 2007). Early experiments indicated that conventional seed treatments are not completely effective in controlling the disease, with significant control achieved only in susceptible varieties (Havis *et al.,* 2008). A number of chemical methods have been used to control seed borne diseases in barley. Treatment with hot humid air has recently been demonstrated to be effective in controlling many seed borne infections in barley (Forsberg, 2004). One of the oldest non chemical treatments is hot water treatment. This does have activity against the fungus *Ustilago nuda* (Loose smut). (Maude & Shuring, 1968) *Pseudomonas chlororaphis* is a soil bacterium which has been developed into a biological seed treatment to control a range of fungal pathogens on barley in Sweden (Johnsson *et al.,* 1998). Another bacteria, *Bacillus subtilis* has also been shown to have activity against cereal root pathogens (Kim *et al.,* 1997). As part of a new LINK funded project a series of field trials were established in order to examine the movement of the fungus and the potential of physical and non chemical seed treatments to control the movement of *R. collo-cygni* and symptom expression in spring barley crops.

MATERIALS AND METHODS

Movement of the fungus from infected seed

Three spring barley varieties with differing susceptibility to *R. collo-cygni* were sown in small hill plots at Drumalbin farm in Central Scotland in 2008. The varieties used were Cocktail (susceptible), Decanter (resistant) and Optic (intermediate for resistance). *Ramularia* DNA levels were measured in seeds prior to sowing (Cocktail 29.1 pgrams, Decanter 20.5 pg and Optic 20.8 pg). After emergence 10 plants were harvested at regular intervals. The plants were split into respective leaf layers and the percentage leaf area infected with *R. collo-cygni* assessed visually. Following this, DNA was extracted from the leaves using the Illustra™ Phytopure™ DNA extraction kit (GE Healthcare, UK). Samples were tested for the presence of *R. collo-cygni* using the real time PCR reaction described previously (Taylor *et al.,* in press). Area under the curve for *Ramularia* DNA was calculated using the trapezoidal rule. A spore sampler and weather station at the site allowed *Ramularia* spore movement to be measured and periods of high leaf surface wetness recorded.

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

Trial A.
Seed samples from three varieties (Cocktail, Optic and Decanter) grown in 2008 spring barley trials were treated with novel seed treatments. The treated seed was sown in field trials at the Bush Estate, Midlothian in April 2009 in a randomised block design.

The seed treatments used were tebuconazole + triazoxide (teb + triaz) (Raxil S ®), Hot water (2 hours at 52 °C followed by 72 hours at 25 °C), Steam (Thermoseed treatment), *Pseudomonas chlororaphis* (Cedomon ®), *Bacillus subtilis* (Subtilex ™).

Trial B.
A second field trial using the same seed varieties was set up at Drumalbin in Lanarkshire. In this trial, untreated and hot water treated seed from 2008 seed stock was compared with commercial treated seed stocks (tebuconazole + triazoxide). Seed was sown in April in a randomised block design.

In both trials the plots received one foliar fungicide application at GS 25-30 (75 g/l metrofenone (Flexity ®) and 80 g/l pyraclostrobin (Comet 200 ®)

Leaf layers were assessed for the severity of RLS throughout the growing season and Area under disease progress curves (AUDPC) values were calculated using the trapezoidal rule. Leaf samples were also taken from the plots and the *Ramularia* DNA levels quantified as described above.

RESULTS

Movement of fungus from infected seed

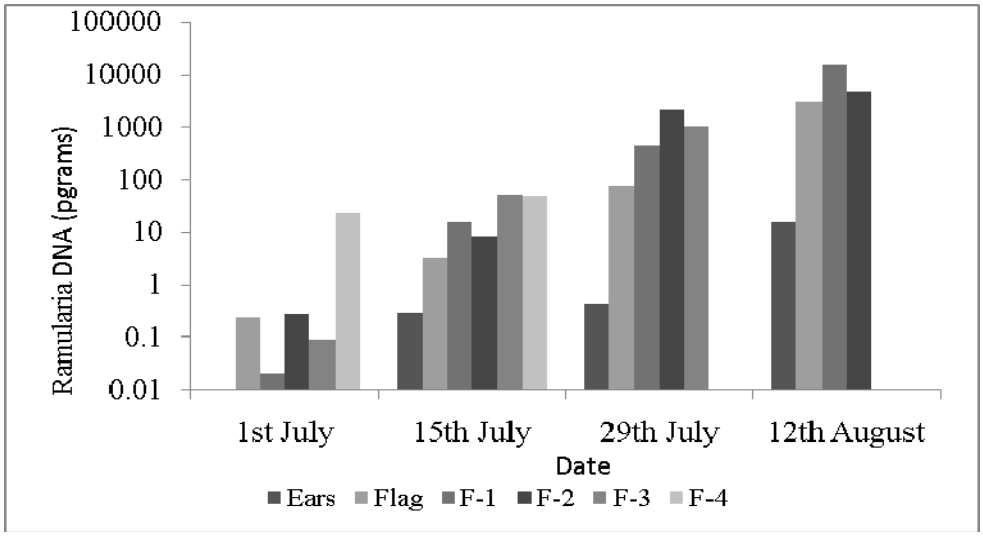

Figure 1. *Ramularia* DNA in cv Optic at Lanark site. The majority of *Ramularia* DNA recorded is in the lower leaves

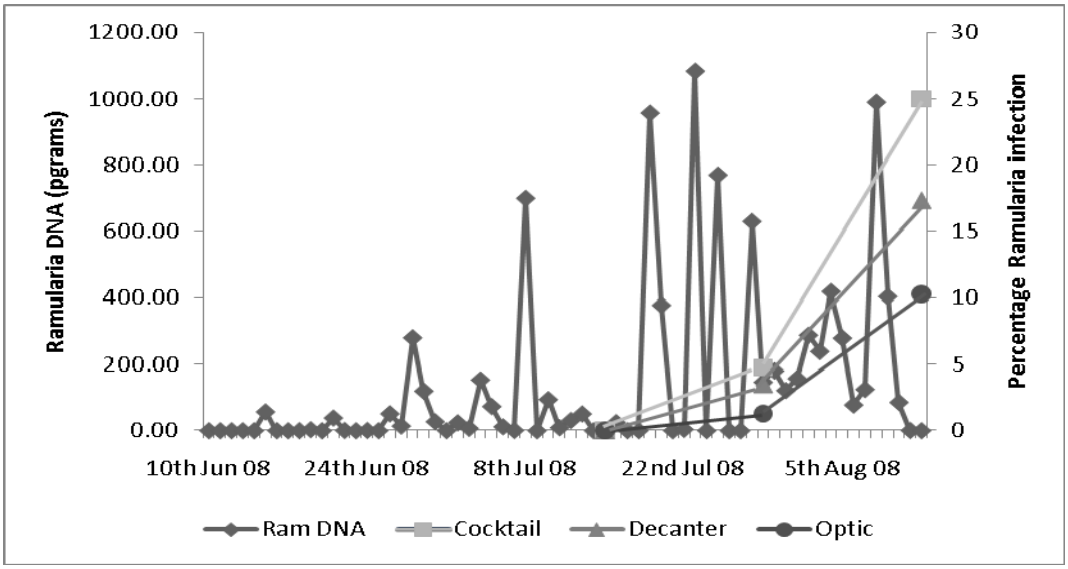

Figure 2 Area and Disease progress curves and *Ramularia* DNA levels from spore tapes at Lanark site in 2008

DISCUSSION

The movement of *R. collo-cygni* from infected seed into developing plant has been reported previously (Havis *et a.l,* 2006). The hill plot trial at Lanark in 2008 is the first time *Ramularia* levels in a developing spring barley crop have been monitored and quantified. Figure 1 shows the results for Optic over mid to late season. 60 days after sowing (1st July) fungal DNA was detectable in the emerged leaves and also the developing ear. The lower parts of the canopy contained more *Ramularia* than the upper canopy. This trend was also observed in the varieties Cocktail and Decanter.

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

Table 1. *Ramularia* AUDPC levels in field trial A

Treatment	Cocktail	Decanter	Optic
Trial A			
P. chlororaphis	335	273	295
Hot water	344	242	285
Teb + triaz	316	250	313
Steam	265	274	285
B. subtilis	375	282	237
Untreated	316	251	316
LSD (P=0.05)	68	68	68
Trial B			
Untreated	407	357	426
Hot water	422	357	491
Teb + triaz	398	366	411
LSD (P=0.05)	84	84	84

Table 2. *Ramularia* DNA levels in cv Cocktail in field trials (19/Jun/09)

Treatment	Flag	F-1	F-2	F-3	F-4
Trial A					
P. chlororaphis	0.24	0.29	0.28	1.43	7.33
Hot water	0.1	0.2	0.12	0.28	0.91
Teb + triaz	0.19	0.11	0.08	0.03	0.52
Steam	0.22	0.41	0.72	0.65	26.6
B. subtilis	0.09	0.28	0.17	0.59	5.38
Untreated	0.18	0.05	0.06	0.44	2.49
Trial B					
Hot water	0.05	0.38	0.94	3.32	1.63
Teb + triaz	0.15	0.11	0.29	0.73	1.79
Untreated	0.36	0.42	1.5	2.03	12.3

Table 3. Grain Yield and screening measurements in field trials

Treatment	Yield (t/ha at 85% DM)			Screenings (>20mm)		
Trial A	Cocktail	Decanter	Optic	Cocktail	Decanter	Optic
P. chlororaphis	6.48	6.65	6.13	88.93	88.87	88.97
Hot water	6.79	6.61	6.47	89.03	88.57	89.83
Teb + triaz	6.56	7.05	6.93	88.77	88.00	89.00
B. subtilis	6.55	6.86	6.99	89.47	88.73	88.77
Untreated	6.39	6.91	6.47	89.30	87.77	88.63
LSD (P=0.05)	0.52	0.52	0.52	3.15	3.15	3.15
Treatment	Yield (t/ha at 85% DM)					

Trial B	Cocktail	Decanter	Optic
Hot water	7.954	8.186	7.04
Teb + triaz	7.427	7.782	6.664
Untreated	7.582	8.058	7.034
LSD (P=0.05)	0.42	0.42	0.42

These three varieties differ in their susceptibility to RLS with Decanter classed as good for resistance, Cocktail intermediate and Optic intermediate to poor. The DNA results from the trial suggest that these differences are not related to inhibition of fungal movement in the plant.

Examination of the results from the spore sampler based at the Lanark site indicates that there a number of *Ramularia* spore release events in late June, primarily from earlier maturing winter barley crops (Figure 2). However, it is unlikely the spores could penetrate down into the spring barley canopy to affect the DNA levels. The major spore release events in mid to late July could contribute to rapid build up *Ramularia* DNA in the Flag and F-1 leaf layers. The additive effect of external inoculum and internal movement is only now being studied. Previous work has shown RLS symptoms in winter barley appear before any spore release has taken place (Havis *et al.,* 2009). This points towards the importance of seed borne *Ramularia* in the early movement of the disease in crops.

The AUDPC figures from the two trials indicate considerable variation from the treatments across varieties (Table 1). In general, *P. chlororaphis* and tebucoazole + triazoxide had little effect on symptom expression in Trial A. Previous work showed that the fungicide treatment can reduce AUDPC in cv Cocktail (Havis *et al.,* 2008). However this control was not observed in 2009. The Hot water treatment produced increases in the AUDPC in cv Cocktail in Trial A and all of the varieties in Trial B. The only two treatments which gave any reduction in AUDPC were Steam with cv Cocktail and *B. subtilis* with cv Optic.

The results from the DNA analysis shows that Steam and *B. subtilis* had little effect on *Ramularia* movement in the trials (Table 2). Only the results for cv Cocktail are shown here but the other varieties gave similar results. *P. chlororaphis* treatment gave a small increase in DNA levels while Hot water treatment did reduce levels lower in the canopy. This response from hot water has also been seen in other laboratories (Frei, 2009). The only treatment which gave consistent reductions in *Ramularia* DNA was tebuconazole and triazoxide. This could explain the previously reported effect of this treatment on AUDPC levels in cv Cocktail.

Yield measurements from the trials show that none of the treatments tested in Trial A gave a significant increase in final yield (Table 3). There were no significant differences in yield in Trial B despite the effect on *Ramularia* movement from the treatments. However, this site did have high late season disease pressure from *B. graminis* and *Rhynchosporium secalis,* which did affect green leaf retention.

This is the start of the project and the results presented give a basis for future work. The use of mixtures of seed treatments and alternative formulation/delivery of the biological agents merits further examination in the glasshouse and in field trials. Any new treatment programmes produced from this project could be important in controlling *R. collo-cygni* in conventional and organic systems.

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RHYNCHOSPORIUM SECALIS: A HISTORICAL PERSPECTIVE

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Summary: *Rhynchosporium secalis* is the most damaging pathogen of barley, and incidences of disease have been high since 1980, with several severe epidemics in the UK. In this paper we discuss the origin of the pathogen, and using archived grain and straw samples from the Rothamsted long term Hoosfield experiment look at its recent history to derive further information about this important pathogen and how agronomic and environmental factors has affected its severity.

INTRODUCTION

Rhynchosporium secalis causes leaf scald on barley, rye, triticale and other grasses and is commonly found where these cereals are grown; yield losses can be substantial, particularly in wetter areas. Yield losses of 10-40% are not uncommon (Shipton *et al*., 1974) and in severe epidemics 100% losses on susceptible cultivars have been reported (Yahyaoui, 2004). Data collected by Crop Monitor (www.cropmonitor.co.uk) based on visual assessments (Fig. 1) have shown that levels of rhynchosporium have been high since the early 1980's, with several years where the disease epidemic was severe (1983, 1998 and 2000); it has been the most important foliar disease of barley in recent years (Fig. 2). The disease is currently controlled by cultivar resistance and fungicides and the data shown in Figure 2 indicate that it may be declining. However, *R. secalis* has been shown to develop fungicide resistance, with resistance to MBC fungicides being common, and it is being closely monitored for resistance to other fungicides.

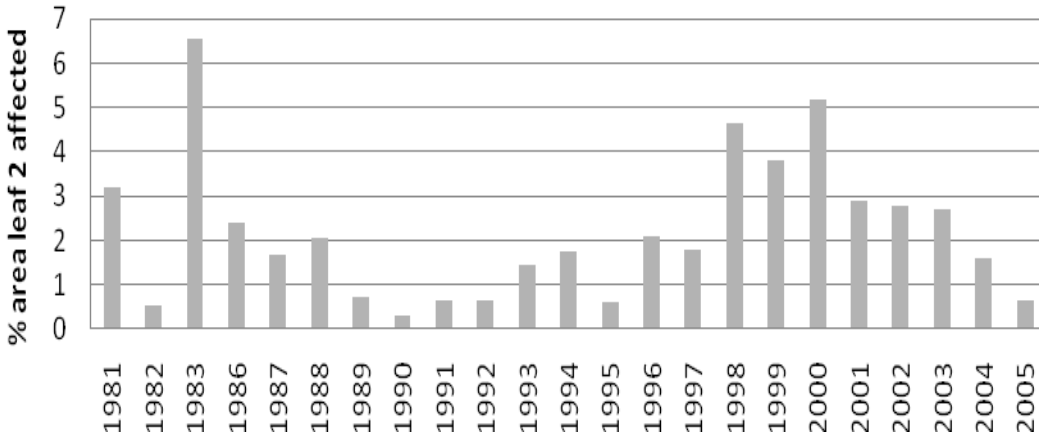

Figure 1. Severity of Rhynchosporium leaf scald from 1981 to 2005 on winter barley. Data provided by Crop Monitor

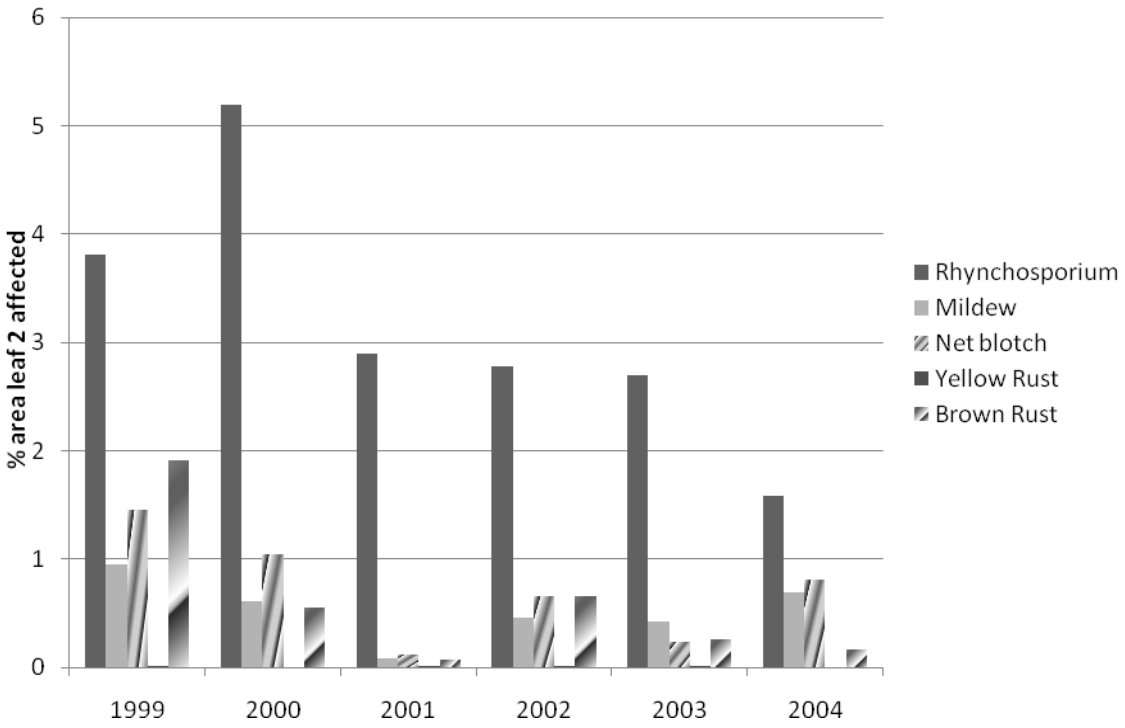

Figure 2. Comparison of diseases on barley between 1999 and 2004. Data provided by Crop Monitor

Yet what do we know about this pathogen? Where did it come from and what are the main drivers for epidemic development? What lessons can we learn from this pathogen that are relevant for other emerging diseases such as ramularia?

It was first thought that *R. secalis* co-evolved with its host, barley, in the Fertile Crescent of the Middle East and Ethiopia as with other pathogens such as *Mycosphaerrella graminicola* on wheat (Banke and McDonald, 2005). Yet recent work (Zaffarano *et al*., 2006; Brunner *et al*., 2007; Linde *et al.,* 2009), has shown that the greatest genetic diversity in *R. secalis* populations can be found in Scandinavia. They hypothesised that the pathogen evolved there on other hosts such as *Agropyron repens,* and shifted host with the introduction of barley into Northern Europe around 3600 BP. Current research is mapping the evolutionary global distribution and migration of this pathogen (Brunner *et al*., 2007; Zaffarano *et al*., 2008; Linde *et al*., 2009) and provides evidence about how the pathogen is able to disperse. Although *R. secalis* has a global distribution, Zaffarano *et al*., (2008) found that the migration pattern for the pathogen did not have a significantly long distance dispersal which would have indicated that it was wind dispersed; therefore, dispersal is hypothesised to be by some other means. Although high levels of genetic variation and equal proportions of both mating types in populations indicate the presence of a sexual stage (Foster and Fitt, 2003; Linde *et al*., 2003), the sexual stage has not been identified yet. Atkins *et al*. (2008) found no evidence for airborne dispersal of sexually produced ascospores over two growing seasons in the UK. The global distribution of the pathogen linked to the low migration pattern indicates another form of dispersal, most likely that of seed borne carriage.

It has long been observed that *R. secalis* can be found on other hosts and has been reported in many investigations. Table 1 lists some of these.

Table 1. Some of the reported grass hosts of *Rhynchosporium* species, on which distinct Rhynchosporium lesions were observed.

Host species	Common name	Reference
Agropyron repens	Couch grass	Lind (1913), as cited in Caldwell (1937)
Agrostis stolonifera	Creeping bent	Sprague (1950)
Bromus sterilis	Sterile brome	Brooks (1928), as cited in Reed (1957)
Bromus inermis	Awnless brome	Drechsler (1921), as cited in Caldwell (1937)
Bromus mollis	Soft brome	Brooks (1928)
Dactylis glomerata	Cocksfoot	Caldwell (1937); Sprague (1950); Brooks (1928); Kajiwara & Iwata (1963)
Danthonia spp.	Oatgrass	Drechsler (1921)
Elymus virginicus	Virginia wild rye	Caldwell (1937)
Hordeum murinum	Wall barley	Bartels (1928)
Hordeum jubatum	Foxtail barley	Caldwell (1937)
Lolium multiflorum	Italian ryegrass	Wilkins (1973)
Lolium perenne	Perennial ryegrass	Bartels (1928)
Phleum pratense	Timothy-grass	Makela (1973)

Several studies have investigated the host range of different *Rhynchosporium* species, often with different results. Caldwell (1937) did one of the first major studies including cross-species inoculation in both field and laboratory conditions. The Caldwell (1937) findings suggested near complete host specificity but this contrasts with other studies. Bartels (1928) used *Rhynchosporium* conidial suspensions sampled directly from *Hordeum murinum* and *Lolium perenne* in experiments. The suspensions were inoculated onto barley, rye, wheat, oats and several grass species, including *Agropyron*, *Bromus*, *Lolium*, *Holcus* and *Phleum*. Each type of conidial inoculum was able to produce typical rhynchosporium lesions on all of these hosts and led Bartels to conclude that grass species could act as inoculum sources for barley rhynchosporium epidemics.

Zaffarano (2007) made a major breakthrough in understanding the host specificity of barley, rye and *A. repens Rhynchosporium* isolates. This study used *R. secalis* isolates from nine hosts, including barley, rye, triticale, *Agropyron caninum* (bearded couch), *A. repens* (couch grass), *Bromus diandrus* (great brome), *Hordeum leporinum* and *H. murinum* (wall barley) and *H. spontaneum* (wild barley). Partial gene sequences of beta-tubulin, actin, EF-1α and ITS were used to construct a phylogenetic tree. The results provided evidence for monophyletic lineages. These lineages divided into three groups, with all originating from a common ancestor. One lineage was found to infect *Hordeum spp*., the next infected rye and triticale with another lineage infecting *Agropyron spp*. When pathogenicity assays were done, it was found that isolates had the ability to produce symptoms only on hosts that were in the same lineage, which was defined by Zaffarano (2007) as species borderlines. These proposed names for the four clades are summarised in Table 2.

Table 2: Summary of the *Rhynchosporium* lineages from Zaffarano (2007).

Lineage	Host	Proposed *Rhynchosporium* species
A	*Hordeum vulgare* (barley)	*R. communis*
B	*Agropyron repens* (couchgrass)	*R. agropyri*
C	*Cereale secale* (rye)	*R. secalis*
D	*Dactylis glomerata* (cocksfoot)	*R. orthosporum*

The work by Zaffarano (2007) suggests that, over time, *Rhynchosporium* has evolved to specialise in infecting specific hosts: implications on how this has affected the pathogen and its ability to reproduce and spread are as yet unknown.

At Rothamsted Research long-term experiments on winter wheat and spring barley are being conducted since 1843 and 1852, respectively. Straw and grain samples have been archived and DNA extracted from these samples can still be amplified. DNA amplification levels can be used to investigate the long-term dynamics of pathogen populations, as demonstrated for the wheat pathogenic fungi *Mycosphaerella graminicola* and *Phaeospaeria nodorum* by Bearchell *et al.* (2005). Preliminary work on detection and quantification of *R. secalis* in the archive samples shows that high levels of *R. secalis* DNA can be quantified in the last three decades (Fig. 3), with highest level of pathogen present in 1981 and 2002. We are now investigating if the introduction of short straw cultivars has caused an increase in pathogen DNA levels in straw at the beginning of the 1960's and study the frequencies of particular traits over time (e.g. fungicide resistance alleles and virulence factors such as NIP1). The importance of climatic factors such as rainfall in predicting epidemics is also under investigation.

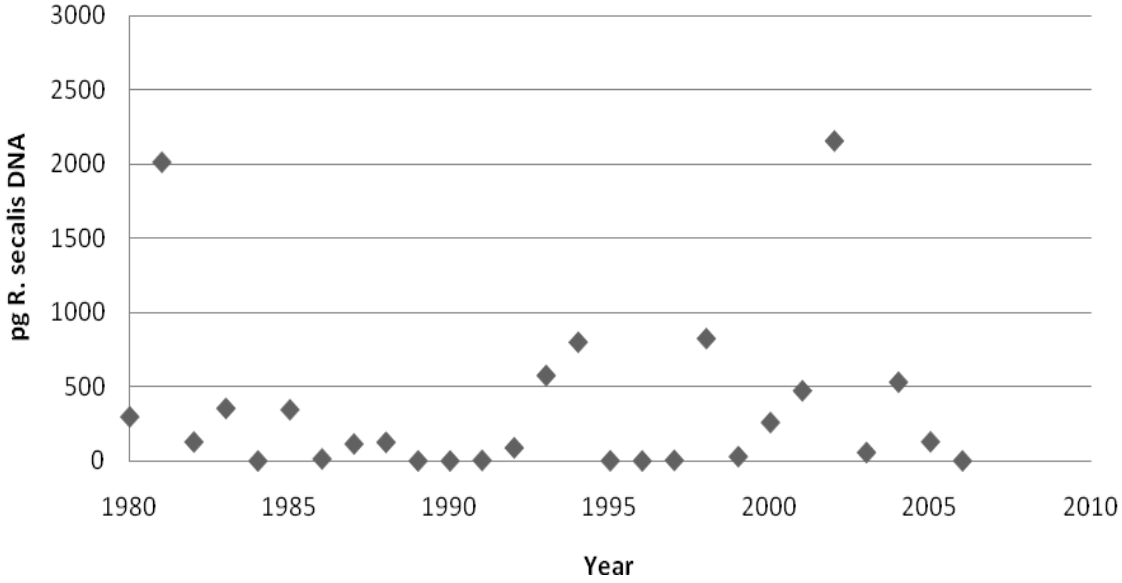

Figure 3. R. secalis DNA levels (in pg from 50 ng straw DNA) from archived spring barley straw from the long term barley trial at Rothamsted Research.

To summarise, *R. secalis* still remains an elusive pathogen. As further research is performed in investigating the organism more questions arise. This paper discusses some of the current areas where researchers have started to understand the biology of this pathogen. Results indicate that airborne spread of the pathogen is not important but seed-borne carriage is. A

long, sypmptomless infection of barley during the cold winter months explains the sudden appearance of lesions in the spring and asexual production of spores that are rain dispersed demonstrates why wet areas are more at risk to infection. Controlling the pathogen with fungicides and cultivar has proven to be effective but is not a long term strategy. The pathogen has demonstrated the ability to shift host and evolve to specialise in colonising, and a change in barley breeding in the 1960's may have increased the incidence of this pathogen. Rhynchosporium still remains the major pathogen of barley today, understanding host pathogen interactions may provide the key to controlling this pathogen

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SENSITIVITY AND STEWARDSHIP OF DMI FUNGICIDES FOR THE CONTROL OF *RHYNCHOSPORIUM SECALIS* IN BARLEY

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Summary: Rhynchosporium is a major pathogen of barley crops in the UK and the fungicide prothioconazole is now widely used on crops because of its efficacy against the pathogen and the yield improvements associated with its use. Isolates of *Rhynchosporium secalis* were sampled from field trials in Scotland and Ireland pre- and post-fungicide treatments and their sensitivity to prothioconazole determined. The results establish a base-line and range of sensitivity for the product. The post-treatment testing demonstrated a shift in mean sensitivity occurred after using the product straight. This shift was not evident where isolates were collected from untreated plots or from plots treated with prothioconazole applied in mixture with fungicides selected from a different activity group. Stewardship options to preserve the activity of the DMI fungicide group are discussed and the use of mixtures to reduce selection pressure and hence maintain the efficacy of the product is promoted.

INTRODUCTION

The development of fungicide resistance in target pathogens has removed or eroded the efficacy of many fungicide groups that are, or were, used in cereal crops to protect crops against key pathogens and to maintain yields. Barley, either winter or spring, is an important crop in rotations in the north of Britain and *Rhynchosporium secalis* is the most economically damaging disease of the crop, with effective control from fungicides adding over a tonne/ha to yield in HGCA trials (Oxley and Hunter, 2005). The fungicides used to manage the disease and to reduce these losses are of vital importance to the margins associated with growing the crop. Fungicides from several FRAC activity groups (FRAC, 2009) have efficacy against the disease and commercial spray programmes are generally based around mixtures of demethylation inhibitors or DMI fungicides (also known as azoles) applied in mixture with strobilurins, aminopyralids, morpholines or chlorothalonil. DMI fungicides are therefore important to the continued yield and economic viability of the crop. The development of resistance in this group of fungicides has been well documented in several cereal pathogens and activity against rhynchosporium has also declined, with some older DMI chemistry now significantly compromised (Hollomon *et al*., 2002). Typically DMI resistance is under the control of multiple genes and therefore resistance becomes evident as a decline in efficacy rather than a single step major gene resistance.

A new DMI, prothioconazole, was introduced to the barley market in 2003. HGCA fungicide trials have demonstrated its efficacy against rhynchosporium (Oxley and Hunter, 2005) and the yield benefits and margins associated with its use on crops. In 2008 89% of the winter barley crop and 55% of the spring barley crop in Scotland were treated with products containing prothioconazole (Reay, 2009). In addition 54% of the winter barley and 46% of the spring crop were treated with other azoles. The reliance of the market on the DMI group and its history of resistance in other pathogen systems meant there was a need to assess the risk of resistance in the key pathogen rhynchosporium and to investigate stewardship options that would reduced the selection pressure for resistance and seek to preserve the efficacy of this key group.

The aim of the work described in this paper was to establish base-line sensitivity data for the product and to determine if there were any shifts in efficacy following usage and if any mixtures strategies would reduce or manage this risk.

MATERIALS AND METHODS

Trials were carried out over three seasons starting in the autumn of 2004 and running to harvest in 2007. There were two winter barley trials and two spring barley trials located at sites in northern and central Scotland and a spring barley trial located in Northern Ireland in each season. The varieties used were susceptible to the disease. The winter barley variety used was Haka and the spring barley varieties were Braemar at the Scottish sites and Annabel at the Irish site. Full details are given in Oxley *et al*. (2008b). Trial design was as randomised blocks and there were three replicates of each treatment. Fungicide treatments were applied at the start of stem extension and the trials were over sprayed with chlorothalonil at booting to suppress late disease and allow for commercially relevant yield information to be attributed to treatment. Fungicides used are shown in Table 1. The rates applied were half of the maximum label rate.

Table 1. Fungicide treatments applied to trials.

Trade name	Code	Active ingredient	Group name	Resistance risk	FRAC code	Application rate (kg ha^{-1})
Proline	P	prothioconazole	demethylatation inhibitor	medium	G1	100
Corbel	C	fenpropimorph	morpholine	low to medium	G2	375
Vivid	V	pyraclostrobin	quinine outside inhibitor	high	C3	125
Unix	U	cyprodinil	aniline pyrimidine	medium	D1	250
Bravo 500	B	chlorothalonil	chloronitril	low	M	500

Before fungicide treatments were applied, leaves with lesions of *R. secalis* were collected from three control plots and from three pre-treatment plots to allow the initial sensitivity of

the *R. secalis* population at each site to be determined. Around 20-30 days after fungicide application and prior to over treatment of plots with chlorothalonil, 50-100 lesion-bearing leaves were collected from each plot. The leaves were air-dried at room temperature and stored in a –20°C freezer to produce one mixed isolate per sampled plot. Active lesions were cut from leaf, leaving a little green leaf area around the lesion. Lesions were then washed by submersing them in sterile distilled water for 10 minutes. In a laminar flow cabinet, leaves were surface sterilised by submersing in 10 % Sodium hypochlorite for 1.5 minutes and then allowed to dry on filter paper for 5 minutes. Segments were then plated onto antibiotic malt yeast glucose agar amended with iprodione (5-6 lesions per plate), ensuring that the lesion was uppermost on the leaf surface. Dishes were sealed, inverted and then incubated at 18 °C for up to three weeks. Starting three days after plates were set up they were examined daily until growth was noted when the top of the growing cultures was picked off under sterile conditions and plated onto fresh potato dextrose agar.

Sensitivity testing in liquid medium

Full testing methods are given in Oxley *et al*. (2008b). Spore suspensions (20 µl) of test isolates were added to each well of 96-well plates. Glucose-gelatin broth (180 µl), amended with fungicide to achieve final concentrations of 10, 10, 1, 0.1, 0.01, 0.001 and 0 mg of active ingredient of the test fungicides fungicide/l, was then pipetted into each well. Each isolate was tested in three replicate wells. Plates were measured for optical density (absorbance at 450nm) on a Labsystems Multiskan plate reader and again after incubation (14 days in dark, 19°C with gentle rocking). From the subsequent differences the ED_{50} values (estimated dose giving 50% control) were calculated in Genstat 10.2.

RESULTS

There was a wide range of sensitivity to prothioconazole - the median ED_{50} was 5.06 ppm, the mean 12.7 ppm and the lower to upper quartile range was 0.29 to 18.2 ppm. The sensitivity data for the isolates showed an unusual distribution and, while the majority of isolates fell closer to the median values there was a long distribution 'tail' to the least sensitive isolates assessed. The full range of sensitivities was 0.01 to 78.4 ppm.

Isolates with reduced sensitivity to prothioconazole also tended to have reduced sensitivity to epoxiconazole. There was a highly significant correlation ($r = 0.714$, $P<0.001$) between the sensitivities of isolates to prothioconazole and epoxiconazole indicating cross resistance, as shown in Figure 1.

There was a significant shift in the sensitivity to prothioconazole of isolates sampled from trials pre- and post-fungicide treatment ($P<0.001$). Where prothioconazole was applied in a two-way mixture the sensitivity of isolates was not significantly different to that in the untreated controls, as shown in figure 2. The sensitivity of isolates had a bimodal distribution. There were more isolates in the higher ED_{50} categories following treatment with straight prothioconazole. This was not the case when the prothioconazole had been applied in mixture with either chlorothalonil (B), cyprodinil (U), pyraclostrobin (V), or fenpropimorph (C).

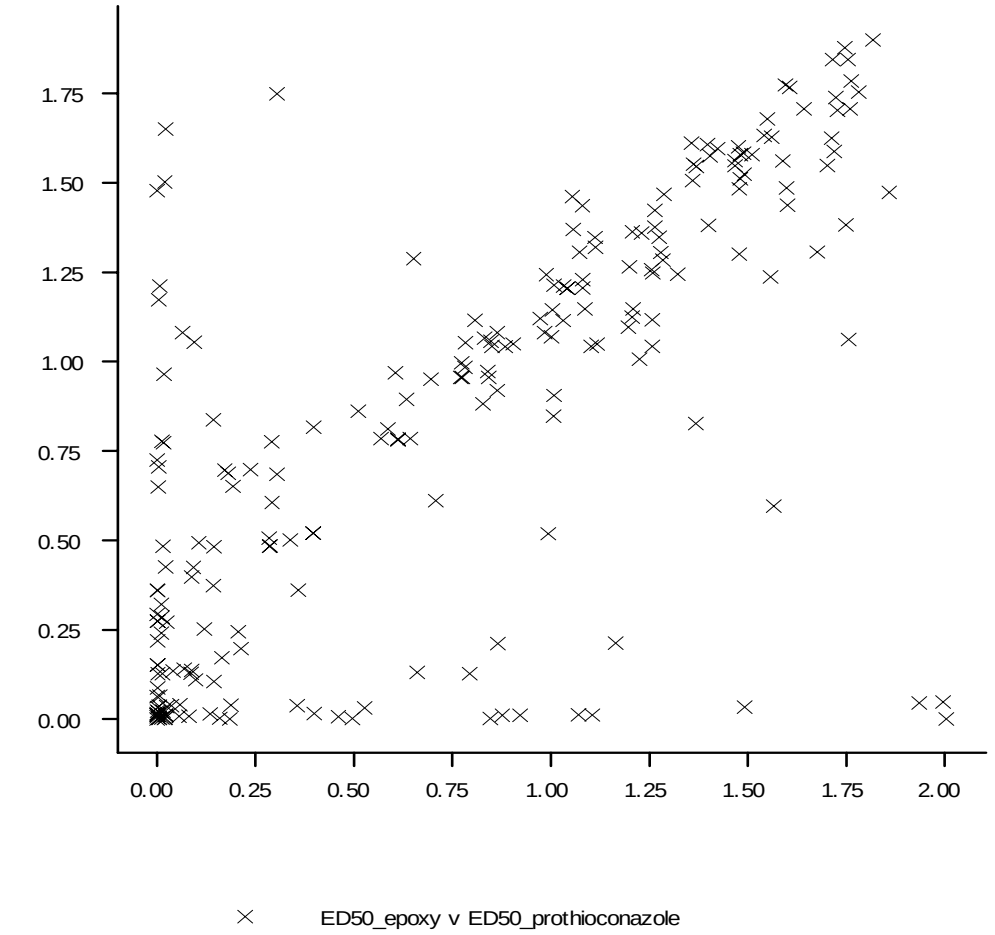

Figure 1. Sensitivity of *R.* secalis isolates to epoxiconazole and prothioconazole showing cross resistance (units as $\log^{10}(ED_{50}+1)$).

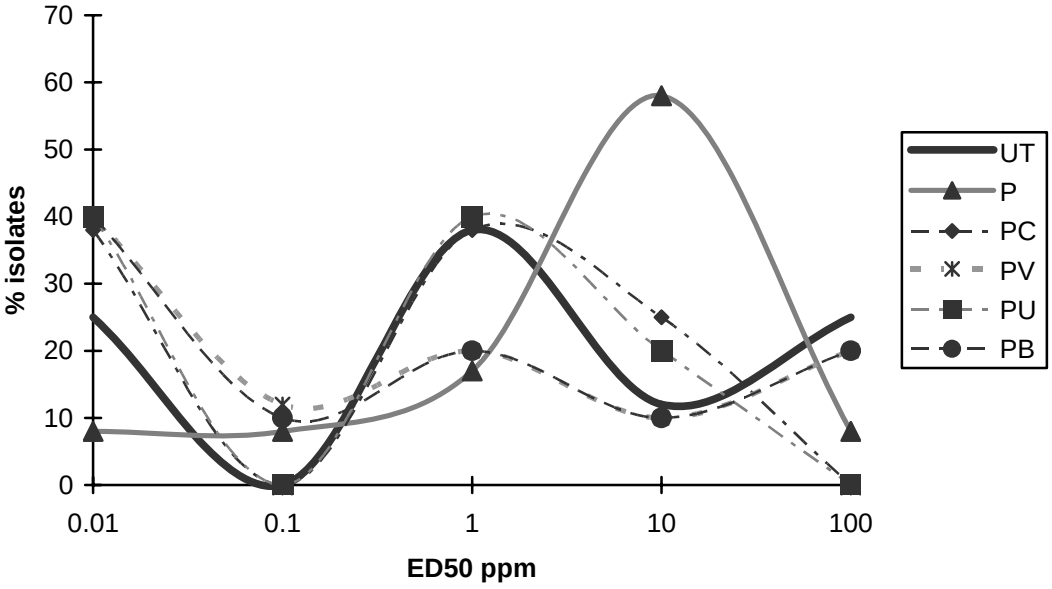

Figure 2. Percentage of isolates in each sensitivity category following prothioconazole treatment straight or in two-way mixture.

DISCUSSION

The results demonstrate that, in common with several other cereal pathogens, *R. secalis* isolates have a wide range of ED_{50} values to prothioconazole with the least sensitive isolates having ED_{50} values 100-fold higher than those most sensitive isolates. Despite the identification of less sensitive strains of the pathogen, efficacy in the field was retained (Oxley *et al.,* 2008b). Cross resistance to epoxiconazole was confirmed and there have been previous reports of reductions in efficacy of epoxiconazole (Cooke *et al*., 2004). Although there was no discernable shift in sensitivity over the duration of the work reported here (Oxley *et al*., 2008b) the data highlights the potential risk to prothioconazole efficacy, particularly given the level of DMI usage on barley crops.

Treatment with prothioconazole applied as a straight product in trials lead to a significant shift in the mean sensitivity of isolates sampled from those treatment plots, demonstrating the risks associated with using the product straight. There was no significant shift in sensitivity, however if the product was applied in mixture with any of the other fungicide groups used as treatments demonstrating that a mixture strategy reduced the risks of resistance to the DMI group. In practice such a strategy offers the additional benefits of a wider efficacy spectrum against other pathogens and the ability to tailor the eradicant or protectant activity needed at spray application. The DMI group, and in particular prothioconazole, are very important to the maintained yield and margins of winter and spring barley crops. Responsible use and proper stewardship of the product are therefore vital both to individual businesses and to the barley industry nationally. In the past there has been little awareness of the importance of properly stewarding products and a track record of applying fungicides in a manner designed to maximise profit in the short term regardless of the risks of resistance arising and the longer term costs associated with that eventuality. Where voluntary resistance management strategies were introduced as happened at the onset of strobilurin resistance these were widely ignored. There are many examples of the industry using and breaking products and moving on to newer actives. It is generally agreed that there will be no extensive pipeline of new products and that those products that do appear will continue to be used with DMI fungicides to maintain yield and disease spectrum. The need for individuals and the industry collectively to take responsibility for stewarding the DMI products, and any following products, is therefore vital.

This paper demonstrates the efficacy of mixing strategies in reducing fungicide resistance risk. This is strategy which growers use widely for reasons of disease spectrum and flexibility and the use of mixtures is therefore likely to be a practical and widely used strategy. The use of straight DMIs should be strongly discouraged. It is likely to increase resistance risk at that particular location and will contribute to the onset of difficulties for the whole industry.

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THE INFLUENCE OF FUNGICIDE ON ALCOHOL YIELD IN DISTILLING WHEAT

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Summary: Two field experiments were conducted in the 2007/08 growing season to assess the effect of fungicide application on grain yield and quality of distilling wheat. The first experiment used the soft winter wheat variety Glasgow recommended for distilling to study the effect of fungicide applied at GS 59. The second experiment also used the variety Glasgow, with the addition of the soft winter wheat variety Ambrosia and studied the effect of fungicide timing. The experiments showed that fungicide applications were important to maximise both grain and alcohol yield.

INTRODUCTION

Distilling wheat in the UK has traditionally been grown for the Scotch whisky industry. Annually this market has a requirement of approximately 700,000 tonnes of wheat, producing in the region of 300,000 litres of alcohol (Smith *et al*., 2006). The area of wheat required for distilling is expected to increase dramatically with the developing bioethanol industry. There are currently three bioethanol plants being constructed in the North of England. The Ensus plant at Teesside is expected to be operational by late 2009 to early 2010, with an annual wheat requirement of 1,200,000 tonnes, making it the largest wheat refinery in Europe (Ensus, undated). The production of potentially large areas of wheat for non-food purposes has raised some concern over the availability and affordability of food. Therefore it is necessary to address these concerns by maximising the efficiency of production, helping to limit land area requirements.

Recent work has shown that crop variety and fertiliser inputs can have a large impact on the potential alcohol yield of the crop (Kindred *et al*., 2008). To maximise efficiency it is important to determine the effects of all crop management decisions on alcohol yields. Studies on the quality of milling wheat have shown that fungicide applications have the potential to affect grain quality by modifying grain protein concentration (Ruske *et al*., 2003a). This has often led to a dilution in grain protein concentration as a result of increased starch accumulation. Increased starch concentration can be correlated to increased alcohol yield (Kindred *et al*., 2008). Therefore it can be hypothesised that the application of fungicides has the potential to increase alcohol production. Two field experiments were conducted to investigate this.

MATERIALS AND METHODS

Field experiments were conducted at Harper Adams University College in Shropshire UK. Experiment One was established using the soft winter wheat variety Glasgow, recommended for distilling (HGCA, 2009). Experiment Two was also established using the variety Glasgow, with the addition of the soft winter wheat variety Ambrosia. Ambrosia was used in this experiment to study the effect of fungicides on a variety with contrasting alcohol yield to Glasgow (HGCA, 2007). Both experiments received a fungicide application to all plots of epoxiconazole 125 g l^{-1} (Opus, BASF, Cheadle Hulme) applied at 0.6 l ha^{-1} + chlorothalonil 500 g l^{-1} (Bravo 500, Syngenta Crop Protection UK Ltd, Fulbourn) at 1.0 l ha^{-1} at GS 32 and 39 (Zadoks *et al*., 1974). The fungicide treatment was prothioconazole + tebuconazole 125:125 g l^{-1} (Prosaro, Bayer CropScience, Milton) was applied at 0.5 l ha^{-1} at GS 59. Experiment One studied the effect of fungicide application at GS 59 by including a control which received no fungicide at this timing (i.e. only received fungicide at GS 32 & 39). Experiment Two studied the combined effect of fungicide at GS 39 & 59 by including a control which received no fungicide at both these timings. In each experiment treatments were replicated seven times in a randomised block design. Each plot was 12.0 x 1.8 metres with a 1.5 metre buffer between replicates and a 0.2 metre buffer between plots within each replicate.

Both experiments were established in early October into a plough based seedbed which consisted of a sandy loam soil using a seed rate of 300 m^2. Crops were established as the first wheat in the rotation after oilseed rape. Visual assessment keys (AgroEvo, 1997) were used to assess foliar disease infection on the crop. Septoria leaf blotch was the only disease present at high enough levels for assessments to be performed. An average of 10 flag leaves assessments was taken for each plot on each of several assessment dates. This technique was also used to study green flag leaf area decline, with assessments being conducted from early July until early August. Harvesting was carried out using a field trials combine; grain from each plot was weighed to enable the yield to be calculated once adjusted to 15% moisture content. A 1 kg sample was kept from each plot within the experiment. The moisture content and specific weight (adjusted to 15% moisture) of each sample was taken using a Sinar 6060 AP analyser. The thousand grain weight (TGW) was measured by weighing a subsample of approximately 25 g and then counting the number of grains. Analysis of the grain starch concentration (GSC) was performed using the total starch assay kit (Megazyme Ltd. Wicklow, Ireland). Samples were analysed three times to provide an accurate average reading. Analysis was conducted in accordance to the method described by Megazyme (undated). Grain protein concentrations (GPCs) were analysed using the Foss Infratec 1241 grain analyser. This device measures the grain nitrogen concentration by near infrared reflectance from the intact grain and the GPC is determined by multiplying this figure by 5.7 (Ruske *et al*., 2003). A calibration has been developed for the grain analyser by The Scotch Whisky Research Institute to give a predicted alcohol yield (PAY). This calibration is now commercially used within Scottish grain distilleries.

The data analysis programme Genstat (version 12.0) was used. Analysis of variance (ANOVA) was performed on all data collected. For Experiment One a one-way ANOVA in randomised blocks was used, Experiment Two was analysed using a two-way ANOVA as the experiment used two varieties. A Gompertz regression model was used to study the effect of fungicides on green leaf decline. The difference between treatments was studied at 37% GFLA (*m*).

RESULTS

The application of fungicides resulted in a highly significantly ($P < 0.001$) reduction in levels of *Septoria tritici* infection and increased green flag leaf retention (Table 1). This resulted in increased grain weight and grain starch concentration and a dilution in the grain protein concentration. These effects upon grain quality resulted in a highly significant ($P < 0.001$) increase in predicted alcohol yield (Table 1) compared to plots receiving no fungicides at GS 59 (Exp. 1) or GS 39 & 59 (Exp. 2). Highly significant ($P < 0.001$) yield increases were also seen in both experiments (Table 1).

Table 1. Effect of fungicide application on predicted alcohol yield (L t^{-1}) and grain yield (t ha^{-1}) (mean ± SE, n=7)

Experiment	Fungicide treatment	Septoria (%)	Yield (t ha^{-1})	PAY (L t^{-1})
1	Untreated (GS 59)	2.51 (± 0.48)	11.99 (± 0.15)	449.50 (± 0.89)
1	Fungicide treated	0.93 (± 0.36)	12.40 (± 0.12)	451.53 (± 0.67)
2	Untreated (GS 39 & 59)	41.04 (± 7.53)	10.22 (± 0.45)	437.9 (± 2.21)
2	Fungicide treated	0.96 (± 0.31)	12.00 (± 0.21)	447.7 (± 0.50)

DISCUSSION

The experiments showed that fungicides were necessary to protect crop yields. When no fungicides were applied at GS 59 in Experiment One a significant yield deficit of 0.41 t ha^{-1} was shown in comparison to fungicide treated plots. This was shown to be the result of a reduction in grain filling caused by increased disease pressure and early crop senescence. This resulted in lower grain starch concentrations, resulting in a high GPC as a result of the lower dilution effect. Given that starch is the substrate for alcohol production the PAY for plots receiving no fungicide at GS 59 was 2.03 LA t^{-1} below that of fungicide treated plots. The results from experiment Two suggest that the GS 39 fungicide application is of a greater importance to both crop yield and alcohol yield. Where no fungicides were applied at GS 39 or GS 59 (Experiment Two), yields were 1.78 t ha^{-1} below fungicide treated plots with the PAY been reduced by 9.80 L t^{-1}. The GS 39 fungicide treatment protects the flag leaf which provides the greatest contribution to photosynthesis and yield (Cooke *et al*., 1999). The results from the experiments showed that fungicide application to distilling wheat crops can play an important part in helping to maximise the efficiency of alcohol production.

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CLUBROOT RISK AND MANAGEMENT IN OILSEED RAPE CROPS IN NORTHERN UK

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Summary: Clubroot is becoming increasingly common in oilseed rape crops in the north of the UK as a consequence of the high proportion of the crop in rotations and the persistence of this soil-borne pathogen in soils. In a survey of soils from oilseed rape crops in Scotland in 2008 and 2009, 54% tested positive in bioassay tests. Clubroot is therefore a major factor limiting yields in oilseed rape crops and management strategies are a requirement for affected fields. Bioassays demonstrated the efficacy of varietal resistance (variety Mendel) in reducing disease. The work also identified that the Mendel resistance is under pressure and that not all strains of clubroot are controlled. A field trial in Aberdeenshire where soil amendments were used with the aim of manipulating pH and calcium levels gave variable results and did not significantly reduce disease, although a negative correlation between disease severity and pH and soil calcium levels was noted.

INTRODUCTION

Clubroot is the most damaging disease of brassica crops globally and accounts for £30 million losses in the UK *per annum* (Donald *et al*., 2006). It persists in soils for upwards of 15 years, with a half life of 3.7 years (Wallenmammer, 1996), and is therefore a major issue in rotations. The introduction of oilseed rape into rotations in the UK in the mid 1980s increased the risk of disease spread and build up. Oilseed rape is particularly important in arable rotations in the north of the UK as other break crop choices become increasingly limited. Rape is commonly grown one year in three in northern arable rotations and one year in two is not uncommon in Aberdeenshire. This frequency in the rotation increases the risk of the disease multiplying and spreading on farms. In the last few seasons, problems with clubroot infection in rape and yield losses in commercial crops have been widely evident. Mendel, a variety with resistance, is widely grown on soils known to be contaminated but this variety requires higher levels of agronomic inputs such as agrochemicals and does not have the same yield potential as newer varieties. The overuse of a single management strategy exerts a strong selection pressure and the race specific nature of the 'Mendel' resistance means it may not be durable (Werner *et al*., 2008). It is under further pressure as it is present in other crops such as swedes, which increases the selection pressure where these are gown in the rotation. The work described here is an early report of an ongoing project aiming to determine how widespread clubroot is in northern rotations and to determine if there are any management strategies that could be used to reduce or manage the problem.

MATERIALS AND METHODS

Survey

In 2008 and 2009 a survey of commercial oilseed rape fields in Scotland was undertaken. A total of 76 samples were collected. Fifty cores were taken from each sampling area to give a soil sample of approximately 2 kg using a narrow bladed fern trowel or auger and the cores were taken at a depth of 15-20 cm. The cores were collected at regular intervals in a "W" or multi "W" pattern. Large stones and plant material were removed from the sample before bagging in a large heavy gauge polythene bag. The soils were mixed by hand and used to fill seed trays as described for the bioassay below into which 20 seedlings of untreated Chinese cabbage variety SB1 Kilo were planted. These were assessed and scored after six weeks for clubroot infection as described below.

Bioassay variety tests

A bioassay of 31 varieties plus a Chinese cabbage control were used to test for varietal resistance to clubroot. Plastic seed trays with drainage holes (20 x 14.5 x 5.5 cm) were filled with John Innes No. 2 potting compost, pH 5.5. Each of these trays was then put into a larger tray with no drainage holes (33.5 x 21 x 5.5 cm). To inoculate the compost, a 50 ml clubroot resting spore suspension containing 10^5 spores/ ml was poured over the top of the soil to give a final concentration of around 10^4 spores/g soil. The spore suspension was prepared by planting twenty seeds of untreated Chinese cabbage var. SB1 Kilo into soil selected from an infected site in Fife into trays as described above. The trays were then to be put on raised benches in the glasshouse and watered daily by pouring water into the larger trays without holes. The glasshouse air temperature was set around 18^{o}C. After 6 weeks, the resultant galls were washed free of soil, homogenised, filtered through 8 layers of muslin using 20 – 25 ml tap water and centrifuged at 100 x g. The pellet from this step was discarded and the supernatant spun at 6000 x g for 15 minutes to pellet the clubroot spores. The spores were then re-suspended in de-ionised water and a haemocytometer used to count the spores in 25 mls solution.

Table 1. Oilseed rape varieties tested for susceptibility to clubroot in a bioassay.

Code number	Variety	Code number	Variety
1267	Mendel	1907	Flash
1355	Toccata	1930	EGC521
1378	Winner	1947	CWH086D
1583	Lioness	1953	Vision
1592	Bravour	1955	WRH 300
1593	Victory	1956	WRH 289
1608	Castille	1963	ANX3506
1684	Excalib	1965	RNX3504
1692	Betty	1970	X05W/085c
1710	Grace	1975	RAPN285
1780	Hornet	1976	LSF0526
1834	Canti	1978	NPZ0527
1857	Catana	1982	NPZ0525
1897	Temple	1983	NPZ0524
1902	PR45D03	1989	MH223
1904	PR46W14	Chinese cabbage	SB I KILO

The varieties tested were sown into inoculated soil at 25 seeds per tray to allow for some losses with the aim of having 20 plants to assess after the growing period. After six weeks growth in a glasshouse as described above, plant roots were assessed for clubbing on a four point scale where 0 was uninfected, 1 = slight clubbing, 2 = moderately clubbed and 3 = severely clubbed. A percentage severity index was then calculated by weighting the incidence of plants in the three positive categories by a factor of one, two or three respectively. Three replicates of each variety were used and arranged in fully randomised blocks.

Field trial

A field trial was also established in Aberdeenshire in autumn 2008 as part of a trial series of which this paper is an early report. A site with a history of severe clubroot was selected and results are reported for the variety Kommando which carries no known resistance to clubroot. Trial design was as four fully randomised blocks. Soil treatments were applied as indicated in Table 2. Products were selected as having shown efficacy in previous work on transplanted vegetable brassicas (Harling, 2006). These were a precipitated calcium carbonate product (LimeX70) and a calcium cyanamid product (Perlka). The former was applied to plots before cultivation and incorporated. The latter was applied after soil preparation for drilling and then incorporated in two of the treatments and not incorporated for a third treatment. Boron was also evaluated as a soil amendment. The boron was applied as Solubor (20.8% boron) using a knapsack sprayer to apply as a soil drench. There were two control treatments, one untreated and a further control to balance the nitrogen in the calcium cyanamide product. Trial design was as randomised blocks and other inputs were as per local practice. Plot size was 30 m^2. Clubroot was assessed in the autumn and the spring by sampling 10 plants at random per plot and assessing on the 0-3 scale described for the bioassay. Soil samples were taken post harvest for analysis of pH and extractable calcium levels. Two replicates were bulked together so that there were two soil samples per treatment.

Table 2. Soil amendments tested in field trials 2008/2009 season.

Treatment	Product and rate
1.	Untreated control
2.	Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha
3.	Calcium carbonate 4 t/ha
4.	Calcium carbonate 8 t/ha.
5.	Calcium cyanamide 250 kg/ha
6.	Calcium cyanamide 250kg/ha – not incorporated
7.	Control with extra 50 kg/ha nitrogen
8.	Boron – 20kg/ha

RESULTS

The survey results showed that in 2008 57% of soils tested positive and in 2009 40% were positive, with a total mean of 53%. The mean infection index after bioassay testing was 8.4 with a range of 1.7 to 38.3. The mean pH of soils was 6.0 with a range of 5.6 – 6.4. The mean extractable calcium level was 1457 mg/l with a range of 671 – 2030. There was no significant correlation between these parameters and the disease index for infected soils.

The bioassay indicated that Mendel was significantly better than all the other varieties tested and that there was no significant difference in susceptibility between these other varieties. The range in clubroot severity for the other varieties tested was 65.0 for Flash and 87.5 for NPZ0527. The mean index (excluding Mendel and the uninoculated control) was 74.1.

Table 3. Oilseed rape varieties tested for susceptibility to clubroot in a bioassay.

Variety	% clubroot index	Variety	% clubroot index
Mendel	26.9	Flash	65.0
Toccata	77.6	EGC521	69.0
Winner	72.5	CWH086D	73.3
Lioness	74.0	Vision	74.7
Bravour	72.0	WRH 300	75.4
Victory	80.5	WRH 289	75.6
Castille	69.8	ANX3506	68.6
Excalib	66.0	RNX3504	85.0
Betty	71.9	X05W/085C	79.7
Grace	66.3	RAPN285	74.0
Hornet	74.4	LSF0526	80.9
Canti	68.6	NPZ0527	87.5
Catana	71.0	NPZ0525	78.9
Temple	77.6	NPZ0524	79.0
PR45D03	71.0	MH223	78.3
PR46W14	65.0	Chinese cabbage	72.9
Untreated control	0		
SED			7.057
P value			<0.001

The field trial results are shown overleaf in Table 4. Disease levels were moderate in the trial; in the spring the mean index in the two control treatments was 9.16. There were no significant differences in disease levels in either the autumn or spring assessments. There was a trend in the autumn and spring for the unincorporated calcium cyanamide treatment to have lower disease levels but this was not significant for either timing. There were significant differences in calcium and pH values from soil samples taken post harvest. The highest extractable calcium levels and pH values were in the calcium cyanamide unincorporated treatment. Many of the soil amendment treatments had significantly higher pHs than the extra nitrogen control treatment, however the value for the untreated control was also significantly higher than this so that interpretation was difficult.

There were significant correlations between disease levels and these soil parameters. The correlation co-efficient between soil pH and disease index in the spring assessment was very highly significant ($r = -0.921$, $P = <0.001$). There was also a significant correlation between extractable calcium levels and disease in the spring ($r = -0.582$, $P = 0.018$).

Table 4. Clubroot indices in autumn and spring assessments and pH and extractable calcium levels (mg/l) post harvest.

Treatment	Disease index autumn	Disease index spring	pH	Extractable calcium (mg/l)
1. Untreated control	3.33	5.83	7.5	3725
2. Calcium carbonate 4 t/ha + Calcium cyanamide 250 kg/ha	5.83	7.50	6.8	2445
3. Calcium carbonate 4 t/ha	12.5	7.50	7.2	3045
4. Calcium carbonate 8 t/ha.	8.33	9.17	7.6	7210
5. Calcium cyanamide 250 kg/ha	15.0	7.50	7.5	4015
6. Calcium cyanamide 250kg/ha – not incorporated	0.00	0.83	7.8	14800
7. Control with extra 50 kg/ha nitrogen	12.5	12.5	6.3	1860
8. Boron – 20kg/ha	2.50	6.67	7.8	5345
SED	8.367	9.247	0.256	1722.1
P value	0.559	0.967	0.006	<0.001

DISCUSSION

A survey of oilseed rape field carried out in 2008 and 2009 showed that 53% of oilseed rape field are infected with clubroot. This makes it a far more significant problem to the industry than was previously appreciated. This figure is far higher than the 2% figure given for England and Wales in CropMonitor data which may be under reported or may be representative of lower risk.

The use of the variety Mendel in the bioassays showed the strength of varietal resistance in managing this disease. The level of control noted was 63.7% compared to the mean of other varieties tested. Whilst this level of control is encouraging it should be noted that it is by no means complete and implies that a portion of the clubroot population which was sourced from a high pressure site in Fife can overcome the Mendel resistance. This is indicative of the

pressure the 'Mendel' gene is under and of the need for new forms of varietal resistance to clubroot. Further erosion of this varietal resistance through the continued widespread use of this gene and resultant selection for strains of clubroot able to overcome it would be of major concern to the industry. Little is known about the pathotypes within the clubroot population with regards to virulence on Mendel or varieties using the same resistance. Previously strains were characterised by the range of species they infected (Buczacki *et al*., 1975) which was a somewhat limited system. The use of molecular markers as a method of characterising strains of clubroot virulent of Mendel (or any following resistant varieties developed) and thereby identifying sites at risk would aid individuals and the industry. The development of alternative forms of resistance would greatly assist.

While there were no significant effects on clubroot levels as a result of the soil amendments tested in the trial reported here, the trend to lower disease in some treatments and the negative correlation between clubroot severity and pH and calcium levels shows potential for this type of approach. The data illustrated the difficulties in altering soil pH or nutrient levels by enough to significantly attribute disease control effects to treatments. The level of control from varietal resistance is always likely to be greater in addition to having less cost or environmental implications. The impact and cost of clubroot in a single season on farm is relatively simple to calculate. The impact in the longer term could be more damaging and future work to properly cost different rotational strategies with regards to the frequency of use of oilseed (and other susceptible crops) would be useful to the industry. It would indicate if the long term profitability of the crop is threatened by the current frequency of this crop in farm rotations.

ACKNOWLEDGEMENTS

This work was funded with a grant from HGCA and soil amendment products were kindly supplied by Plant Solutions Ltd and British Sugar. The authors wish to thank SAC colleagues for field trial management, soil sampling and bioassay testing and the growers who took part in the survey. SAC receives support from Scottish Government RERAD.

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CONTROL OF BROMUS SPP. AND AVENA SPP. WITH PYROXSULAM + FLORASULAM

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Summary: Broadway Star herbicide (formulation code GF-1364) was launched in the UK in spring 2009 for the control of *Avena spp*, *Bromus spp*, *Lolium spp* and a range of broad leaved weed species in winter wheat. Containing pyroxsulam plus florasulam and the safener cloquintocet-mexyl, it can be safely applied at 265 g/ha from GS11 (first leaf unfolded) to GS32 (2nd node stage) of winter wheat. Used as part of a spray programme it achieves >98% control of *Avena* and *Bromus spp*. The very short half life of both pyroxsulam and florasulam allows all major rotational crops to be grown with no carry over risk and without cultivation restrictions.

INTRODUCTION

GF-1364 is a water dispersible granule (WG) containing 7.08% w/w pyroxsulam, 1.42% w/w florasulam and 7.08% w/w of the crop safener cloquintocet-mexyl. The chemical, toxicological and environmental characteristics of GF-1364 have been outlined (Harris *et al.,* 2009). By utilising the grass weed activity of pyroxsulam and combining with the proven broad-leaved weed activity of florasulam (Thompson *et al.*, 1999), GF-1364 provides UK farmers with an effective solution for the control of *Avena spp*., *Bromus spp., Lolium spp.* and a number of commonly occurring broad-leaved weeds. It offers farmers excellent control at low usage rates as well as highly flexible following crop options and no cultivation restrictions.

Data is available from winter wheat selectivity trials conducted in the UK between 2003 and 2007(Harris *et al.*, 2009. The maximum label rate for GF-1364 will be 265 g/ha for the control of grass weeds. GF-1364 is not selective to barley or oats.

GF-1364 controls *Avena spp*. and *Anisantha sterilis* (previously known as *Bromus sterlis*) and *Bromus spp*. at a rate of 265 g/ha plus adjuvant. As is typical with ALS inhibitors, control is best when applied to small, actively growing plants. GF-1364 has the flexibility to be used in the autumn or the spring with early removal of weeds being advantageous to minimize the effects of crop competition and yield loss. However it is recommended as part of a spray programme of non ALS, residual acting products either applied before or in combination with GF-1364. These recommendations support the advice of Weed Resistance Action Group (WRAG) of utilizing chemical products with different modes of action as well as adopting cultural control methods to minimize the impact of weed resistance and slow its spread.

Though GF-1364 will give high levels of control of *Lolium spp*., this paper concentrates on the dominant grass weed species in Northern Britain of *A. sterilis* and *Avena spp*.

MATERIALS AND METHODS

Trial design and crop safety

Field testing of GF-1364 began in 2004 and all grass weed data presented are from UK trials. Initial studies were conducted in mixture with an esterified rapeseed adjuvant, however later studies were conducted such that a range of adjuvants are supported. Treatments were applied using precision small plot sprayers at application rates of 100 to 200 litres per hectare through flat fan nozzles at fine to medium spray quality. Label rate and double label rate were tested in combination with an esterified rapeseed oil adjuvant at 1 litre/ha and 2 litres/ha respectively and recorded equivalent yield to the untreated control when autumn or spring applied.

Efficacy assessments

Grass weed control data are from head count data (4-5 quadrat counts per plot) expressed as % control relative to the untreated, where 0% represents no control and 100% represents total control.

To demonstrate the activity against grass and broad leaved weeds so as to secure a registration, GF-1364 was applied as a single spray application, though the product is recommended for use in spray programmes.

RESULTS AND DISCUSSION

Control of *Avena spp (*wild oats*)*

Figure 1 shows the combined data for *A. fatua* and *A. ludoviciana* control. GF-1364 achieved 98% control of *Avena spp.* applied up to GS30, equivalent to mesosulfuron-methyl + iodosulfuron-methyl-sodium (meso+iodo) 98.6%. Both treatments achieved significantly higher control than clodinafop-propargyl + an adjuvant containing 60% mineral oil and 40% surfactants (88%).

Autumn applications of GF-1364 achieve very high levels of control yet benefit from the adoption of a spray programme using either pendimethalin + flufenacet (pdm+flu) or flufenacet + diflufenican (flu+dff) as the pre-emergence product. The pre-emergence product introduces additional modes of action for grassweed control, thereby reducing the selection pressure on the ALS inhibitor product. The overall effect was high and consistent levels of control as shown in Figure 2 below.

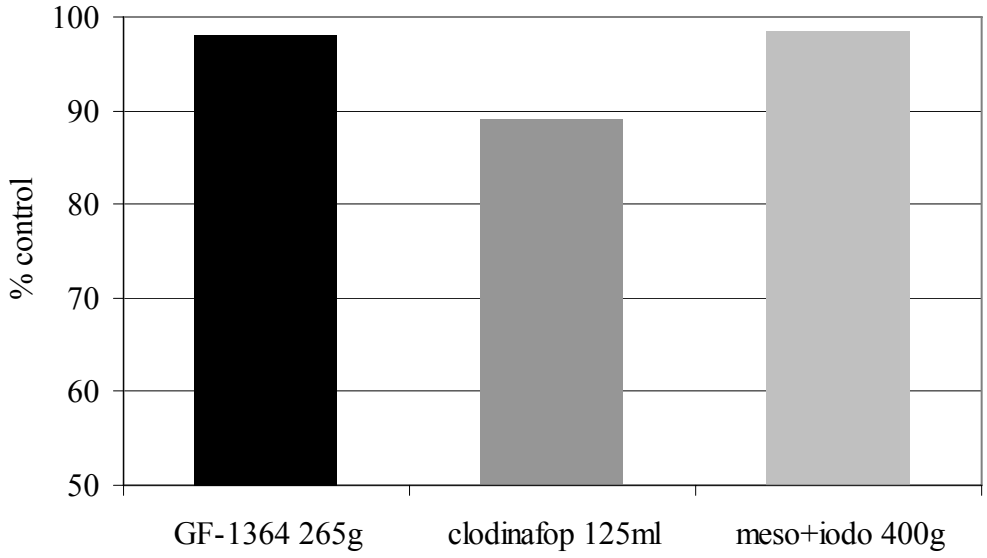

Figure 1. *Avena spp* control from spring applied treatments. UK 2004-2009 (mean of 16 trials, LSD = 1.97, p=0.05)

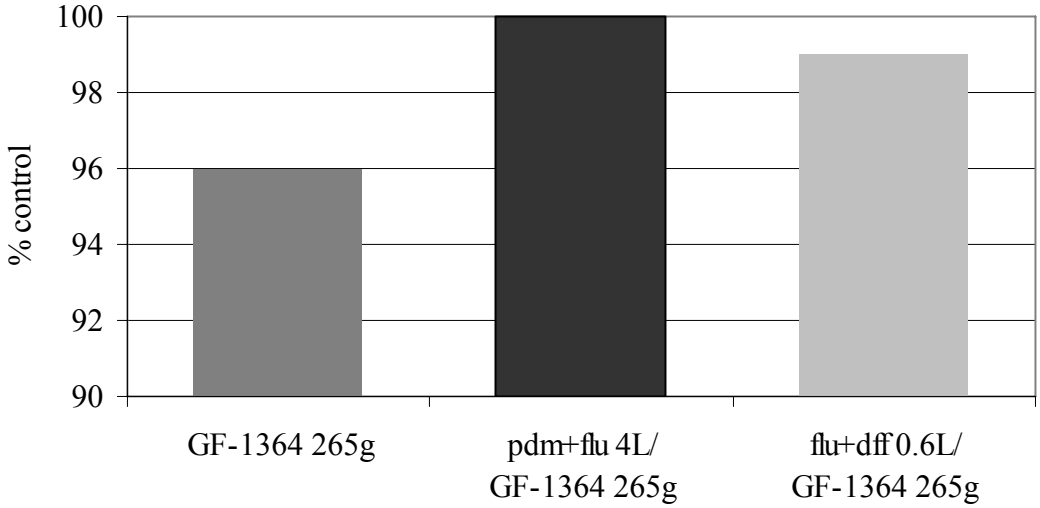

Figure 2. Autumn *Avena spp* control from GF-1364 alone or following a pre-emergence treatment (mean of 2 trials, LSD 3.18, p=0.05)

Control of *Anisantha* and *Bromus spp*

A. sterilis remains a difficult weed to control as much a consequence of plant physiology (leaf hairiness) as plant species. Initial studies concentrated on applying GF-1364 in the spring and are presented in Figure 3, with GF-1364 at 265 g/ha achieving 77% control significantly higher than the reference products, sulfosulfuron at 25 g/ha and meso + iodo at 400 g/ha.

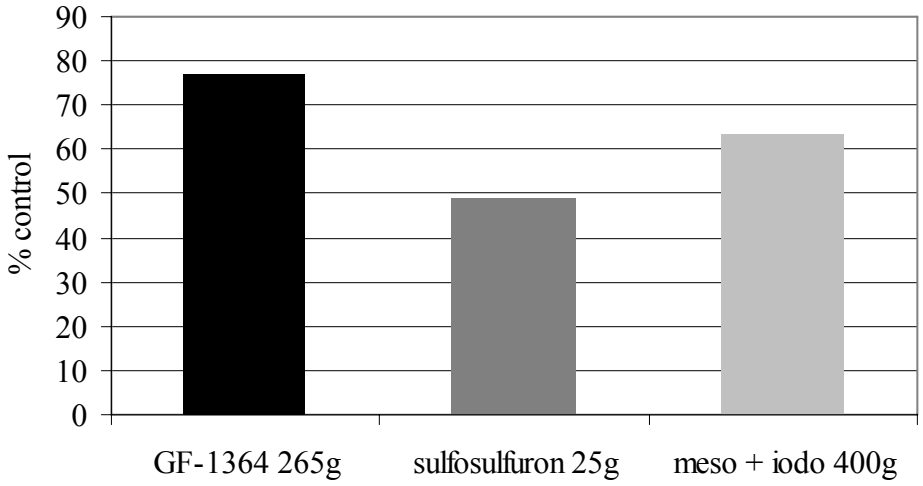

Figure 3. *A. sterilis* control from spring applied treatments. UK 2004-2008 (mean of 12 trials, LSD 1.97, p=0.05)

Data are presented in Figure 4 from 3 non replicated studies conducted in 2007 and 2008 investigating *A. sterilis* control from a programme of a pre-emergence treatment (pdm+flu or flu+dff) followed by an autumn application of GF-1364 at 265 g/ha + 800 g ai/ha pendimethalin (pdm) applied to *A. sterilis* at GS 10-13. Data clearly shows the benefit of a spray programme, with the pdm+flu followed by GF-1364 + pdm giving 99.4% control, compared to 93.8% control without a pre-emergence treatment. Where a pre-emergence treatment was followed by a spring application of GF-1364 control was 78.3%.

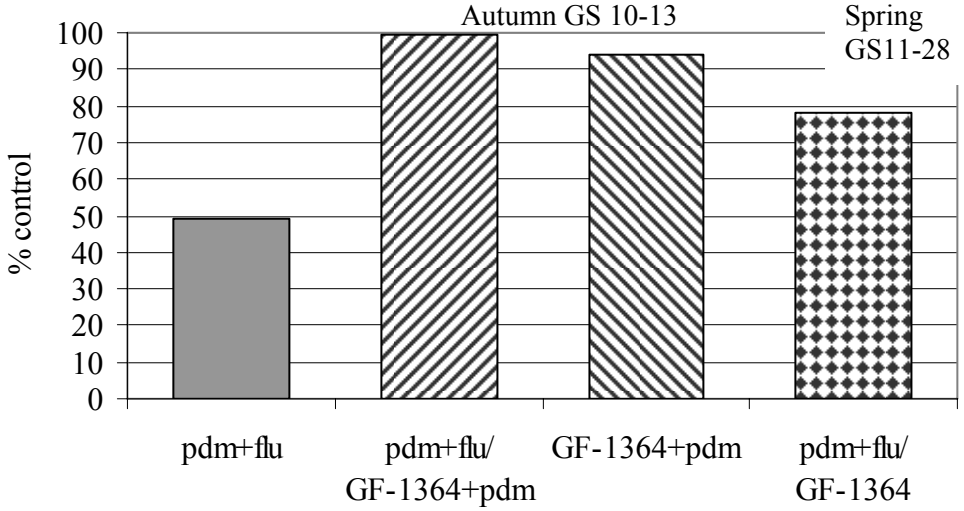

Figure 4. *A. sterilis* control from an autumn spray programme or a spring application (mean of three trials, LSD 2.30, p=0.05).

Broad leaf weed control

Pyroxsulam is unique amongst the triazolopyrimidine sulfonanilide group of herbicides in having both grass weed and extensive broad leaf weed activity. Florasulam is a proven dicotyledonous herbicide. GF-1364 therefore controls many of the commonly occurring broad leaf weeds in winter wheat and a summary of the available data are presented in Figure 5.

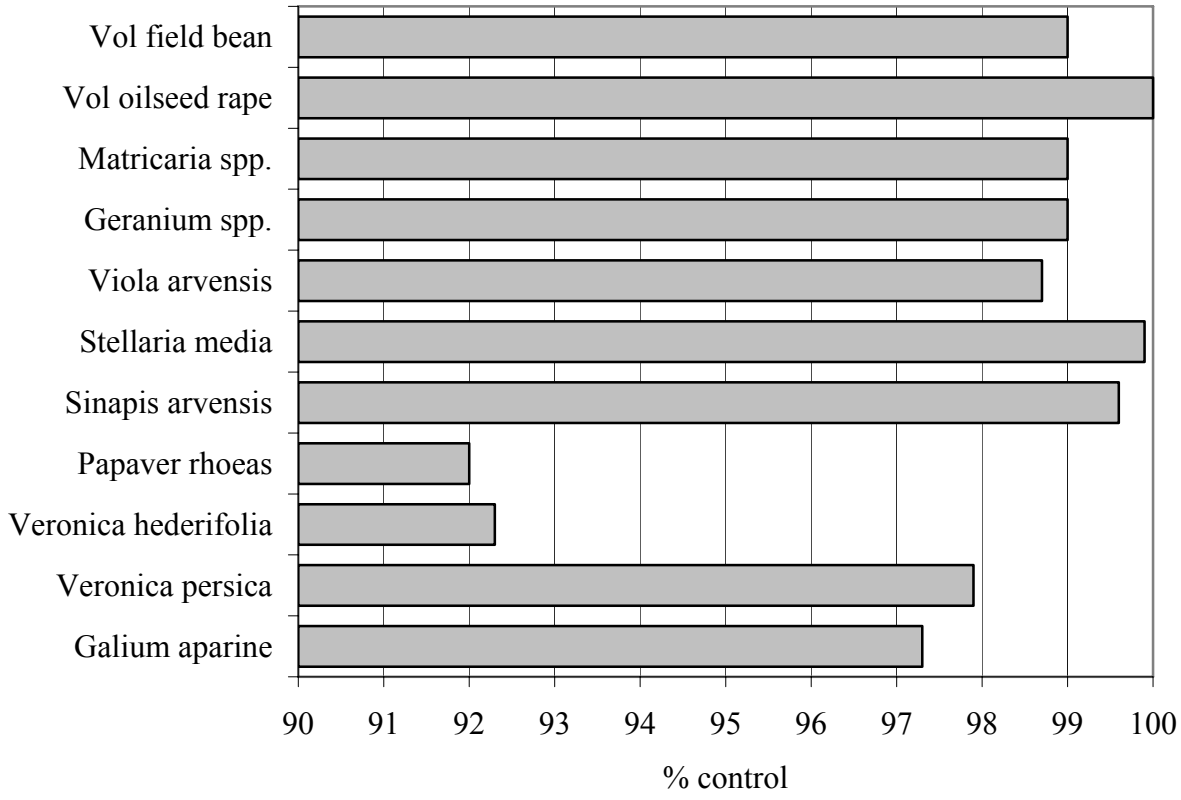

Figure 5. Broadleaved weed control from spring applications of GF-1364 applied at rates between 170 – 210 g/ha

Following crops

Rotational crop safety from carryover is an important attribute for any cereal herbicide. The soil half life (DT_{50} Lab) of pyroxsulam is 3 days, similar to that for florasulam (<5days), (Thompson *et al.,* 1999). Extensive field testing of GF-1364 was conducted on a broad range of soil types, climatic conditions, cultivation techniques and planting intervals to investigate whether any effects would be observed in following crops. No effects have been observed on the following crops after an application of GF-1364 to winter wheat:- autumn sown cereals, winter oilseed rape, field beans, brassica transplants and grass or spring planted cereal crops, oilseed rape, field beans, peas, sugar beet, potatoes, grass/clover, maize, linseed, brassica

transplants, carrots and parsnips. All these crops can be established using either ploughing or minimal cultivation.

CONCLUSION

GF-1364 at 265 g/ha plus an approved adjuvant can be applied to winter wheat from GS11 to GS32 and will control wild oats (including ACCase target site resistant populations), bromes and a range of broad leaved weeds. As an ALS inhibitor, resistance management practices determine that the preferred product recommendation is for use in sequential spray programmes and the need to implement good cultural techniques recommended within the WRAG guidelines.

GF-1364 delivered 98% control of *Avena spp* and applied following a pre-emergence application of pendimethalin + flufenacet control rose to 100%. Although it is the most effective product for *A. sterilis* control when applied in the spring (77% control), studies have identified that an autumn timing applied at GS 10-13 of the weed can achieve 99% control when used in combination with a pre-emergence treatment and residual partner product. Where conditions for a pre-emergence treatment were unfavourable, an autumn application of GF-1364 + a residual delivered 94% control. The addition of florasulam to pyroxsulam strengthens the herbicide activity and in the absence of grass weeds, control for most commonly occurring broadleaf species was >92% at rates of 170-210 g/ha. Comprehensive field testing in weed free sites across many different soil types has shown excellent crop selectivity in winter wheat with no yield reduction. GF-1364 can be tank mixed with a wide range of fungicides, insecticides, herbicides and plant nutrients/trace elements, though mixtures with organophosphate insecticides are not recommended. With a soil half life of <5days, fields treated with GF-1364 can be safely rotated to all major crops with no cultivation restrictions.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the assistance of all Dow AgroSciences colleagues during the development of GF-1364.

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THE CHARACTERISATION OF ALS TARGET SITE RESISTANCE IN *STELLARIA MEDIA* (CHICKWEED) BIOTYPES FROM THE UK AND THEIR CONTROL BY ALS FORMULATIONS INTENDED FOR GRASSWEED MANAGEMENT

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Summary: ALS inhibiting herbicides are a fundamental part of most broad-leaved weed control strategies. *Stellaria media* showing resistance to ALS inhibitors was first identified in 2000 in the UK. Recent work has shown that resistance is due to single nucleotide changes at the P197 or W574 positions of the ALS gene. Cross resistance patterns varied depending on the particular resistant allele with W574 R alleles providing higher levels of resistance to sulfonylureas than those at P197, along with additional cross resistance to the triazolopyrimidine florasulam. ALS inhibiting formulations mainly intended for grass weed control (in particular the mesosulfuron + iodosulfuron mixture 'Atlantis') provided surprisingly high levels of control against confirmed P197 (but not W574) resistant biotypes emphasising the importance of chemistry, dose rate and formulation against target site changes often assumed to confer "total" resistance.

INTRODUCTION

Stellaria media (common chickweed) is among the most important broad leaved weeds in the UK, being very prolific and causing severe yield loss in cases of high infestation. It can flower and set seed very rapidly, in some cases completing a whole life cycle in as little as six weeks (Grime *et al*., 1988) and normally flowers from early spring until late autumn making cultural control difficult. Herbicide resistant biotypes of *S. media* were first reported in Denmark and have now been confirmed across 10 different countries including the UK, Ireland, Sweden and Norway (Heap, 2009). In the UK ALS resistant biotypes were first detected in 2000 (Moss *et al*., 2005). A research project on herbicide resistant broad leaved weeds including *S media* and *Papaver rhoeas* (common poppy) in the UK has recently been completed (Marshall, 2009).

The cross resistance patterns of confirmed resistant *S. media* biotypes to different ALS inhibiting broad leaved weed and grass weed formulations is of interest for several reasons. These include predicting the efficacy of alternative ALS formulations against resistant biotypes and selection for resistance in non-target weed species.

The objectives of this research were as follows: (1) To determine the baseline levels of ALS resistance in several *Stellaria media* biotypes and to identify the mechanisms responsible (2) To examine the extent of cross-resistance to alternative herbicides and to ALS inhibitors intended for grass weed control, and to assess the possible impact of such issues on continuing management strategies.

MATERIALS AND METHODS

Seeds of two suspected resistant *S. media* biotypes were obtained from field sites in Perthshire (SCOT), and Aberdeenshire (ABER). Both biotypes were collected from fields where farmers or consultants reported failure of control with ALS inhibiting herbicides. Sequencing of the ALS gene from both resistant biotypes showed that SU resistance segregated with two separate biotype specific single nucleotide changes in the conserved regions of the ALS gene conferring predicted amino acid changes at P197 (Pro to Gln, SCOT) and W574 (Trp to Leu, ABER) of the ALS enzyme (Marshall 2009). A susceptible standard biotype (UKA) was included for comparison. The ALS gene sequence of this susceptible standard showed no SNPs conferring potential amino acid changes in the conserved regions of the ALS gene.

Baseline dose response assay

Plants from the SCOT and ABER biotypes, along with the UKA susceptible standard, were grown in a glasshouse to the rosette stage (8-15 cm) before spraying with a number of different herbicides appropriate for spring-time control of *S. media*. Spraying was performed using a track sprayer delivering 246 L spray solution ha^{-1} at 210 kPa through a single 'Teejet' TP110015VK flat fan nozzle at a range of different doses including the usual field rates (in bold). Treatments were metsulfuron-methyl at 12, **6**, 3, 1.5, 0.75, 0.375 and 0.1875 g a.i. ha^{-1}; florasulam at 10, **5**, 2.5, 1.25, 0.625, 0.3125 and 0.15626 g a.i. ha^{-1}, fluroxypyr at 400, **200**, 100, 50, 25, 12.5 and 6.25 g a.i. ha^{-1}, and mecoprop-P at 2400, **1200**, 600, 300, 150, 75 and 37.5 g a.i. ha^{-1}. A total of 10 reps with a single plant per 5 cm pot were included at each dose along with a total of 20 untreated controls per biotype. Pots were randomised after spraying. Plant harvests were carried out 20 days after treatment and fresh foliage weights were recorded. Four parameter logistic curves of the type $E(y) = A + C/ (1 + EXP(-B(x – M))$ were fitted to fresh weight data compared to untreated plants using MLP v3.09 (Ross, 1987) and $\log_{10}$ ED_{50} values were calculated. Resistance indices were calculated as the ratio of ED_{50} values relative to the susceptible standard.

Outdoor container experiment

This experiment was designed in order to examine the resistance profile of resistant *S media* biotypes with confirmed target site resistance to a variety of different ALS grass weed herbicides under simulated field conditions.

The experiment was set up in early March as a randomised block design with five treatments (including nils), three biotypes (SCOT, ABER, UKA), and three replicates. Each replicate block contained 15 containers, with 12 plants per container grown initially in small pots and then transplanted. Herbicide treatments were the sulfonylaminocarbonyltriazolinone propoxycarbazone-sodium at 70 g a.i. ha^{-1}, the sulfonylureas iodosulfuron-methyl at 9.6 g a.i. ha^{-1} and mesosulfuron-methyl + iodosulfuron-methyl mixture at 12 + 2.4 g a.i. ha^{-1}, and the imidazolinone imazapyr at 375 g a.i. ha^{-1}. The adjuvants "Biopower" at 0.5 % total volume, and "Comulin mineral oil" at 1 L ha^{-1} were included with the mesosulfuron + iodosulfuron and propoxycarbazone treatments respectively. Imazapyr was included as a representative of imidazolinone chemistry although it is not a selective grass weed herbicide and is not used in the UK. Metsulfuron was not included in this experiment, but a previous study had shown very poor (0 – 20 %) control of the SCOT and ABER biotypes at the field rate (6 g a.i. ha^{-1}).

Containers were randomised by replicate block under protective netting with watering provided daily. Containers were sprayed as described previously. All containers were harvested 51 days after treatment when foliage weights were recorded and the number of surviving plants counted. Data were subjected to analysis of variance using percentage reductions in fresh weights relative to untreated plants and reduction in plant numbers.

Further dose response assay

A whole plant dose response assay was performed in order to further characterise the response to several of the ALS inhibiting herbicides tested in outdoor containers.

S. media plants from the SCOT and ABER resistant biotypes, along with the UKA susceptible standard were grown in a glasshouse to the rosette stage (8-15 cm) before spraying as described above at a range of different doses including the usual field rates (in bold). Treatments were mesosulfuron + iodosulfuron mixture at 0.375 + 0.075, 0.75 + 0.15, 1.5 + 0.3, 3 + 0.6, 6 + 1.2, **12 + 2.4**, 24 + 4.8 and 48 + 9.6 g a.i. ha^{-1}; iodosulfuron at 0.075, 0.15, 0.3, 0.6, 1.2, 2.4, 4.8, **9.6**, 19.2 and 38.4 g a.i. ha^{-1}; and imazapyr at 5.859, 11.719, 23.438, 46.875, 93.75, 187.5, **375** and 750 g a.i. ha^{-1}. A single field rate (6 g a.i. ha^{-1}) dose of metsulfuron was also included to confirm resistance. The adjuvant "Biopower" at 0.5 % total volume was included with all iodosulfuron and mesosulfuron + iodosulfuron treatments. A total of 10 reps with a single plant per pot were included at each dose along with a total of 20 untreated controls per biotype. Pots were randomised after spraying. Plants were harvested 32 days after treatment and foliage weights were recorded. Four parameter logistic curves were fitted to percentage reduction in fresh weight data as before.

RESULTS

Baseline dose response assay

High levels of resistance to the sulfonylurea herbicide metsulfuron were observed in all resistant biotypes compared to the susceptible standard UKA. The highest dose of twice field rate was not sufficient to cause reductions in fresh foliage weight for both resistant biotypes. The calculated metsulfuron ED_{50} value for the UKA susceptible standard was 0.25 g a.i. ha^{-1}. It was not possible to calculate ED_{50} for the two resistant biotypes, but both were substantially greater than 12 g a.i. ha^{-1}, the highest dose used. Consequently RI (resistance index) values for the resistant biotypes were each greater than 48 compared to the susceptible standard. Resistance to the ALS inhibiting herbicide florasulam was observed in the ABER biotype with an ED_{50} of 163 g a.i. ha^{-1} and an RI of $\geq$1046. ABER was the only biotype demonstrating significant levels of resistance to florasulam (RI for the SCOT biotype was $\geq$1.3). There was no evidence of significant resistance to mecoprop-P or fluroxypyr. Resistance indices were highest for the SCOT biotype (1.3 fold difference after treatment with mecoprop-P, 1.5 fold difference with fluroxypyr) and the ABER biotype (1.4 fold difference with fluroxypyr), respectively.

Outdoor container experiment

Good control of all resistant and susceptible biotypes was achieved using imazapyr, while propoxycarbazone offered almost no control in terms of fresh weight reduction (Table 1).

Table 1. Analysis of variance comparing mean percentage reduction in *S. media* plant weights.

	Mean % reduction in fresh weight compared to untreated			
Biotype	Propoxycarbazone	Iodosulfuron	Meso + iodosulfuron	Imazapyr
UKA (S)	-1.0	99.1	99.2	99.4
SCOT (P197)	-2.4	47.2	95.3	99.2
ABER (W574)	-0.3	28.2	54.4	95.1
S.E. ±		4.8		
LSD ($P \leq 0.05$)		14.0		

The grass-weed SU type ALS inhibitors (iodosulfuron and iodosulfuron + mesosulfuron mixture) were more effective against confirmed ALS target site resistant *S. media* than the broad leaf SU herbicide metsulfuron at field rates. Previous work with metsulfuron in containers showed only 0 – 20 % control (Marshall, 2009). The Pro-197-Gln SCOT biotype was well controlled by mesosulfuron + iodosulfuron (> 90 % reduction in fresh weight compared to control), while iodosulfuron caused significant foliage damage. Comparing survivor numbers showed the SCOT biotype was significantly more resistant to mesosulfuron + iodosulfuron than the susceptible standard UKA (data not shown), but all surviving SCOT plants were badly damaged. Metsulfuron and florasulam resistant ABER plants with confirmed Trp-574-Leu target site resistance were not completely controlled by mesosulfuron + iodosulfuron mixture but were severely damaged before growing back (54 % fresh weight reduction compared to control). Iodosulfuron failed to provide useful levels of control against the ABER biotype at field rate. Imazapyr provided the best levels of control overall with fresh weight reductions > 90 % for all biotypes. Comparing surviving plant numbers showed a small but significant decrease in control with imazapyr for the ABER biotype.

Further dose response assay

All herbicide treatments gave good control of the susceptible UKA biotype at the minimum applied dose; ED_{50} was lower than the minimum dose in all cases. The calculated mesosulfuron + iodosulfuron ED_{50} for the Pro-197-Gln SCOT biotype was 2.5 + 0.5 g a.i. ha^{-1}, well below field rate (Figure 1). Lower levels of control were achieved for the Trp-574-Leu resistant ABER biotype with mesosulfuron + iodosulfuron giving no control even at the highest dose of 48 + 9.6 g a.i. ha^{-1}, four times field rate. In comparison field rate application of metsulfuron (6 g a.i. ha^{-1}) provided > 98 % control of the UKA susceptible standard but only 1.9 and 10.4 % control of the SCOT and ABER populations, respectively.

Iodosulfuron alone provided lower rates of control compared to the mesosulfuron + iodosulfuron mixture for the SCOT biotype on an iodosulfuron dose for dose basis with a calculated ED_{50} of 1.2 g a.i. ha^{-1} providing evidence that mesosulfuron provides part of the broad leaf weed activity observed in the mixture. Higher dose rates of iodosulfuron had a measurable effect on foliage weights of the ABER biotype with an iodosulfuron ED_{50} calculated as 20.4 g a.i. ha^{-1}. The non selective imidazolinone herbicide imazapyr provided the highest levels of control with both UKA and SCOT biotypes fully controlled at the lowest dose (Figure 2). ABER was the only biotype to demonstrate resistance to imazapyr with an ED_{50} around 300 g a.i. ha^{-1}.

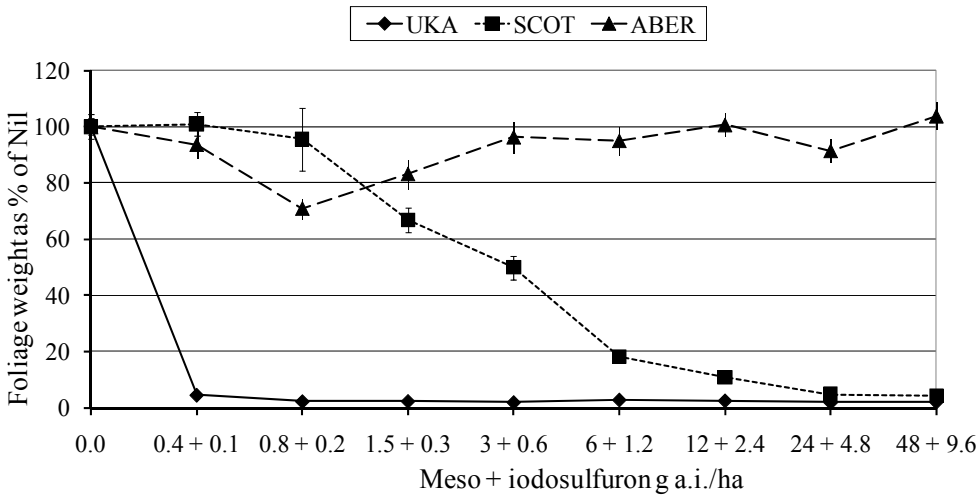

Figure 1. The effect of mesosulfuron + iodosulfuron on three *S. media* biotypes.

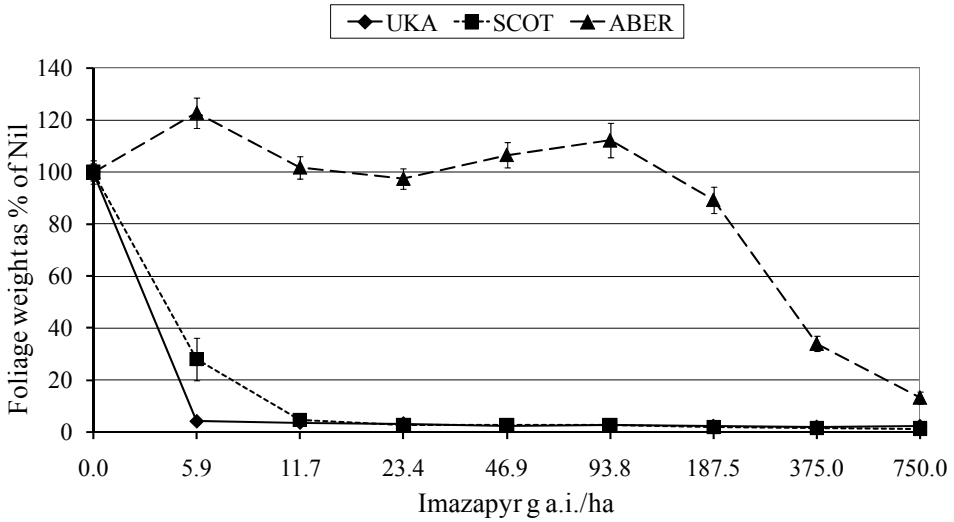

Figure 2. The effect of imazapyr on three *S. media* biotypes.

DISCUSSION

Cross resistance patterns in ALS target site resistant *Stellaria media* appear to be more complicated than previously thought. It is usually assumed that an amino acid substitution conferring resistance to one ALS inhibitor will also provide resistance to others of the same chemical class but results show this is not always the case. Control of confirmed ALS target site resistant *S media* may be possible using alternative herbicides, even of the same chemical class (e.g. sulfonylureas).

The activity of field rate mesosulfuron + iodosulfuron mixture against confirmed ALS target site resistant *S. media* was unexpected: the mixture proved to be significantly more effective than iodosulfuron alone, and markedly more effective than metsulfuron against the Pro-197-Gln resistant SCOT biotype. Unpredictable cross-resistance patterns to different ALS inhibiting chemistry were demonstrated using ALS formulations intended for grass weed control and a wide range of applied doses. Target site changes at P197 are usually assumed to confer resistance to sulfonylureas but not to imidazolinones, while those at W574 are thought to confer broader spectrum resistance including sulfonylureas, imidazolinones, triazolopyrimidines and sulfonylaminocarbonyltriazolinones (Tranel and Wright, 2002). In our studies resistance was shown to be dependent on dose and specific active ingredient with higher doses overcoming resistance in both P197 and W574 resistant biotypes using both sulfonylurea and imidazolinone chemistry. Different levels of control were also observed at field rates in glasshouse pots and outdoor containers for some herbicides, emphasising the importance of realistic field of simulated field conditions in assessments of herbicide efficacy.

In practical terms this work illustrates that effective control of confirmed P197 resistant *S. media* is possible by switching to triazolopyrimidine chemistry (e.g. florasulam), by applying a more active sulfonylurea formulation (for example mesosulfuron + iodosulfuron) where this is possible, or by switching to non-ALS herbicides such as fluroxypyr or mecoprop-P. Control of W574 resistant biotypes is more challenging and is best tackled by switching to a non-ALS herbicide.

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INTERACTIONS BETWEEN BARLEY CULTIVARS AND SOIL TILLAGE - EFFECTS ON YIELD AND DISEASE

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Summary: Four winter barley cultivars, selected by different rooting characteristics, and their mixtures were trialled under five tillage techniques (zero and minimum tillage, plough, deep plough, and plough with compaction). Mixtures gave yield increases and disease reduction in most treatments, demonstrating their practicality in a wide range of farming systems. A further 120 cultivars were trialled subsequently showing differential adaptation to the tillage treatments. Sub-sets were trialled again to determine the traits responsible for these performance differences.

INTRODUCTION

There have been several comparisons of yield performance of cereals under different tillage regimes and disease incidence has been monitored for some of these. Such research is vital to understand crop responses to modern farming practices, such as the shift towards reduced- or zero-tillage (Cannell & Hawes 1994). Most breeding trials are based on soils cultivated to at least 20 cm depth with a pass from both plough and harrow, but over 100 million ha of farmland worldwide is tilled using single pass minimum tillage implements to <10cm or direct-drilling (Verch *et al*., 2009). Less intense tillage changes the root environment, particularly mechanical impedance, aeration and plant available water in soil (Betz *et al*., 1998). Plant traits that respond well to these conditions may be different than suggested by breeding trials using intensive tillage. For instance, reduced tillage preserves continuous macro- and bio-pores in soil, which some cereal cultivars can exploit better than others (McKenzie *et al*., 2009). Another major difference between ploughing and reduced tillage is the distribution of plant debris on the soil surface and in the topsoil (Wang & Dalal, 2006).

Mixtures of cultivars have been grown for many years but they have only found widespread acceptance in a few markets. In Poland they are grown extensively (Gacek *et al*., 1996), primarily because their enhanced stability characteristics are particularly valued. Their yield and disease control benefits, especially for winter barley, have been demonstrated in the UK (Newton *et al*., 2009), and they are being increasingly grown for winter feed and distilling quality crops. There is also a perception that mixtures are particularly appropriate for low-input and organic systems. Whilst the disease reduction characteristic is particularly desirable in such systems where other options are restricted, there is clear evidence that mixtures perform particularly well in intensive systems (Finckh *et al*., 2000, Newton *et al*., 2009). In this paper we report experiments designed to determine whether both varieties and mixtures perform similarly with different tillage.

MATERIALS AND METHODS

Cultivars were selected for the tillage method trial on the basis of seedling rooting trait differences. A range of winter barley varieties were characterised for total root length, average longest root, average shoot length, and average root angle. Together with UK Recommended List of Cereals published variety characteristics of height, standing power, earliness, yield sensitivity, powdery mildew resistance and rhynchosporium resistance, four cultivars, Sumo, Pastoral, Fanfare and Pipkin, were selected with contrasting traits. Following uniform cultivation and a spring barley crop, 15 blocks measuring 33 x 33m were marked out in an even grid with five blocks in each of three north-south columns representing the three treatment replicates.

Five tillage treatments were established in autumn 2003 that imposed different levels of soil disturbance: Zero-Tillage (Zero), Minimum Tillage to 7 cm depth (Minimum), and ploughed treatments followed by power harrowing consisting of Conventional plough to 20 cm depth (Conventional), plough to 20 cm followed by Compaction by wheeling the entire plot with a Massey Ferguson 6270 tractor fitted with 16.9R-38 rear tyres (Compact) and Deep Plough to 40 cm depth (Deep). These treatments were selected to provide different physical constraints to root impedance and water availability. Blocks were separated from each other by 3m wide strips which were sown with grass seed after the first trial year was sown. Within each of the 15 blocks a split block trial was sown.

There were 15 trial entries comprising the four monocultures, all six two-component, all four three-component and the four component mixture in equal proportions adjusted by thousand grain weights. Plots measured 1.55m wide by 6.0m long and were sown at 360 seed/m^2 using an eight-row 'Hege' plot drill with five plots per bed. Three replicates and two fertiliser levels were used, n1 = half rate and n2 full rate applied as two top dressings in April and May. The trial was sown in five successive years and whilst the randomisations were different for every block, identical randomisations were used each year so that the same monoculture or mixture was sown in the same place every year.

In the following (sixth) trial year, 64 winter barley and 56 spring barley cultivars were trialled under the same conditions, except that only the full nitrogen rate was used and plots measured only 2.0m long. A sub-set of 20 winter and 10 spring cultivars was trialled using 6m plots again in the subsequent year. Diseases were scored on a 1-9 scale where 1 was susceptible and 9 had no undiseased leaf, converted to percentage, the Area Under the Disease Progress Curve (AUDPC) calculated and analysed by ANOVA. Plots were harvested when ripe, grain was dried to 9% moisture and weighed. Contrasts were calculated to compare mixtures with monoculture means. Bulk density in each of the 15 blocks was measured just after harvest on triplicate 56 mm diameter x 40 mm height cores taken from 2-6 cm depth.

RESULTS

Over the five years of this experiment, differences in the bulk density of the seedbed soil (2-7 cm) developed (Table 1). There were also evident differences from a visual assessment of soil structure, with plough pans evident at 7 cm in Minimum Tillage, 20 cm under plough and plough-compaction and at 40 cm under deep plough (per. comms. Bruce Ball). The depth of soil disturbance also influenced microbial communities (data not shown).

Table 1. Bulk density of the seedbed after harvest (mean ± s.e.).

Year	Zero Tillage	Minimum Tillage	Plough	Compaction	Deep Plough	*P*
2004	1.32 ± 0.03[a]	1.30± 0.02[a]	1.32± 0.01[a]	1.33± 0.02[a]	1.33± 0.02[a]	n.s.
2008	1.34 ± 0.05	1.25 ± 0.02	1.22 ± 0.05	1.27 ± 0.08	1.32 ± 0.07	<0.001

In the first two years, levels of rhynchosporium were moderate but in subsequent years all disease was very low. Yields were similar for the three high soil disturbance treatments (plough, plough-compaction and deep-plough) but declined over the years for the low disturbance treatments (zero- and minimum-tillage). Differences between the performance of the five cultivars for yield were small but changed in ranking in response to the treatments as the soil conditions developed (Figure 1). The mixtures, represented by the 4-component mixture in Figure 1, showed similar responses. The mixtures showed substantial reduction in rhynchosporium infection compared with the mean of the monocultures, and in proportion to their complexity. The reductions were similar for all soil tillage treatments (Figure 2).

In the subsequent trial of cultivars using mature soil tillage conditions four years later, about two thirds of the winter barley cultivars showed significantly different yield responses with a trend towards higher yield under high soil disturbance being correlated with poorer yield under low soil disturbance (Figure 3). With a few exceptions, these differences were confirmed in the subsequent trial with larger plots and increased replication. Amongst the spring barley cultivars, fewer showed differences in response to soil tillage treatment (data not shown).

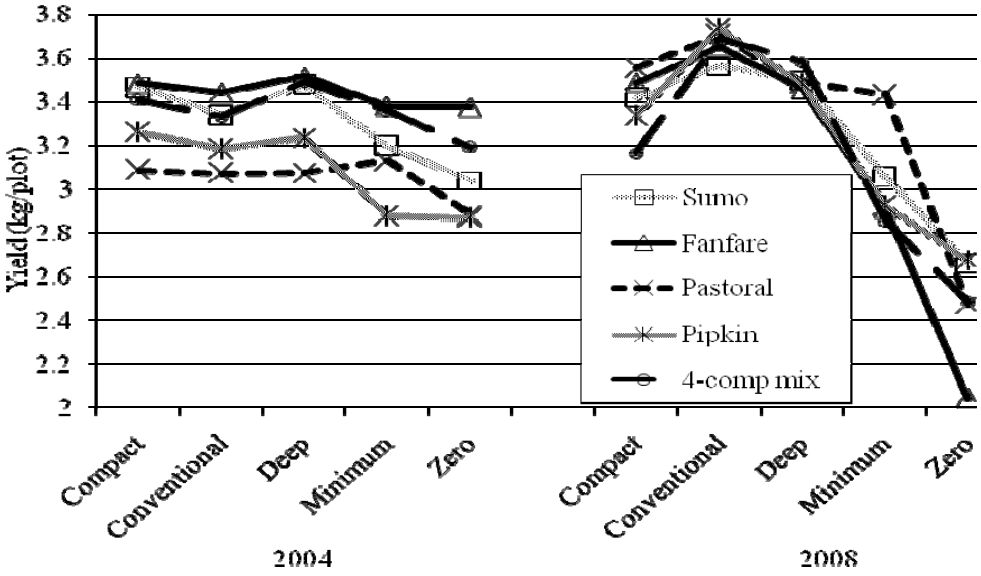

Figure 1. Cultivar ranking changes between 2004 and 2008 in response to soil tillage conditions. LSD = 0.402

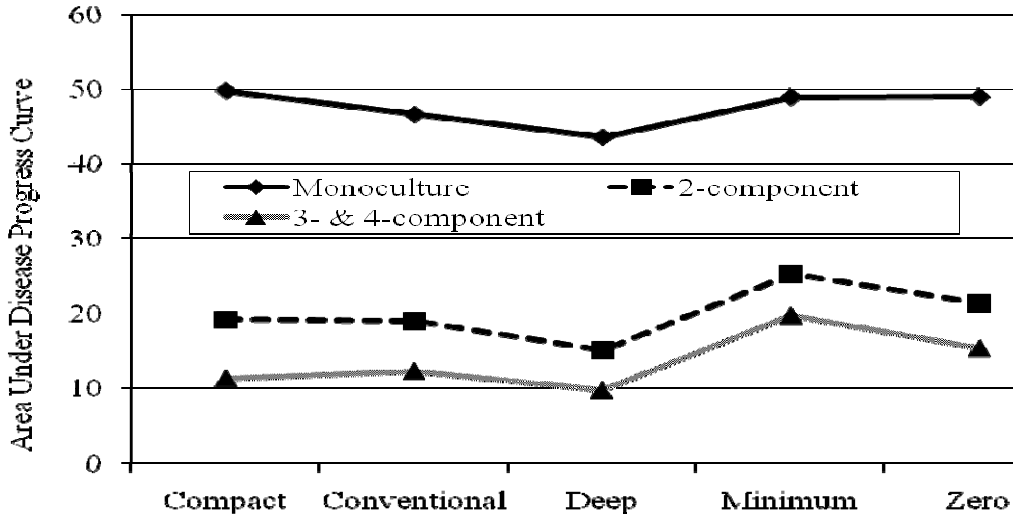

Figure 2. Comparison of the efficacy of mixtures of different complexity under different soil disturbance conditions.

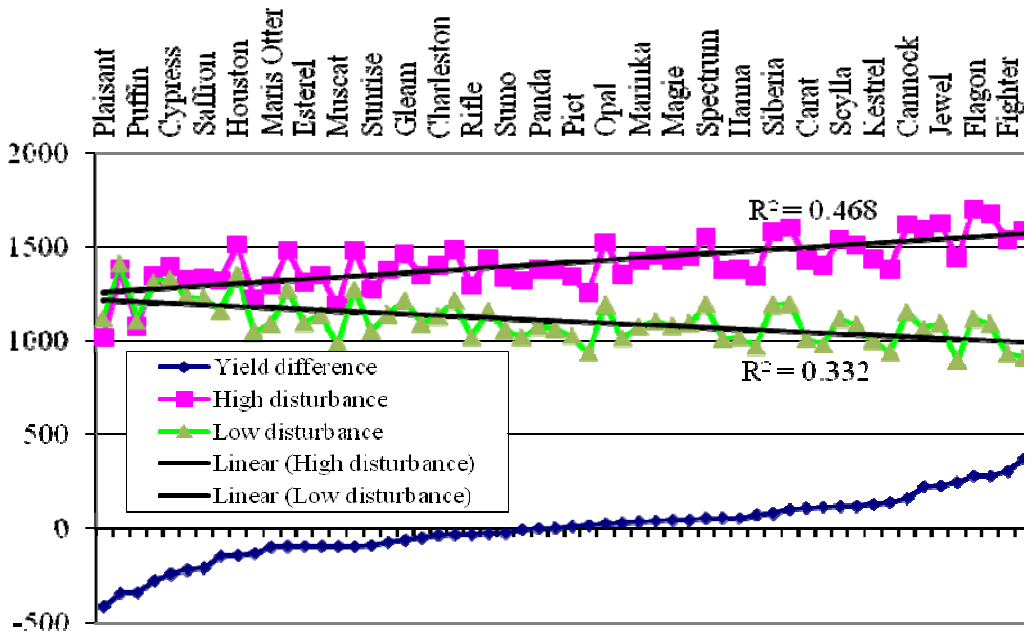

Figure 3. Yield of 64 (half labelled) winter barley cultivars ranked by difference in yield between performance under low and high soil disturbance conditions. LSD = 374

DISCUSSION

Over the five year period of this experiment, constraints to rooting and soil microbial structure followed trends anticipated for the different depths of soil tillage. There were also some differential responses amongst the four cultivars used in their yield response to treatment. For example, Fanfare performance decreased under zero tillage. Mixtures showed small significant increases compared with monoculture means (~2-4% - data not shown) and substantial disease reductions (~60-80%). However, there was no significant response to soil disturbance or nitrogen x soil disturbance treatment in mixtures efficacy for either yield or disease reduction, performing equally well under all treatments. No correlations between rooting characteristic differences and cultivation treatments were observed yet.

Grouping soil disturbance into low (zero- and minimum tillage) and high (plough, deep plough, and plough-compaction) classes produced interesting trends in yield response for a wide range of winter barley cultivars. The best yielding varieties under high disturbance, a tillage regime typical of breeding trials, had a tendency to be the worst yielding varieties under reduced tillage (Figure 3). These data demonstrate the massive impact of the soil environment on cultivar response and suggest that breeding trials need to incorporate and appreciate the impact of reduced tillage systems. In 21st century agricultural production, reduced tillage is increasingly common, so basing variety selection on trials from ploughed soils may not identify the best performers for reduced tillage.

Overall we can conclude that mixtures offer advantages for growing winter barley and are suited to a wide range of farming conditions including intensive systems. The advantages are in

terms of yield increase, disease reduction and general crop stability and resilience. Under mature differences in soil cultivation conditions, some genotypes are unsuited to low disturbance conditions in particular, and these tend to be the better-performing cultivars under high disturbance conditions.

ACKNOWLEDGEMENTS

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EFFECT OF MECHANICAL TINING ON WEED CONTROL IN ORGANIC SPRING BARLEY AND OATS IN NORTHERN IRELAND

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Summary: Weed growth in organic cereals is probably the main factor limiting their production in Northern Ireland. A three-year study of mechanical tining in organic spring barley and oats using two models of tiner, on farmers' crops indicated that, although numbers of both broad-leaved and grass weed were decreased with the best treatment by around 30% when assessed about 6 weeks after tining, that effect was reduced by the time of the second assessment around 4 weeks later. There was neither a significant reduction nor an increase in the biomass of crop heads, although there were indications of greater crop stem weights following tining. In a second three-year study with the same two tiners, two tining dates and two spring barley cultivars, earlier tining generally proved more effective in weed removal as measured at the first assessment, although any effects had disappeared by the time of the second assessment. Tining had no overall effect on crop biomass or yield, but did reduce numbers of crop plants and dry weights of stems in one year. There were no clear-cut effects of tiner type or crop variety. Individual weed species that responded to tining were broad-leaved dock and white clover.

INTRODUCTION

Organic cereal production in Northern Ireland is a relatively small-scale undertaking, but could be considerably expanded to serve the organic milk and beef industries. However, although conventional cereals grow well in N. Ireland and can give high yields, growing them organically can be problematic, particularly where weed control is concerned. Preliminary studies on weed control in spring barley, using mechanical tiners, were reported previously (Mercer and Morgan, 2008) and suggested that, although some measure of weed control could be achieved, there was little improvement in grain yield. However, because of a lack of suitable alternatives, a three-year study was set up in 2007, using farmers' fields and a research station site, to look at the effectiveness of two models of tiner, timing of treatment, and crop variety on weed removal and crop and grain yield.

MATERIALS AND METHODS

Trials were carried out from 2007 - 2009 on the farms of organic cereal producers and on a 70 ha organic farmlet attached to Greenmount Agricultural College in Co. Antrim. Two models of tiner were used, both designed to be towed by a quad-bike. The smaller model was a spring tine harrow (Model DTC, SCH Supplies Ltd., Ipswich, UK) in which numbers of tines could be varied, as could the ground pressure by adjusting the volume of water on an onboard tank. Preliminary work (Mercer & Morgan, 2008) indicated optimum results with a full set of tines and full water tank towed at a speed of ca. 4 km/h and given a single pass of the crop.

The second tiner was a larger spring tine machine (Logic LSH 150/200 super harrow). In 2007 and 2008, five farmers' fields were used and in 2009, four. Crops were confined to either spring barley or spring oats (2007 and 2008 – 2 barley, 3 oats; 2009 – 3 barley, 1 oats). There were three unreplicated treatments – small tiner, large tiner and untined, applied on a dry day as close as possible to two-leaf emergence of the crop, around three weeks after sowing.

At Greenmount Agricultural College, a single trial in each of the years 2007- 2009 compared the two tiner models at two tining dates ("early" – shortly after two-leaf emergence and "late" about two weeks later) on two varieties of spring barley, Optic and Riviera. As for the growers' trials, tining was carried out on a dry day. There were five replicates.

Crops at all sites were assessed for tiller, grass and broad-leaved weed numbers at the beginning of July and end of July/beginning of August and samples were taken using quadrats in early/mid-August for crop and weed biomass. The Greenmount trials were also combined and yields and grain weights estimated.

RESULTS

Growers' trials

When growers' trials were analysed either each year or all years together, numbers of both broad-leaved and grass weeds at the first assessment were generally lower following tining, while numbers of crop plants were broadly similar. However, the only statistically significant effect was a reduction in broad-leaved weeds and a close to significant reduction (P = 0.059) in grass weeds following treatment in 2008. However, when results for the same treatments from the Greenmount trials (*i.e.* two sets of barley plots) were added to those of the growers, overall

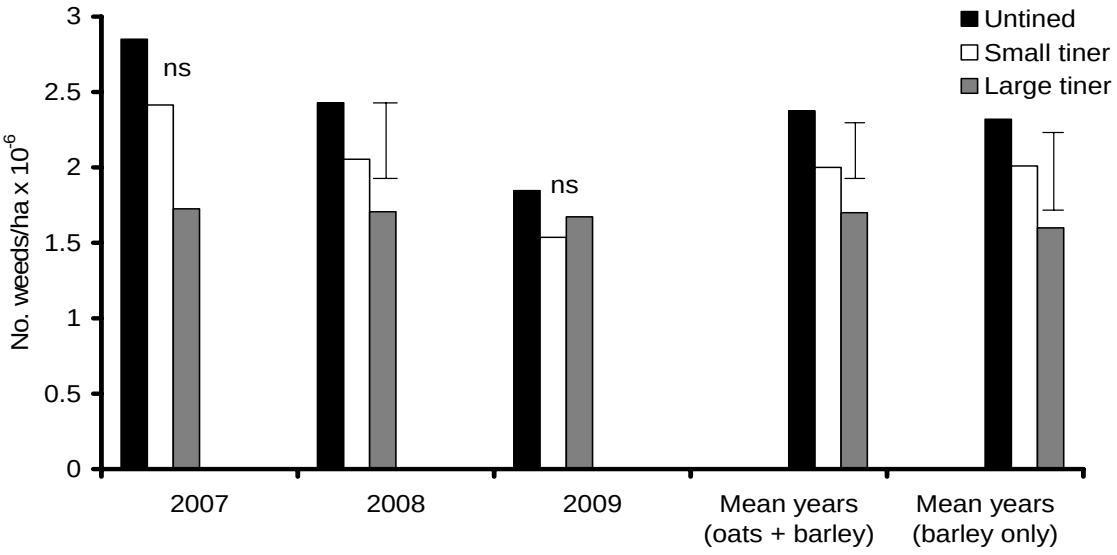

Figure 1. Effect of tining on total number of broad-leaved weeds at first assessment, (oats and barley combined), 2007 – 09. L.s.d. bar at 5% probability.

significance was achieved for broad-leaved and grass weeds, both when analysis was carried out on oats and barley combined and on barley alone, but not on oats alone. Broad-leaved weeds (Fig. 1) were significantly reduced by both tiner models, 28% and 13% for the large and

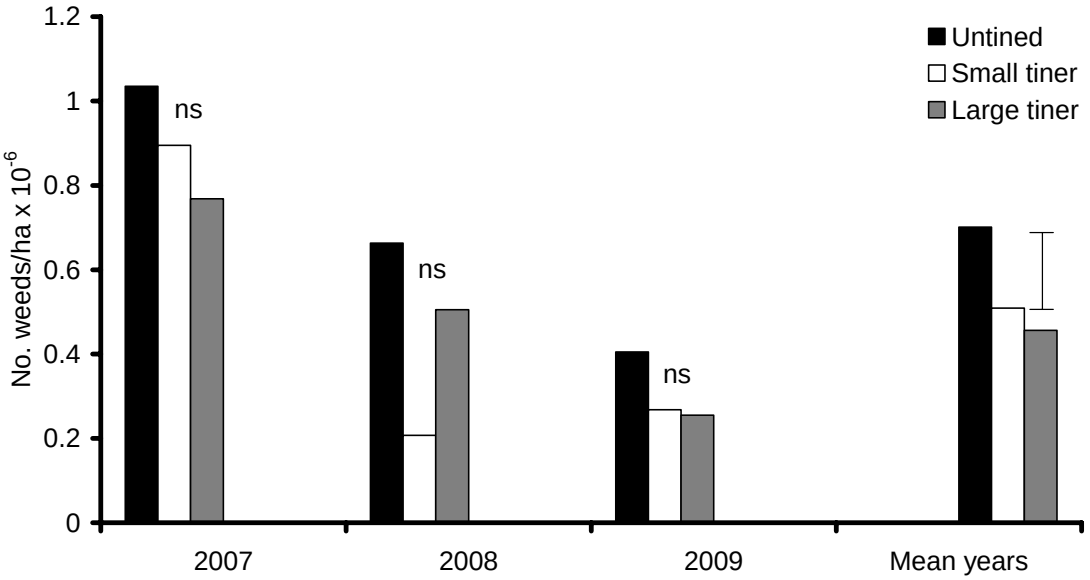

Figure 2. Effect of tining on total number of grass weeds at first assessment (oats and barley combined), 2007 – 09. L.s.d. bar at 5% probability.

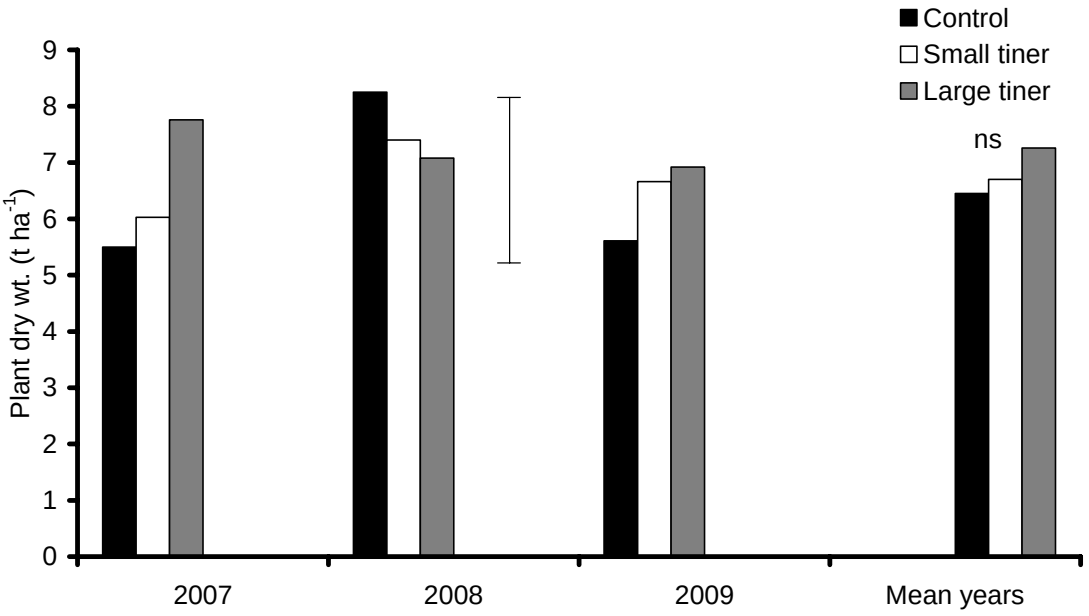

Figure 3. Effect of tining on total dry weight of crop stems in biomass (oats and barley combined), 2007 – 09. L.s.d bar at 5% probability for comparison amongst years and treatments.

small tiners respectively (oats and barley combined). Grass weeds were reduced significantly by both tiners (Fig. 2) – 35% for the large tiner and 27% for the small tiner. When crops were assessed a second time about a month later, although trends were similar to those at the first assessment, there was no longer any statistical significance.

When crop and weed biomass were analysed towards the end of August, there was no effect of treatment on the weight of crop heads. However, there was an indication of an effect on the weight of crop stems (Fig. 3), although this varied significantly between the years, being higher with treatment in 2007 and 2009 and lower in 2008. There was also a significant reduction in broad-leaved, although not grass weed biomass (Fig. 4) with the large tiner, although not with the small tiner.

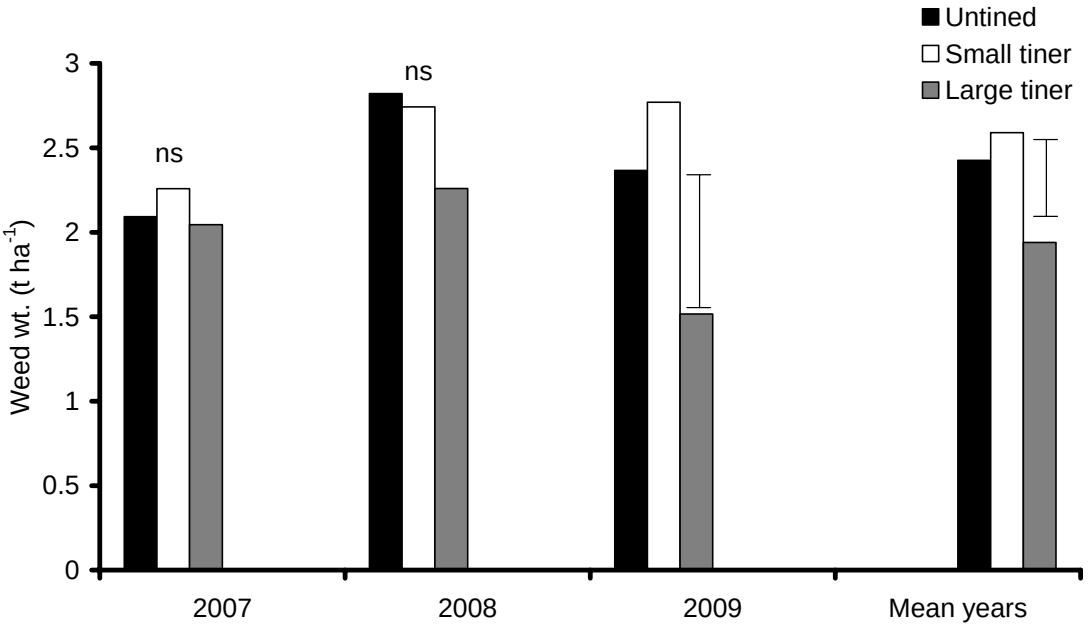

Figure 4. Effect of tining on total wt. of broad-leaved weeds in biomass, (oats and barley combined), 2007 – 09. L.s.d. bar at 5% probability.

Greenmount Agricultural College trials

At the time of the first assessment of the Greenmount trials there was no effect on numbers of crop plants or grass weeds, but for broad-leaved weeds there was a significant interaction between the time of tining and the year (Fig. 5). On average, the earlier tining was more effective, particularly in 2008, when there was a reduction of 28%. At the time of the second assessment there were no significant effects. When crops were assessed for biomass at the end of August, there was no significant effect of tining on numbers of crop plants over the years, although late treatment almost significantly reduced the dry weight of crop stems (9.73 vs. 10.6 t ha^{-1} (l.s.d. 1.02)) and there was a significant reduction in numbers of crop plants in 2008 with early tining (5.69 vs. 6.92 x 10^6 ha^{-1} (l.s.d. 1.13)). There was no significant effect on the dry weights of grass or broad-leaved weeds. When crops were harvested there was no overall

effect over the years on grain yield. There were no clear cut effects of tiner type or crop variety on any variate at any of the assessments or at harvesting.

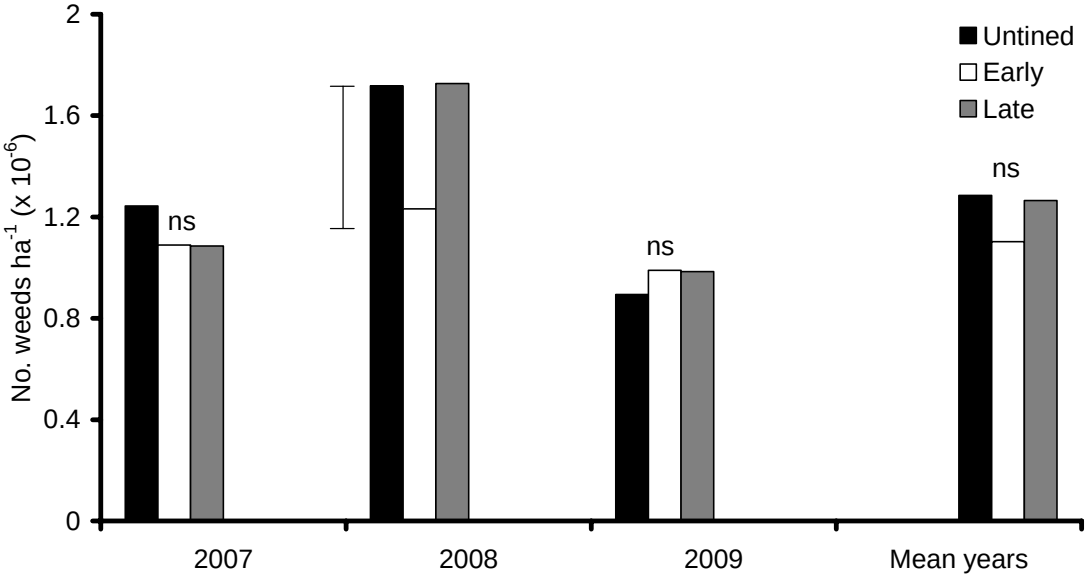

Figure 5. Effect of time of tining on total no. of broad-leaved weeds at first assessment, at Greenmount, 2007 – 09. L.s.d. bar at 5% probability.

Weed species varied between years, the most common being chickweed, broad-leaved dock, knotgrass, white clover, field bindweed, redshank and annual meadowgrass. Although there generally were no significant effects of treatments on individual weed species across the years, broad-leaved dock and white clover were significantly reduced by tining in 2008 - 8.6 vs. 20.8 x 104 ha-1 (l.s.d. 7.2) and 2.2 vs. 4.3 x 104 ha-1 (l.s.d. 1.7) respectively.

DISCUSSION

Although there was a clear reduction in broad-leaved and grass weeds in the growers' trials and a reduction in broad-leaved weeds in the Greenmount trials at the time of the first assessment, the overall effect was only of the order of 30% and any effects at the time of the second assessment, four weeks later, were much smaller. The effect on the crop itself was rather equivocal, occasionally appearing to increase biomass and, at other times, to reduce it, although overall the effect was broadly neutral. This contrasts with work by Lunkvist (2009) in Sweden on spring wheat and oats, where although much greater reductions in weed numbers (ca. 65%) were achieved with a combination of pre- and post-emergent tining, these treatments also resulted in reductions in crop yield of 12 – 14%. Leblanc and Cloutier (2004) in Canada also found a reduction in bread wheat yield (8%) following tine-harrowing at the two-leaf stage. Lunkvist makes the point, however, that even though cereal crop yields may be lower following tining, the subsequent reduction in numbers of weed seeds may benefit crops at other stages of the rotation. On the other hand, in the N. Ireland context, it is likely that reductions in weed number of more than the current 30% would be necessary before this would happen.

In the current work, there was considerable variation between years, which was probably due to a combination of weather, the place of the crop in the rotation and the main weed species. For example, the Greenmount trial in 2009 followed grass, was sown late and had a high infestation of broad-leaved dock which proved difficult to control. Earlier tining did appear to have an advantage, particularly in 2008, and it may be that pre-emergence tining could improve effectiveness further. However, weather is likely to be a limiting factor - Cirujeda and Taberner (2004) in Spain recommended 15 dry days after tining of winter cereals for the best control of field poppy and 15 consecutive dry days are relatively uncommon in N. Ireland. Wider row spacing and precision-guided weeders could be effective, but it is unlikely that they would be economic with the relatively small field size in N. Ireland. Another possibility is the use of minimum tillage, which would avoid the bringing up of weed seeds on ploughing, and this will be examined in the coming year.

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PRE-TREATMENT AND SEED RATE EFFECTS ON THE ESTABLISHMENT, GROWTH AND NUTRIENT CONTENT OF CALIENTE (A BIOFUMIGANT CROP) GROWN NEAR ARBROATH

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Summary: A number of companies are developing crop mixtures, predominantly of brassica origin, to use as more environmentally acceptable alternatives to chemical soil sterilisers in order to reduce disease and pest pressure on high value crops such as potatoes. Optimising the agronomy of the biofumigant crop, in order to obtain adequate establishment and maximise growth and active compound and nutrient contents of the crop prior to its incorporation, is an important element of the system. This paper quantifies several of these factors derived from a trial involving a Caliente crop established at three different seed rates on two pre-cropping treatments (potato or strawberry) in 2008.

INTRODUCTION

Soil-borne pests and diseases are particular problems of high value horticultural crops. In the past, synthetic fumigants (e.g. methyl bromide) have been used to sterilise soils in attempt to limit such problems, but recent legislative changes are severely reducing the control options available to growers. Environmentally acceptable, but effective methods of control would be welcomed by growers of such crops. Investigations of the beneficial effects that plant extracts and crop residues may be able to play in this role have been undertaken in recent years. One of these approaches is 'biofumigation'. Angus (1994) and Kirkegaard & Sawar (1998) defined biofimigation as the pest suppressive action of decomposing Brassica tissues. Hiladago-Diaz & Kerry (2007) expanded this definition to include decomposition of animal and a wider range of plant residues, not just Brassicas, in this role. Bello *et al*. (2002) broadened the definition still further to include the action these volatile substances had on weeds as well as pests and diseases.

For the purposes of this paper, the role of brassica biofumigant crops will be described in more detail. Brassicas are known to produce glucosinolates, stable sulphur compounds stored in the plant cell vacuoles (Brown & Morra, 1997). Myrosinase, a group of hydrolytic enzymes is also produced by brassicas, but this is stored separately from the glucosinolates, although it moves freely in the cell cytoplasm (Rosa *et al*., 1997). The typical approach to activate the release of these compounds from the crop in the field would be to macerate the fresh material in situ and incorporate the residues into the soil, followed by rolling, preferably within 20 minutes of chopping to reduce loss of the biofumigant gases being released (Plant Solutions, 2009). The resulting damage to the crop material enables the myrosinase and glucosinolates to come into

contact with each other when a hydrolysis reaction can take place assuming moisture levels are sufficient. This reaction releases a variety of biologically active products which include isothyocyanates, organic cyanides, oxazolidinethiones and ionic thiocyanates (Brown & Morra, 1997).

Of course, for any of this to work in practice, the crop needs to be established and grown such that it can produce sufficient biomass with adequate concentrations of these active components present in its tissues. The biofumigant crop also needs to slot efficiently into the rotation in terms of (a), allowing adequate growth of the biofumigant crop prior to incorporation, and (b) its position in the rotation where the biofumigation activity can be maximised in order to reduce the target organism load. This paper describes results from a field trial which compared aspects of establishment and growth of a Caliente crop (a biofumigant mustard blend) sown after either strawberries or potatoes at three sowing densities.

MATERIALS AND METHODS

A Caliente crop (Brand 99; Plant Solutions Ltd) was established towards the end of May 2008 in a field near Arbroath, Scotland, that had been used to grow potatoes on one half and strawberries on the other half during the previous season. Three different sowing densities (8kg, 10kg and 15kg ha^{-1}) of the Caliente were used on the two areas which had previously been under either strawberries or potatoes. The area was ploughed and harrowed prior to sowing, at a drill width of 12.5cm and maximum depth of 10mm prior to rolling. Fertiliser was applied a few days after sowing the crop at 120kg N ha^{-1} and 20kg S ha^{-1}. No other management practices were undertaken until the Caliente crop was ready for chopping and incorporating in the autumn. A series of measurements were made on the 26th August 2008, just prior to incorporation. Plant counts were undertaken at three random points within each treatment using a 50cm x 50cm quadrat. Plant heights were measured at three random points within each treatment. Biomass (total) was taken from the three quadrats per treatment used during the plant count assessment, with fresh weight and dry weights being measured for each of these. The Caliente material sampled was analysed for its nutrient content, in order to give an estimate of the green manure potential of the crop, in addition to its main purpose as a biofumigant crop, although this latter role was not investigated in the present paper.

Experimental design and statistical analysis

The treatments within the trial were un-replicated. This was a relatively small, on-farm experiment undertaken by the farmer, with guidance from Plant Solutions Ltd, who wanted to get an approximate measure of the crop in terms of ease of production and its potential effectiveness at reducing disease and pest issues after growing high value, susceptible crops, in this case potatoes and strawberries. Technical guidance on the appropriate agronomy for Caliente was provided by Plant Solutions Ltd. In order to make some kind of estimate of treatment effects and the variation within each treatment three measurements / samples were taken within each of the treatments, with a general ANOVA used on the data sets.

RESULTS

The higher seed rate always produced a greater plant density than the lower seed rate treatments ($p \leq 0.05$) irrespective of the previous crop (either potatoes or strawberries). For each of the sowing rates treatments, establishment tended to be greater after potatoes compared to after strawberries, although this difference was not statistically significant (Figure 1).

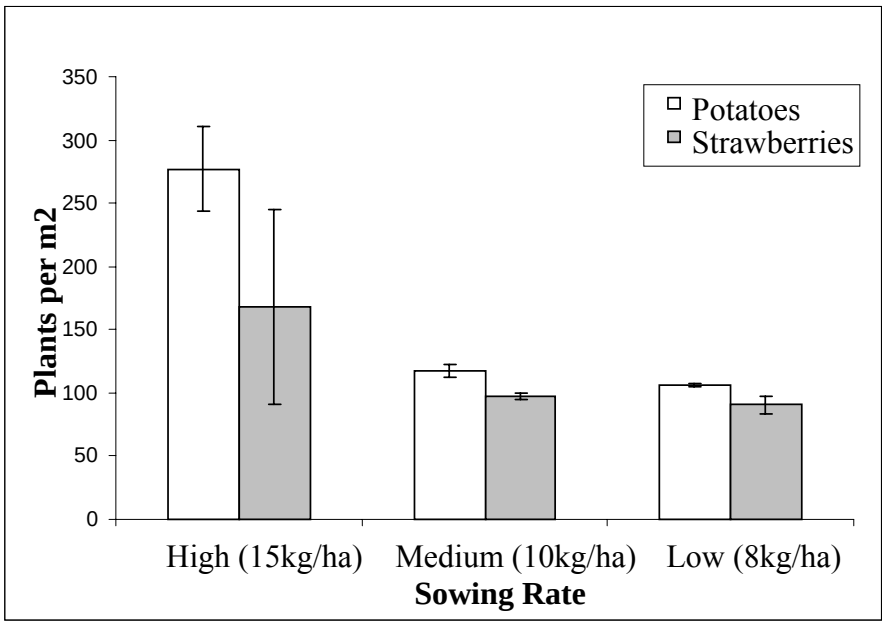

Figure 1. Plant density of Caliente established at three sowing rates on land used to grow either potatoes or strawberries in the previous season. Error bars represent SEM (n=3).

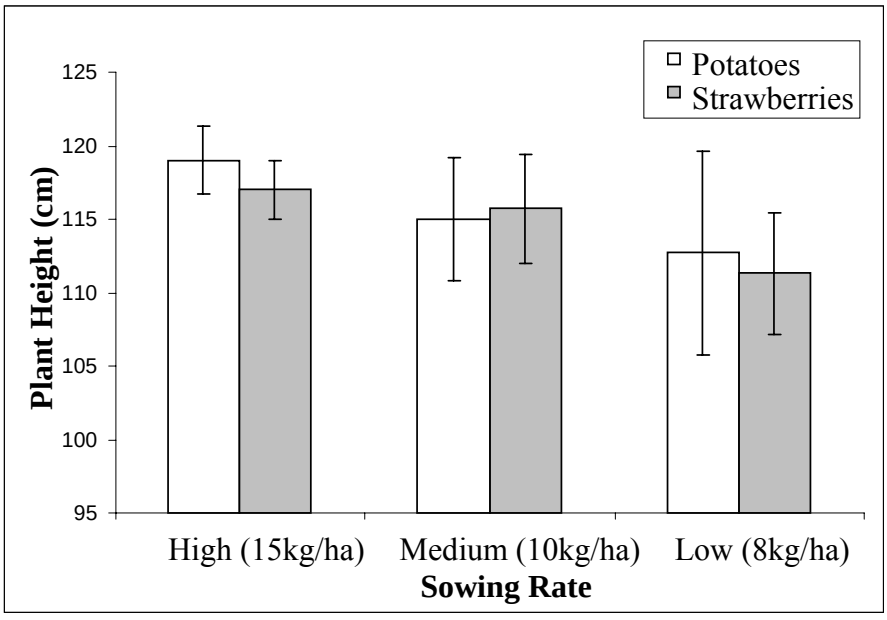

Figure 2. Plant height (just prior to chopping and incorporation) of Caliente established at three sowing rates on land used to grow either potatoes or strawberries in the previous season. Error bars represent SEM (n=3).

As sowing rate increased, plant height also tended to increase, although not significantly ($p \leq 0.05$). However, pre-cropping appeared to have little effect on plant height as there was no obvious difference between plant heights of the Caliente established at the same seed rate, but on ground which had previously grown either potatoes or strawberries (Figure 2).

Compared with the medium sowing rate treatment, biomass was significantly greater ($p \leq 0.05$) for the high and low sowing rate treatments, although no significant difference in biomass was observed between the two extreme sowing rates. Pre-crop treatment had no significant effect on biomass irrespective of sowing rate (Figure 3).

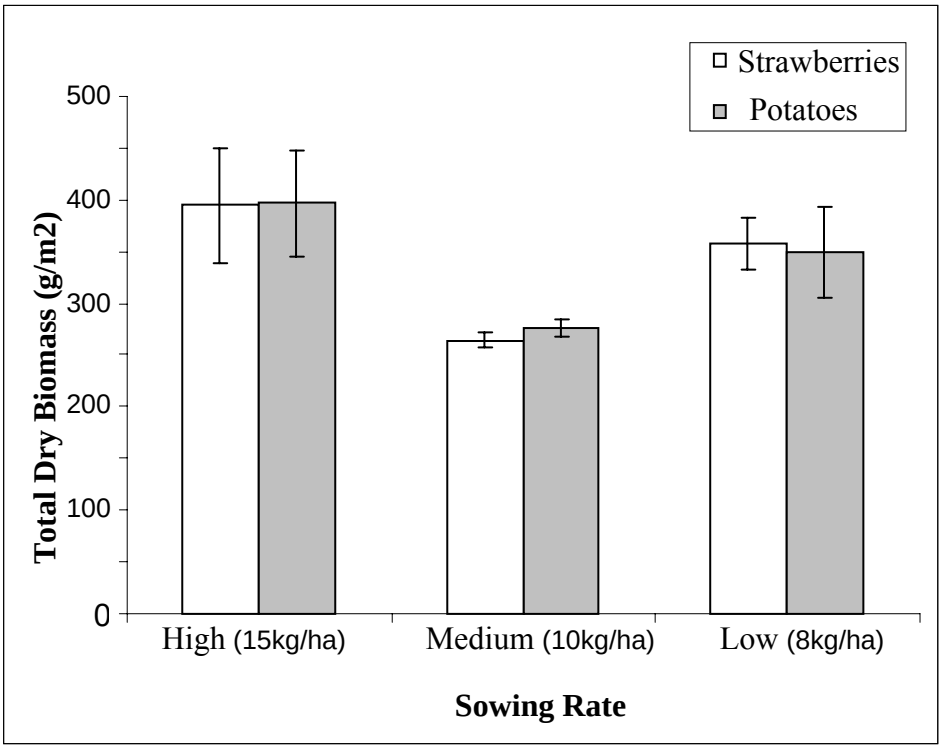

Figure 3. Total dry biomass (just prior to chopping and incorporation) of Caliente established at three sowing rates on land used to grow either potatoes or strawberries in the previous season. Error bars represent SEM (n=3).

Table 1 highlights the nutrient value of the crops, with this confirming that at least 75% of the N applied as fertiliser is taken up by the crop, and in the majority of treatments, this figure is closer to 90%. The major difference between treatments with regard to N uptake appeared to be that the medium sowing rate (10kg ha^{-1}) was by far the least efficient treatment, with this being the case for both the pre-treatments (either potatoes or strawberries). A similar trend was observed for the uptake of the other two major nutrients (P and K). There were no apparent differences observed between N, P and K uptakes at the two extreme sowing rates (8 and 15 kg ha^{-1}), with this true for both the potato and strawberry pre-crop treatments, although these observations were not tested statistically.

Table 1. Major nutrient (elemental N, P and K) contents of the Caliente biomass at sampling (just prior to chopping and incorporation). Figures in parentheses represent SEM (n=3).

	Potatoes (pre-crop)			Strawberries (pre-crop)		
Seed rate	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
High 15 kg ha^{-1}	120.7 (17.1)	13.4 (1.9)	175.2 (24.8)	120.4 (21.8)	13.0 (2.3)	185.8 (33.6)
Medium 10 kg ha^{-1}	80.9 (2.1)	8.2 (0.2)	115.6 (3.1)	74.6 (2.9)	8.8 (0.3)	103.6 (4.1)
Low 8 kg ha^{-1}	103.5 (7.4)	11.8 (0.8)	164.7 (11.8)	98.7 (9.8)	13.8 (1.4)	173.5 (17.2)

DISCUSSION

Higher seed rates would typically be expected to provide higher plant densities, as was the case here, with the highest seed rate providing approximately double the plant numbers of the two lower seed rate treatments. The pre-crop treatment results showed that establishment after potatoes, where large amounts of cultivation had taken place the previous year compared to the strawberries, as well as the impact of straw incorporation (on the strawberries) with its very high C:N ratio may have influenced these results. Soil structure will be damaged by large amounts of cultivation especially in wet conditions (The Scottish Government, 2005) and high C:N ratios of incorporated residues (e.g. straw) reduce initial N availability to the following crop (Silgram & Chambers, 2002) even if N fertiliser is applied. Both these factors had the potential to impact on establishment of the following crop.

Plant heights of the Caliente increased as sowing rate increased, a typical response (e.g. Blumethal *et al*, 1988), which can probably be associated with intraspecific competition (Begon *et al*., 1996) between the Caliente plants as they attempt to modify their growth in an order to improve light interception. The taller, 'leggier' plants of the high seed rate treatment (15 kg ha^{-1}) and the shorter, 'stockier' plants of the low seed rate treatment (8 kg ha^{-1}) produced similar biomass, with both treatments producing greater biomass than the medium sowing rate treatment (10 kg ha^{-1}). It is possible that the two extreme sowing rate treatments were able to access resources (primarily Photosynthetically Active Radiation) more effectively as a result of their contrasting growth habits compared to the middle sowing rate.

In addition to its potential as a biofumigant, the incorporation of the Caliente material will have a green manuring effect resulting in a range of nutrients being returned to the system. The treatments that produced the greatest biomass (the tall, 'leggy' high sowing rate treatment and the short, 'stocky' low seed rate treatment) both contained the greatest N, P and K content compared to the middle sowing rate treatment. Almost all of the applied N could be accounted for in the two extreme sowing rate treatments compared to only around 75% in the middle one. This can potentially be attributed to a number of reasons including the better combination of resource capture exhibited by the two extreme sowing rate treatments compared to the middle

one. In this instance, light (PAR) interception is a primary example, which is likely to influence both nutrient and water availability and uptake, through its influence on the growth rate and structure of the roots during the season (Hoad *et al*. 2004).

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BIOFUMIGANT CROPS AND THEIR POTENTIAL TO REDUCE NEMATODE PEST AND WEED PROBLEMS IN AN ORGANIC GLASSHOUSE CROPPING SYSTEM

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Summary: There is an increasing focus on more sustainable methods of agriculture, with reduced levels of chemical inputs (e.g. fertilisers and pesticides) frequently targeted in order to help achieve this. A range of alternative approaches to the management of these systems are likely to be required in order to overcome the challenges associated with these aims. One approach, more usually associated with control of soil-based plant pathogens, is biofumigation. This paper describes a pot experiment using four different biofumigation crops, with cucumber as a test crop, undertaken in an organically managed glasshouse system on the Mediterranean island of Crete. In this experiment, the potential for biofumigation to reduce nematode pest and weed control issues in similar systems was investigated. The results suggest specialist biofumigant mixtures, in particular, have the potential to act in this role.

INTRODUCTION

Soil-borne pests and diseases, in conjunction with weeds, are particular problems of vegetable crops which tend to be of high value. Typically, these issues have been controlled with synthetic fumigants (e.g. methyl bromide) and herbicides, although legislation changes are becoming increasingly strict, severely limiting the control options available to growers. Over recent years, the use of plant extracts and crop residues to fulfil this function, at least in part, in an environmentally acceptable manner has received wider interest from the agricultural and research community. The term 'biofumigation' can be used to describe these approaches and Kirkegaard & Sawar (1998) and Angus (1994) defined it as the pest suppressive action of decomposing Brassica tissues, with this later being expanded to include decomposition of animal and other plant residues in this role (Ciancio & Nukerji, 2007). In a slightly different definition, Carcia Alvarez *et al*. (2002) viewed biofumigation as the action of volatile substances from the biodegradation of organic matter as a fumigant to control plant pathogens and weeds.

The incorporation of biofumigant crops into the soil releases biocide substances which affect soil-borne pests and diseases and weeds (Sanders & Reyes, 2005). The majority of biofumigation research has concentrated on the effects of various Brassica crops on a range of crop plant pathogens. Brassicas are able to produce glucosinolates which are stored in the plant cell vacuoles where they remain stable (Brown & Morra, 1997). The plants also produce myrosinase, a group of hyrdrolytic enzymes which are stored separately from the glucosinolates, and moves freely in the cell cytoplasm (Rosa *et al*., 1997). The typical procedure to stimulate the release of the bioactive compounds from the biofumigant Brassica

crop is to chop up and incorporate the residues into the soil. This action damages the tissues, cells and vacuoles allowing the myrosinase and glucosinolates to come into contact and, in the presence of water, the hydrolysis reaction that takes place releases a variety of biologically active products. These include isothyocyanates, organic cyanides, oxazolidinethiones and ionic thiocyanates (Brown & Morra, 1997).

This paper concentrates on the effects of three Brassica biofumigant crops on weed germination / suppression and reduction in problems linked to the root knot nematode (*Meloidogyne* spp.), a particular problem for organic glasshouse growers producing tomatoes, peppers, aubergines and cucumbers in the Mediterranean. The three biofumigant crops used in the experiment were chosen based on local familiarity with the crop, potential effectiveness against nematodes and weeds (based on a literature review and discussions with local growers) and commercial availability of the crops.

MATERIALS AND METHODS

The experiment was undertaken over the winter period (2007-2008) in an organic grower's glasshouse in Crete. 5L pots with a surface diameter of 20cm and a depth of 20cm were each filled with 5kg semi-saturated soil. The soil used was from an area that had grown aubergines the previous season, and was known to have been infected by root cyst nematodes (evidenced by the plants' above ground appearance and nematode cysts on the roots of the plants when removed from the soil). Roots from the infected aubergines were also cut into small pieces (<2cm) and 80g of this material was incorporated into each of the pots in order to increase the nematode content as the roots contained eggs which could potentially develop into juveniles capable of infecting the plant roots. To confirm nematode infection of the soil, soil samples taken prior to the application of infected roots were analysed at the National Institute of Agricultural Research (Greece). After a two week incubation period in modified Baerman funnels, this pre-treatment soil was found to have 20 juveniles kg^{-1} soil. This independent laboratory analysis did not highlight estimates of error.

Biofumigant treatments

Five treatments were used: (1) *Raphanus sativus* (RS), (2) *Brassica juncea – Sinapis alba* mixture (MX), (3) *Sinapis nigra* (SN), (4) Oxamyl – a nematicide (N) and (5) Control – no input (C). Sowing rates were double the recommended rate, with thinning taking place in the weeks after germination until the plant density recommended by the seed supplier was attained. These were 6-7 plants pot^{-1} for RS, 24 plants pot^{-1} for MX (20 *Brassica juncea* and 4 *Sinapis alba*) and 27 plants pot^{-1} for SN. Control treatments (N and C) had no plants at this stage of the experiment. Pots were placed on a plastic sheet in order to prevent infection of healthy soil.

Nutrition

A drip fertigation system was installed which provided 4 L hr^{-1} of water as well as essential nutrients permitted for use under organic certification. Over the eight weeks that the biofumigant crops were growing, all pots received 12g of potassium and 6g of nitrogen through the fertigation system. Other nutrients were supplied from the soil reserves.

Procedures and measurements

An assessment of the weed species density in each pot was not made until four weeks after sowing; from this point onwards no changes were observed in weed density.

Eight weeks after sowing, the weeds were removed from the experimental system and the biofumigant crop in each pot was destroyed, cut into small pieces (<2cm) and re-incorporated back into the soil of the pot it was removed from. It was left in this condition for six weeks, with the only input to the system over this period being water applied at 1L hr^{-1} in order to aid the reaction between glucosinolates and myrosinase which requires moisture. Weed numbers per pot over this six week period were recorded.

Two weeks after the biofumigant crops were incorporated, cucumber seeds were propagated ready for transplanting into the treatment pots in a further four weeks time (i.e. six weeks after incorporation of the biofumigant material). The reason for using cucumber as a test crop is that they are highly sensitive to many plant parasitic nematodes, which in turn are a major pest of commercial cucumber growing operations.

For the control treatment using a nematicide, the oxamyl (DuPont) was applied to a depth of ~ 7-8cm, 3 days before transplanting in accordance with the recommendations of the pesticide company.

Cucumbers were grown for six weeks after transplanting, although not through to full maturity due to time constraints. At this point, plants were removed from the pots and the intensity of nematode infection was assessed using a root-knot rating chart (Bridge & Page, 1980).

Experimental design and statistical analysis

A randomised block design with five replicated blocks was used. Results were analysed using ANOVA to determine differences between treatment means at the 5% level (n=5).

RESULTS

During the eight weeks that the biofumigant crops were grown, they produced approximately 475g fresh, above ground biomass per pot, with no significant differences in biomass observed between treatments (Table 1). No biofumigant plants were sown in the two controls (N and C), and hence no biomass was produced in these treatments.

Weeds started to germinate two days after the biofumigant crops were sown. The first weeds to germinate were *Chenopodium murale*, whereas the last to germinate were the *Trifolium spp* and *Solanum nigra*. There was generally little difference between the species germinating and their populations between all treatments with the exception of the biofumigant mixture (MX). This treatment had exceptionally low populations of weeds developing during the initial eight weeks of the experiment when the biofumigant crops were being grown (Table 2). This treatment showed a minimum 90% reduction in weed numbers compared to the other treatments and appeared to be particularly effective against *Theligonum cynocrambe* and *Poa annua*. In all treatments, the weeds that did germinate exhibited vigorous growth, with rapid development.

Table 1. Above ground fresh biomass produced by each treatment (not including weeds) which was subsequently incorporated back into the soil after cutting into <2 cm pieces.

Treatment	Mean biomass (g fresh weight pot^{-1})
RS	484
MX	508
SN	438
N	0
C	0
l.s.d. (P≤0.05)	136.7

Table 2. Range and population of weeds during biofumigant crop growth. Number of weeds pot^{-1} eight weeks after the start of the experiment. SEM (n=5) is shown in parentheses.

Weeds*	Treatment				
	RS	MX	SN	N	C
CM	1.4 (0.75)	0.4 (0.40)	0.2 (0.20)	1.4 (0.68)	1.0 (0.45)
RF	1.2 (0.73)	0.0 (0.00)	1.2 (0.73)	2.2 (0.66)	1.8 (0.37)
S	0.6 (0.40)	0.2 (0.20)	0.0 (0.00)	0.8 (0.37)	0.0 (0.00)
TC	4.4 (1.47)	0.6 (0.40)	4.8 (1.24)	6.2 (1.69)	5.4 (0.51)
T	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.2 (0.20)	0.0 (0.00)
P	5.0 (2.24)	0.0 (0.00)	6.4 (3.08)	8.6 (2.38)	11.4 (1.57)
SM	0.4 (0.24)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
SN	0.4 (0.24)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
Total	13.4	1.2	12.6	19.0	20.0

*Weed list: (CM) *Chenopodium murale*; (SN) *Solanum nigra*; (RF) *Ranunculus ficaria*; (S) *Setaria spp*; (TC) *Theligonum cynocrambe*; (T) *Trifolium spp*; (P) *Poa spp*; (SM) *Stelaria media*

Table 3 shows the cumulative mean total number of weeds per pot for each treatment after the biofumigant crops had been incorporated. All the biofumigant treatments significantly reduced weed numbers compared to the controls (C and N), which had around three times as many weeds in them at this stage of the experiment.

Table 3. Mean weeds number in each treatment after incorporation of the biofumigant crops.

Treatment	Mean weed number per pot
RS	2.60
MX	2.00
SN	3.60
C	11.60
N	10.20
l.s.d. (P≤0.05)	2.20

The effect of the biofumigant treatments on the nematodes is shown in Table 4. The control without any inputs at all (C) showed significantly greater nematode damage on the roots, even at this relative early stage of growth for the cucumber test crop. All the biofumigant treatments had comparable nematode control with that shown by the nematicide treated control (N) at this stage of growth. There was some indication that the biofumigant mixture (MX) was the most effective nematode control, although this was only significant compared to the control (C).

Table 4. Nematode index of each treatment on a 0-10 scale, where 0 = no knots on roots and 10 = all roots severely knotted (after Bridge & Page, 1980).

Treatment	Nematode index (0-10)
RS	0.80
MX	0.20
SN	0.80
C	2.20
N	1.00
l.s.d. (P≤0.05)	0.98

DISCUSSION

The specialist biofumigant mixture (MX) proved to have several beneficial properties. It was the most effective weed suppressor, both during its eight week growing period as well as after its incorporation. This latter point agrees with elements of Carcia Alvarez *et al*. (2002) and suggests that as its residues broke down, they released antagonistic compounds that affected the germination and growth of several weed species, although its effects were more dominant for the *T. cynocrambe* and *P. annua* species highlighted earlier. In addition to this, MX appeared to reduce root-knot nematode damage to a level comparable with the other biofumigant treatments and, perhaps more importantly, the nematicide treatment. This is of particular relevance given that growers face increasing restrictions on pesticide use and the biofumigant approach outlined here has potential for both organic and non-organic growers. It must be acknowledged that this experiment was undertaken in a glasshouse, with fertigation / irrigation. This enabled relatively good control of the soil / crop environment, compared to a true field situation where e.g. water relations and temperature, both important in the breakdown and release of biofumigant compounds (Brown & Morra, 1997; Rosa *et al.*, 1997) may be more difficult to optimise. However, the results are extremely encouraging as a possible option for reducing weed and root-knot nematode problems, as long as the establishment and breakdown phases of the biofumigant system can be suitably incorporated into the grower's rotation.

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THE INFLUENCE OF SPRAY APPLICATION VOLUME FOR VOLUNTEER POTATO CONTROL

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Summary: During the development of Galaxy herbicide, GF-1374 (100 g a.e./l fluroxypyr + 80 g a.e./l clopyralid + 2.5 g a.i./l florasulam) evidence of a possible response to spray application volume for *Solanum tuberosum* (Potato) control was observed. Five trials were established in 2008 to investigate the control of volunteer *S. tuberosum* by GF-1374 applied at 1.5 litres/ha at either 200 or 400 litres/ha application volume. Applied at 200 litres/ha, GF-1374 achieved 88.7% mean control. This increased to 93.2% when applied at 400 litres/ha.

INTRODUCTION

The inevitable consequence of growing potatoes *(S. tuberosum)* is the resulting occurrence of volunteers in the following crop. Volunteer potatoes result in yield losses through crop competition, act as a source of inoculum for potato late blight, and can be an aphid source for other crops on the farm. Therefore reducing the number of volunteers in the cereal crop is an important option and minimises cross contamination, particularly where seed crops are grown. GF-1374 herbicide, an EC formulation containing 100 g a.e./litre fluroxypyr + 80 g a.e./litre clopyralid + 2.5 g a.i./litre florasulam was introduced in 2008 (Harris, 2008) for use in winter and spring wheat and barley up to GS 37 and in winter wheat and spring oats up to GS 31 for the control of a range of broad-leaved weeds including *Galium aparine, Stellaria media, Matricaria spp.* and *Cirsium arvense*. A constituent of GF-1374, Clopyralid, has been shown to have activity against *S. tuberosum*, Five trials were conducted in 2006 and 2007 and showed GF-1374 to achieve 81% control of volunteer potatoes (Harris, 2008).

MATERIALS AND METHODS

Trial design

All trials utilized a randomised complete block design consisting of four replicates and a minimum plot size of 12m^2. Treatments were applied using precision small plot sprayers at 200 or 400 litres application volumes per hectare through flat fan nozzles using a medium spray quality.

Efficacy assessment

Weed control was assessed at given intervals after treatment and expressed as % control where 0% represents no kill and 100% represents total kill.

Application timing

Applications were made from 9th May to 27th June 2008 at crop GS 32-45. *S. tuberosum* growth stages at application were GS 12-18 (2 to 8 true leaves) with a height of 10-20 cm and a density of 13-18 plants m^{-2}

Table 1. Summary of application details for the five *S. tuberosum* trials

Treatment	Rate /ha	Application volume (litres/ha)	Crop growth stage	Weed growth stage
GF-1374	1.5 L	200	GS 32-45	GS 12-18
GF-1374	1.5 L	400		
fluroxypyr + metsulfuron-methyl	200 g a.e./ha + 4 g a.i./ha	200		
fluroxypyr	400 g a.e./ha	200		

RESULTS

Figure 1 summarises control from the five *S. tuberosum* trials conducted in 2008 where GF-1374 at 1.5 litres/ha was applied at an application volume of 200 or 400 litres/ha. The comparison treatments were 400 g a.e./ha fluroxypyr (fluro) or 200 g a.e./ha fluroxypyr (fluro) + 4 g a.i./ha metsulfuron-methyl (met). These treatments were applied using an application volume of 200 litres/ha. Due to trial limitations these treatments were not applied at 400 litres/ha.

Of the three treatments applied at 200 litres/ha, GF-1374 at 1.5 litres/ha achieved the highest control at 88.7% control. This was significantly higher control than 200 g a.e./ha fluroxypyr + 4 g a.i./ha metsulfuron-methyl (79.7%) (LSD 1.99, p = 0.05). Fluroxypyr at 400 g a.e./ha recorded 86.2% control.

Comparing the control of *S. tuberosum* when treated with GF-1374 at 200 or 400 litres/ha, a 4.5% improvement in control was observed (88.7% vs 93.2% respectively). Analysis of the replicate data showed a greater degree of consistency in the data from the 400 litre/ha volume, with data ranging from 85-99% compared to 60-99% for the 200 litres/ha rate.

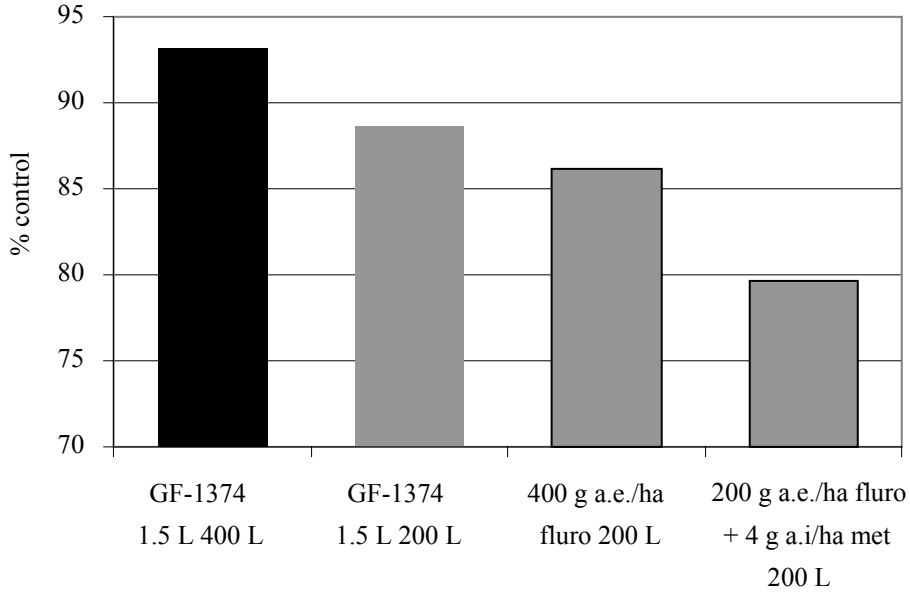

Figure 1. The effect of application volume on *S. tuberosum* control by GF-1374 (mean of 5 trials, LSD 1.99, p = 0.05)

The higher water volume provided greater leaf coverage and improved penetration of the product through the plant canopy. At 200 litres/ha insufficient penetration through the crop canopy can markedly affect control of volunteer potatoes. Leaf physiology may have prevented adequate coverage at the lower application volume due to beading of the spray droplets on the leaf hairs.

DISCUSSION

As growers have sought to increase productivity there has been a trend to reduce the application volume of spray solutions. In many circumstances this does not have deleterious effect, however where crop canopies are dense it has always been recognised that higher application volumes are required. GF-1374 at 1.5 litres/ha was the most efficacious of the tested treatments for the control of *S. tuberosum* achieving 88.7% control at 200 litres/ha application volume, significantly higher than the comparison treatment of 200 g a.e./ha fluroxypyr + 4 g a.i./ha metsulfuron-methyl (79.7%). Increasing the water volume from 200 to 400 litres/ha demonstrated a small response with control from GF-1374 at 1.5 litres/ha increasing from 88.7% to 93.2%.

The data demonstrated that where growers have a volunteer potato problem in winter or spring cereals, GF-1374 provided an effective solution at 200 litres/ha and increasing the application volume to 400 litres/ha delivered improved control. It was concluded that GF-1374 provides an effective solution for the control of *S. tuberosum* and in circumstances of a dense crop canopy increasing the water volume to 400 litres/ha brings improved control.

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NEW PEST RISKS FOR POTATO

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Summary: New pests that may present a risk to potatoes in Northern Britain arise regularly as a result of factors such as: international trade in potato and other members of the Solanaceae; the potential for polyphagous pests to transfer from other hosts; and population increases and natural spread of pests through changes in climate. Some new potential pest risks to potato are described including *Epitrix similis*, pospiviroids and *Candidatus* Liberibacter psyllaurous (syn *Ca.* L. solanacearum).

INTRODUCTION

Many pests affect potatoes and as a result potato production is highly regulated. There are a number of pathways by which new pests of potato can be introduced, including import of potatoes for planting, import of ware potatoes for the fresh or processing market, contact with contaminated containers or conveyances, during the production of protected crops and as a result of cultivation of new crops (Chard & Jeffries, 2006). The aim of this paper is to provide information on some pests presenting potential risks, which have arisen over the last three years.

LEGISLATION

A number of highly damaging pests of potato are listed as quarantine organisms in the Plant Health Directive (PHD, 2000/29/EC) and there are European Community (EC) control directives for four of them (*Clavibacter michiganensis* subsp. *sepedonicus* (ring rot), *Globodera rostochiensis* and *G. pallida* (potato cyst nematodes, PCN), *Ralstonia solanacearum* (brown rot) and *Synchytrium endobioticum* (wart disease).

In assessing whether new pests or increasing findings of existing pests present risks to potato production in Northern Britain, it is important to consider the regulatory context and the patterns of trade (pathways for potential introduction). Import of potatoes for planting from outside the European Union is prohibited, apart from through potato quarantine or under a specific derogation from Canada. Ware potatoes are permitted from a number of countries in the Mediterranean region, mainly to satisfy early potato requirements. Plants for planting of Solanaceae are also prohibited from outside of the European and Mediterranean region, although seeds (apart from true potato seed) are permitted and there are specific requirements for tomato (*Lycopersicon esculentum*). Fruit of Solanaceae e.g. tomatoes and aubergines (*Solanum melongena*) are permitted from anywhere in the world.

In the last three years, new control measures for PCN (Council directive 2007/33/EC) were published and come into force on 1 July 2010 (Pickup 2008). Emergency legislation (Commission decision 2007/410/EC) was passed to address specific threats associated with findings of *Potato spindle tuber viroid* (PSTVd) in ornamental plants (see below for further detail). In addition, Commission directive 2009/7/EC added *Scrobipalpopsis solanivora* (syn. *Tecia solanivora,* Guatemalan potato moth) as a quarantine organism to Annex IIAI of the PHD. As well as being reported from Central and South America, this organism has been found in the Canary Islands.

Many pests and pathways for introduction of pests mentioned in the previous review (Chard & Jeffries 2006) remain, but there are some new organisms or reports of increasing prevalence of existing pests that could present new risks.

NEW PESTS IN EUROPEAN AND MEDITERRANEAN REGION

Epitrix similaris

Damage to potato tubers has been reported in Portugal since 2004 and the causal organism was confirmed as *E. similaris* in 2008 (Boavida 2009, EPPO, 2009a). The larvae cause corky lesions and superficial warty growths on potato tubers and the adults, small black beetles, cut holes in leaves. The main impact is the loss of commercial value of the tubers; damaged tubers are rejected. Yield does not appear to be affected. The organism has spread throughout Portugal rapidly; the adults are active fliers and it is possible that juvenile stages or adults may be spread with tubers or soil.

Tuta absoluta (tomato leaf miner)

Tuta absoluta was recommended by EPPO in 2004 for listing as a regulated pest. It is a serious pest of tomatoes, but potato is also reported as a host (EPPO 2005). It has recently caused outbreaks in tomatoes in a number of European countries, including in glasshouse tomato crops in England in 2009. The economic impact is greater for tomatoes than potatoes, because tubers are not attacked. It is considered a pest of warmer climates (the International Potato Centre classified it in 1999 as a serious foliage pest of potato in warm zones) (EPPO 2005), so it might not present a major risk to potato production in Northern Britain, unless the climate becomes warmer.

Tetranychus evansi (a red spider mite)

This red spider mite is a pest of tomatoes and solanaceous crops and was added to the EPPO Alert list in 2004 after it was found to be spreading in Mediterranean countries. It has been found in a number of European countries and a PRA by Defra in 2005 concluded that it was a risk to glasshouse crops in the UK. It has also been found in Asian and African countries, Southern US, Puerto Rico, the Virgin Islands, Brazil, Argentina and Hawaii. *Solanum nigrum* is a preferred host and it has been found on this host in Europe. It was recommended for regulation by EPPO in 2008 following a pest risk analysis (PRA) (EPPO 2008a). It is considered to be a threat to production in Mediterranean countries and glasshouse crops.

PESTS PRESENT IN THE EUROPEAN AND MEDITERRANEAN REGION

Potato spindle tuber viroid and related pospiviroids

Occasional outbreaks of PSTVd in potato and tomato had been reported in the EU in the early 2000's (Chard & Jeffries 2006), but findings of PSTVd in the Netherlands in two ornamental plants (*Brugmansia* spp. and *Solanum jasminoides*) led to the introduction of emergency EC legislation in 2007. This included a requirement for member states to undertake surveys of host plants in their territories. In addition to the hosts above, PSTVd was subsequently reported in *Petunia* sp., *S. muricatum, S. rantonnetti*, *Streptosolen jamesonii*, and recently in *Physalis peruviana* (Cape gooseberry) (de Hoop *et al.* 2008*,* Mertelik *et al.* 2009 and Verhoeven *et al.* 2009). Infected plants have been destroyed and efforts have been made to trace the sources of the infections and eradicate the organism. These findings have prompted questions on the significance of infected ornamental plants as a threat to tomato and potato production. A recent report of a finding of PSTVd in tomatoes close to infected *S. jasminoides* plants (EPPO 2009b) and the experimental transmission of pospiviroids to weeds (Matousek *et al.* 2007) increases the possibility that ornamentals and weeds may pathways for transmission.

In addition to PSTVd, there have been findings of related pospiviroids in ornamental plants including *Tomato chlorotic dwarf viroid* (James *et al*. 2008), *Columnea latent viroid, Citrus exocortis viroid* and *Tomato apical stunt viroid* (de Hoop *et al.* 2008). Severe outbreaks of *Columnea latent viroid* were found in tomato crops in the UK and elsewhere in 2007 (Nixon *et al.* 2009). Although none of these viroids has been found naturally infecting potato and they appear to present more of a risk to glasshouse grown tomato crops in Northern Britain, questions still remain.

A EUPHRESCO project is currently underway on the epidemiology and diagnosis of PSTVd and other pospiviroids to answer some of the questions arising from these findings (https://secure.csl.gov.uk/euphresco/public/calls/topic2.cfm). A PRA on tomato viroids will be co-ordinated by the European and Mediterranean Plant Protection Organization (EPPO) in 2009.

Clavibacter michiganensis subsp. *sepedonicus* (ring rot) and *Ralstonia solanacearum* (brown rot)

Outbreaks of ring rot and brown rot continue to occur in the EU and these diseases therefore continue to present a risk to potato production in Northern Britain.

For ring rot, the numbers of outbreaks have remained similar for the last few years, but outbreaks occur in different countries in different years. Since publication of the ring rot control directive, it has never been found in Ireland, Luxembourg, Malta, Portugal and Slovenia. In 2008-9 there were a few outbreaks in Germany, the Netherlands, Italy and Spain, but higher numbers in some Eastern European countries. Within the UK, ring rot has never been found in Scottish potatoes.

EU ring rot surveillance in 2007-8 resulted in 24 positives out of 48,034 samples of seed potatoes and 385 out of 19,510 ware samples These results do not include samples from Polish potato production, which has separate requirements for testing lots before movement out of the country.

For brown rot, EU-wide surveillance in 2007-8 resulted in 7 positive seed potato samples out of 50,925 and 40 positives out of 30,138 samples from ware crops. Since publication of the brown rot control directive, brown rot has not been recorded in Bulgaria, Cyprus, the Czech Republic, Denmark, Estonia, Finland, Latvia, Lithuania, Luxembourg, Malta, Poland, Romania and Sweden. Within the UK brown rot has never been found in Scottish potatoes. The organism continued to be found in EU river water surveys in 2007, but mainly in areas already demarcated as infected. There was a case in tomatoes and also in wash water. The organism was intercepted in imported ware potatoes in 2008-9.

Synchytrium endobioticum (wart disease) in Turkey

There have been sporadic outbreaks of wart disease in Europe over the last fifteen years, though none in Scotland. The most recent new cases were in Turkey in 2003 (Çakır *et al.* 2005) and Bulgaria in 2004 (EPPO 2008b). The Turkish authorities suspected that the outbreaks were linked with imports of seed potatoes from Europe several years previously (Basim *et al.* 2005). Recently the Bulgarian plant health service reported a number of interceptions of wart on Turkish ware potatoes (EPPO 2009c). These cases indicate the potential risks of introducing wart in seed and ware potatoes.

Candidatus Phytoplasma solani (potato stolbur phytoplasma)

Potato stolbur is a major problem for potato production in Eastern and Southern Europe (Secor 2007), but it has also been recorded in Germany (EPPO 2006). Warmer summers may increase the likelihood of the vectors of stolbur and other potato phytoplasma diseases becoming more prevalent and therefore increasing the risk of these diseases becoming established in Northern Britain.

Meloidogyne minor (a root knot nematode)

This pest is closely related to *M. chitwoodi* and *M. fallax*, both listed as quarantine organisms in the PHD. It has mainly been found associated with golf courses, football pitches and sand dune habitats and may be a native species in the UK. It has been found on potato very rarely on potato in Europe, although in one case damage to the potatoes was significant. A joint PRA was done by Fera and the Dutch plant protection service and recommended no statutory action should be taken (CSL/Netherlands Plant Protection Service 2007).

Dickeya species

There is increasing interest in *Dickeya* spp. (syn. *Erwinia chrysanthemi*) as a result of outbreaks in a number of European and Mediterranean countries (see paper by Cahill *et al.* in this volume for further information).

NEW PESTS OR INCREASED FINDINGS OF PESTS OUTSIDE EURO-MED REGION

Candidatus Liberibacter psyllaurous (syn. *Ca.* L. solanacearum)

In New Zealand, a new disease of tomatoes was observed in January 2008 and subsequently found causing disease in potato and pepper (*Capsicum annuum*) and symptomlessly in *Solanum betaceum* (tamarillo) and *Physalis peruviana* (EPPO 2009d).

It is highly likely that the organism is transmitted by the potato/tomato psyllid, *Bactericera cockerelli* (syn. *Paratrioza cockerelli*). The psyllid has the same distribution and is known to complete its life cycle on Solanaceae, Convolvulaceae and Lamiaceae.

Ca. L. psyllaurous has now also been found to be associated with the "zebra chip" symptoms in potato described in the 1990's in the USA. The geographical distribution also includes Canada, Mexico, Honduras and Guatemala. EPPO has added the organism to the Alert List and it recommends that countries prevent the entry of this organism and its vector into Europe (EPPO 2009d).

***Leucinodes orbonalis* (eggplant fruit borer)**

The eggplant fruit borer is primarily a tropical pest of aubergine from Africa and Asia, but it can also attack potato (EPPO 2008c). Since 2004 it has been intercepted many times in the EPPO region. The larvae attack leaves and young shoots as well as boring into aubergine fruit. It is unlikely to survive outside in Northern Britain, but could become a pest in protected crops. It has been intercepted in infected fruit but the probablity of transferring from infected fruit to protected cropping is low.

***Keiferia lycopersicella* (tomato pin worm)**

This pest *(*syn. *Phthorimaea lycopersicella)* causes leaf mining, folding and fruit boring in tomato (60-80% of fruit can be affected), but it will attack potato. It is a pest of warm climates, being present in Mexico, Southern USA, Hawaii, Bermuda, Jamaica, Cuba, Costa Rica, Haiti, Colombia, Venezuela and Bolivia (CABI 2007). It could potentially spread in infected tomato fruit, but is likely to be a threat only under glasshouse conditions in Northern Britain.

CONCLUSION

New risks arise from pests of potatoes, tomatoes and ornamentals and as a result of a number of potential pathways. Vigilance by government and industry continues to be required to prevent their introduction and establishment in Northern Britain and to preserve the high plant health status of Scottish potatoes.

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RECENT FINDINGS FROM THE *DICKEYA* SURVEY AND MONITORING PROGRAMME

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Summary: During the last five years, Dutch and Israeli potato production losses attributable to *Dickeya* spp. have risen alarmingly. *Dickeya* spp. cause wilt, blackleg-like symptoms and tuber rots. Although *D. dianthicola* has long been considered the principal species affecting potatoes in Northern Europe it is clear that a new species, provisionally named *"D. solani"*, has emerged. Since 2006, a monitoring programme has been running to ensure that Scottish potato production remains free of all *Dickeya* species. This programme targets imported potatoes for immediate processing, non-Scottish origin seed potatoes for planting, blackleg-affected stems taken from a range of seed and ware crops and water samples from sources of irrigation. Results confirm that seed potatoes of Scottish origin are free from *Dickeya* spp., but that potatoes infected with *"D. solani"* have entered Scotland for processing and planting. The presence of "*D. solani*" has also been confirmed in one river, with a further two rivers containing other *Dickeya* species. The implications of these findings are discussed.

INTRODUCTION

The bacterium *Pectobacterium atrosepticum*, previously *Erwinia carotovora* subsp. *atroseptica,* has long been recognised as the principal cause of potato blackleg and tuber soft rot in Northern Europe. Close relatives in the genus *Dickeya* can also cause similar symptoms on potato (Samson *et al*., 2005), with *Dickeya dianthicola*, previously *Erwinia chrysanthemi*, being the principal species affecting the region. Although *P. atrospeticum* is widespread in Scotland, *D. dianthicola* has never been found, despite the latter causing sporadic problems in some European countries since the 1970s, and in England and Wales since 1990. In recent years however, a new *Dickeya* species has emerged, which is more aggressive than both *D. dianthicola* and *P. atrosepticum*. This species has rapidly become a major problem in many European countries (Belgium, Finland, France, Poland, The Netherlands and Spain) and Israel (Palacio-Bielsa *et al*., 2006; Laurila *et al*., 2008; Tsror *et al*., 2009; Sławiak *et al*., 2009a, b). It was detected for the first time in 2007 in potatoes grown in England and Wales (Parkinson *et al*., 2009) and although it has yet to be formally named, the name "*Dickeya solani"* has been proposed.

In the growing crop, *D. dianthicola* is normally associated with a slow wilt with internal stem necrosis, leading to eventual desiccation of the infected stem. It tends to be problematic when temperatures exceed 15°C (van der Wolf and De Boer, 2007). In contrast, early reports of symptoms caused by "*D. solani*" may more closely resemble typical blackleg symptoms and

although evidence also suggests a preference for warm conditions symptoms can be expressed over a wider temperature range (Toth, personal communication). Wilting can be rapid with black soft rotting extending internally up the vascular system of the stem from the infected seed tuber. In badly affected crops, progeny tubers have been observed to rot before harvest (Tsror *et al*., 2009). In store, symptoms are identical to soft rot disease on potato tubers.

To comply with EU directives, Scottish seed and ware potato crops are surveyed annually for the presence of *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp. *sepedonicus,* as are sources of irrigation water for *R. solanacearum*. Since 2006, a subset of seed and ware crops and all irrigation samples have been tested for the presence of *Dickeya* spp., in addition to samples of ware imports and a limited number of blackleg-affected stems drawn from a wide variety of Scottish seed and ware crops. The results of the first three years of the *Dickeya* survey are presented here.

MATERIALS AND METHODS

Survey of imported ware for processing

Eighty-one bulk samples (200 tubers) were drawn from the grading line, as were any out-grade/rotten tubers which were dealt with separately. Spent wash water used in the grading process was sampled directly from the grading line's waste treatment plant at a rate of 3x250ml per week during the period imported tubers were being processed.

Bacteria were isolated from bulk tuber samples using two methods; a mechanical peeling method adapted from Perombelon and van der Wolf (2002) and a stolon-end tissue sampling method, based on the Commission Directives 2006/63/EC and 2006/56/EC for the detection of Ring/Brown Rot, prior to plating onto CVPM medium (Ahmed, 2001). Out-grade tubers were cut, diseased tissue excised, suspended in Ringer's Solution, allowed to stand for 10 min, then streaked onto CVPM. Water samples were tested within 24 hours of receipt, initially clarified by low speed centrifugation (180g) followed by concentrating the bacterial fraction by centrifugation at 10,000g. Serial dilutions were prepared on the resuspended pellet prior to plating onto CVPM medium.

Survey of irrigation sources

Two hundred and thirty watercourses used to irrigate potato crops were surveyed. Information on irrigated crops was identified during crop inspections. Water samples were collected in sterile bottles (250ml) from a range of sampling sites from each watercourse and delivered to SASA in cool boxes where they were processed within 24 hours. Samples were subdivided into aliquots of 40ml, clarified by centrifuging at a low speed (180g), then 20ml of supernatant were mixed with an equal volume of Pectate Enrichment Medium (Meneley & Stanghellini, 1976) and incubated in an anaerobic chamber at 36^{o}C for 48 hours. Liquid cultures were then centrifuged at high speed (10,000g) to concentrate the bacterial fraction. Serial dilutions were prepared on the resuspended pellet prior to plating onto CVPM medium.

Tuber survey

Bulk samples (200 or 600 tubers) were taken from crops at harvest or drawn from stores. Crops were targeted to include samples from stocks which had the greatest risk of carrying or contracting the disease. These included all crops produced from non-Scottish seed, all irrigated crops and those grown in the vicinity of a *Dickeya*-infested watercourse, as well as a percentage selected at random. Tubers were kept in paper sacks and delivered to SASA for testing. Bacteria were isolated using the stolon-end sampling method described above.

Survey of blackleg-affected stems

Stems of affected plants exhibiting mild symptoms were identified during field inspections. Stems were wrapped in crystal paper and delivered to SASA for testing. Samples were processed as described above for out-grade tubers.

Detection of *Dickeya* and confirmation of "*D. solani*"

CVPM plates were incubated at both 25°C and 36°C for 48h following the method of Kerr *et al*. (2009). *Dickeya* spp. were identified using PCR (Nassar *et al*., 1996), by initially making a pre-selection of colonies that formed characteristic pitting in the media at 36°C, tyical of *Dickeya* spp.. The identity of *"D. solani"* and other *Dickeya* species was confirmed by *rec*A sequencing (Kowalewska *et al*., 2010).

RESULTS

Survey of imported ware for processing

In 2006, two positive results were obtained; one from a sample of out-grade tubers and the other from a bulk sample, the latter showing no obvious signs of rots. In 2007, 3 further positive were found; two from bulk lots and the other from out-grade tubers. In each case the isolates were confirmed to be *"D. solani"* by *recA* sequencing (data not shown). No further positives were found in 2008 & 2009, nor were *Dickeya* spp. ever detected in spent wash water samples from the grading process.

Table 1. Annual survey of imported ware for processing. Samples of bulks, out- grade tubers and water examined for the presence of *Dickeya* spp., positive findings given in parentheses. * ND, not determined

Year	Number of bulk samples (positive findings)	Number of out-grade samples (positive findings)	Number of spent wash water samples (positive findings)
2006	3 (1)	4 (1)	5 (0)
2007	59 (2)	29 (1)	49 (0)
2008	19 (0)	21 (0)	70 (0)
2009	*ND	6 (0)	ND

Survey of irrigation sources

Only three rivers from the 230 watercourses tested over 4 years were found to be infested with *Dickeya* spp. In each case, once the river tested positive the infestation was found to persist in each of the subsequent years of study, although the exact sampling point at which a positive finding was made varied from year to year. The positive finding in 2006 was attributable to an unidentified *Dickeya* sp., whilst positives in 2007 and 2008 were attributable to *"D. solani"* and *D. zeae* respectively (data not shown). Only one species was ever found at each of the infested rivers, which remained consistent throughout this study (data not shown).

Table 2. Results of annual survey for the presence of *Dickeya* spp. in watercourses used to irrigate seed crops. * Positive findings were confirmed in each subsequent year after the initial discovery

Year	Number of Watercourses (sampling points)	Number of new positive rivers*
2006	66 (91)	1
2007	63 (78)	1
2008	36 (53)	1
2009	65 (94)	0

Tuber survey

In all, 523 samples drawn from seed and ware crops after harvest were tested: 291 in 2006/2007; 101 in 2007/2008; 131 in 2008/2009. No *Dickeya* spp. were found in any of the samples, these included 26 non-Scottish origin crops in 2006/2007, 27 in 2007/2008 and 47 in 2008/2009.

Survey of blackleg-affected stems

Approximately 200 crops, over a three year-period, that exhibited blackleg symptoms identified during growing crop inspections were sampled and subsequently tested for *Dickeya* spp. Only two crops were found to be infected with *Dickeya* spp.; both ware crops grown from non-Scottish origin seed. In both cases the species identity was confirmed as *"D. solani"* by *recA* sequencing (data not shown).

Table 3. Limited annual survey for the presence of *Dickeya* spp. in potato plants showing blackleg symptoms identified during growing crop inspections.

Year	Number of crops studied	Number of positive findings
2007	22	0
2008	2	0
2009	174	2

DISCUSSION

It is clear from the data presented here that the majority of Scotland's potato imports, crops and watercourses used to irrigate potatoes are free of *Dickeya* spp. It is also clear that all seed and ware crops of Scottish origin (423 were tested as part of the tuber survey and 190 tested as part of the survey for blackleg-affected stems) were found to be free of *Dickeya* spp. It is of some concern that "*D. solani*" was found in imported ware on a number of occasions however it is encouraging that this bacterium has never been found in the waste treatment plant attached to the grading line. As there is strict separation observed between seed and ware potatoes in Scotland (as required by Regulation 6 of the Seed Potato (Scotland) Regulations 2000) it is likely that the importation of ware potatoes for immediate processing poses minimal risk to the Scottish industry, regardless of whether they carry a *"D. solani"* infection or not. What is of major concern however, is the finding that one river and two ware crops, albeit grown from non-Scottish origin seed, were infested/infected with *"D. solani"*. In the case of the former, growers in the vicinity of the affected watercourse were notified and advised not to irrigate from this source, whilst the ware growers concerned in the latter cases were advised not to retain seed from the two affected crops and to apply strict hygiene and separation protocols to halt the spread of infection. Further research is required to determine the risks associated with the two other rivers found to be infested with *Dickey*a spp., other than *"D. solani"*. In the interim a precautionary approach was adopted and affected growers advised not to irrigate from these sources.

The positive detection of *"D. solani"* in one Scottish watercourse and two ware crops, coupled with the explosive rise of this pathogen in other European countries and Israel (Laurila *et al*., 2008; Tsror *et al*., 2009; Sławiak *et al*., 2009a, b) highlight that Scotland may have a very small window of opportunity to ensure that this pathogen is kept out of our seed production system. As a consequence, the Scottish Government is considering possible options to revise the Seed Potato Certification Scheme (SPCS), and plant health legislation, to ensure that relevant controls are in place to maintain the high-health status of Scottish potatoes. A full consultation will take place with the Scottish potato industry on any proposed new measures.

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HEALTHIER SEED POTATOES: A PROGRESS REPORT

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Summary: Our understanding of seed borne diseases has improved much over recent decades but some continue to be of concern. A stricter tolerance for powdery scab appears a valid option for powdery scab but difficult to implement without a better risk assessment associated with a particular level. The extent of seed borne transmission of late blight is not known but may be significant. Seed crops should therefore receive the best protection. A better understanding of the epidemiology of blackleg, silver scurf, skin spot, black scurf and stem canker has led to improvements in crop management, store hygiene, store design and ventilation. Although this has led to healthier seed, it is not always known which practices have been instrumental in reducing pathogens loads and what the benefits from investments in seed crops have been to the ware crop. Modern diagnostics and crop monitoring are likely to provide the answers to these questions.

INTRODUCTION

The introduction of Virus Tested Stem Cuttings as a method for initiating new seed potato stocks during the sixties was a landmark in seed health management. In Scotland all seed lots entered for certification had to be derived from stem cuttings from 1970 (Jones 1991). It was the culmination of efforts to eliminate seed borne virus through selection and certification. By propagating directly through a stem cutting, the method also offered a prospect of preventing the transmission of other tuber born diseases, for example blackleg (*Pectobacterium atroseptica*), skin spot (*Polyscytalum pustulans*), silver scurf (*Helminthosporium solani*) and black scurf and stem canker (*Rhizoctonia solani*). It soon became apparent that this measure alone was insufficient to prevent the recontamination of seed stocks during the normal sequence of multiplication (Pérombelon *et al.* 1976; Hide 1978, Carnegie *et al.* 1981). However, this development was followed by further research into seed borne diseases, which combined with technical advances in production methods, was aimed at further improvements of seed health. The Rothamsted group, working in the period when seed derived from stem cutting was first introduced, referred to their experimental seed stocks derived from stem cuttings as 'healthier' seed. Ware growers, who wish to realise the maximum potential of their crops, continue to seek health standards over and above what is assured within the seed classification schemes. It is appropriate to ask what impact the knowledge which has been accumulated over the last three decades has had on seed health and what this has contributed to the performance of ware crops.

POWDERY SCAB (*Spongospora subterranea*)

Powdery scab probably presents the most intractable problem today. Its incidence appears to vary greatly between seasons, it is very hard to predict and it is perceived as a serious quality deficit in seed by ware producers. Seed regulations in Great Britain permit 1% in pre-basic and 3% in basic and certified grades (Anon 2000, 2006). However tubers with less than 1/8th of the surface affected will be deemed unaffected. Clearly 1/8th of the tuber surface is capable of carrying much inoculum. Merz & Falloon (2009) have argued for a common practical tolerance limit to be adopted by certifying authorities, in order to limit further spread of the pathogen. Some suppliers already offer terms which include a lower tolerance than the statutory level, and this is likely to reduce the risk to the crops and land of ware growers. To make the case for action there is a need to establish what level would significantly protect the interests of ware growers but the scientific evidence is conflicting. Brierley *et al.* (2008) reviewed the relationship between inoculum levels on seed or in soil and disease and concluded that the lack of understanding was due to a complex interaction between environmental conditions, root infection, primary and secondary infection cycles, host resistance and disease. The recognition of root infection as an important stage in the life cycle (Burnett 1991, Nakayama *et al.* 2007, Nitzan *et al*. 2008) has been an important step towards our common understanding of this disease.

LATE BLIGHT (*Phytophthora infestans*)

Blight epidemics in 2007 and 2008 have been unusually severe in the UK. This could be attributed to weather but also coincided with the predominance of identifiable aggressive strains in Great Britain (Cooke *et al.* 2008). What is not clear is to what extent the spread of these strains was exacerbated by seed borne outbreaks. In detailed surveys of primary sources and first outbreaks in The Netherlands over a 6 year period, 36% of outbreaks could be attributed to infected seed (Evenhuis *et al.* 2007). Most seed borne outbreaks occurred in the South West (53%) and least in the South East (24%), with intermediate values for the North West (28%) and North East (37%). This shows that seed borne transmission may vary in different environments. *P. infestans* is capable of surviving asymptomatically for extended periods in cold stores (Johnson & Cummings 2009). Latent infection has also been demonstrated in sprouts and stems (Adler *et al.* 2001, Appel *et al*. 2001) making it possible for infection foci to emerge in ware crops at any time after emergence. It is critical that tubers in seed crops, which may have been exposed to blight through light infections or arrested outbreaks, are protected. Several products, applied to foliage, provide better than average protection of tubers (Bradshaw 2007). What is less clear is how effective these are in preventing seed borne transmission if, as is often the case, they are applied during only part of the period during which crops are at risk. Sensitive and specific PCR-based methods for detecting *Phytophthora infestans* in tubers exist (Hussein *et al.* 2007, Appel *et al.* 2001) but a practical approach for screening seed lots has not yet been developed.

BLEMISH DISEASES AND BLACKLEG

Although the introduction of seed stocks derived from stem cuttings did not eliminate the pathogens causing blackleg, skin spot, silver scurf and black scurf and stem canker, it was clear that recontamination occurred over several generations. Subsequently the introduction of in

vitro multiplication and the production of a first generation of 'minitubers' in a protected environment led to changes, whereby seed sold for ware production may be just four field generations old and five to six field generations are common. A better understanding of the epidemiology of these diseases has led to practical measures. These normally include store disinfection, drying of crops prior to storage, refrigerated stores and a variety of options for store design aimed at optimal ventilation to prevent condensation and excess humidity. These factors have undoubtedly contributed to better management of blackleg, silver scurf and skin spot. Seed for seed crops is routinely treated with one of several available fungicides to control *Rhizoctonia solani*, the cause of black scurf and stem canker, thereby reducing its incidence.

Although silver scurf is not currently the focus of attention, there are still unresolved questions. For example, the mechanism by which *H. solani* moves from seed to daughter tubers is still unknown (Erampelli *et al.* 2001). Stroma of *H. solani* may resemble microsclerotia of black dot, caused by *Colletotricum coccodes*. Because of this fact and the prevalence today of black dot, the amount of silver scurf in ware crops may therefore be under reported. Skin spot is appreciated mainly as a blemish in a small number of susceptible cultivars. Skin spot may also kill growing points thereby affecting stem numbers in other cultivars and tell-tale brown lesions on stems suggest that many seed stocks are infected. It is not known what the impact is on crop performance from these infections. Transmission of *R. solani* from seed to daughter tubers may be substantially reduced by fungicides but microscopic examination of seed tubers suggests that infection of seed lots is not a rare occurrence. This contamination may be the result of poor fungicide application or soil born infection. Although black dot in ware is mainly associated with soil borne infection (Wale *et al.* 2008), seed lots are from time to time infected, posing a special challenge to the management of this disease in the ware crop.

Although the management of these diseases has improved greatly, there is also considerable variation between the incidence of seed borne disease in high grade seed lots and between management practices by different seed producers. The practical measures, which undoubtedly increase the likelihood of healthier seed, are often time consuming and expensive. A proper cost benefit analysis takes into account the costs of these practices and the benefits in economic terms. For the ware grower these would be expressed in yield, size fractions and quality factors as these affect the sale price of the crop. Currently quantitative data appear to be scarce but ware growers increasingly maintain databases for test digs and quality assessments at harvest, often in considerable detail. These may help indicate the real benefits of seed health in terms of improved productivity. Benefits may also be considered in epidemiological terms. Methods for detecting pathogens using molecular techniques have been developed for all of these pathogens (Brierley *et al.* 2008). Monitoring seed health by assessing pathogen loads using molecular diagnostic techniques would provide the seed producer with valuable feedback to tell whether efforts have been worth wile.

DAMAGE AND ROTS

The wound parasites *Phoma foveata*, which causes gangrene, and *Fusarium coeruleum*, which causes dry rot, are no longer prevalent as they once were. Much has been achieved to reduce damage in seed at every stage of handling between harvest and planting. Hygiene and a managed environment in the store have also contributed to this improvement. The most vulnerable stage is transport between the seed producer and the ware grower and the preparatory stages immediately preceding. When tubers are cold and turgid they are more

likely to suffer mechanical damage or the crescent shaped cracks also known as thumbnail cracks. The latter may be superficial, but occasionally may be accompanied by deep splits under the surface. Transport may cause existing wounds to become exacerbated, presenting a worse picture at delivery than at dispatch. The damaged seed tubers present a risk depending on the presence of pathogens. For example, in a survey of *Fusarium* species in the UK causing dry rot in the UK, it was found that *F. sulphureum* was most pathogenic but also least common. The incidence of this and other dry rot pathogens was highly variable between stocks (Peters & Lees, 2004). Thus the risk of breakdown of planted seed may depend on the coincidence of damage after transport and certain, more aggressive, pathogens. The practical outcome is often seen only as a higher than expected incidence of missing plants, by which time the seed tuber has decomposed or is missing altogether and the cause can no longer be identified.

DISCUSSION

The Seed Classification Schemes continue to provide a robust protection to many virus diseases and Plant Health legislation aims to prevent the transmission of *Ralstonia solacearum,* the cause of brown rot, *Clavibacter sepedonicum* the cause of ring rot and Spindle Tuber Viroid. New challenges arise from the pressure of international trade, climate change and commercial pressures, for example the expansion of ware potato production in traditionally seed producing regions. (Crops grown for the purpose of producing seed and ware, basic seed production in ware growing regions of the UK and the use of farm saved seed each have their rationale but present specific challenges). However, the knowledge we now have of many diseases is extensive. Despite their complexity, clear advice about best practice can and in many instances is made. The challenge which remains is to measure the benefits of such advice in terms of reduced pathogen loads and improved production. The means to do so is within our grasp with the development of modern diagnostics and monitoring ware crop performance in relation to seed sources.

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DETERMINING THE DISTRIBUTION PATTERN OF SOIL-BORNE *RHIZOCTONIA SOLANI* AG3

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Summary: A field with a history of *Rhizoctonia solani* was intensively sampled by collecting 100 soil cores within a 4 ha area. The soil samples were individually tested by quantitative PCR. Contour plots of Kriged estimates for levels of *R. solani* AG3 DNA showed that there was one main cluster of inoculum stretching across the length of the sampled area. An analysis of the Ripley's K-function, a measure of the degree to which inoculum is aggregated showed that inoculum was highly aggregated for samples collected 90 to 140 m apart (P<0.05). This represents an area of approximately 2 to 3 Ha. The data presented here supports the current sampling strategy of 100 cores from a 4 Ha area for the detection and quantification of *R. solani.*

INTRODUCTION

Robust quantitative molecular diagnostic tools and soil DNA extraction techniques have recently been developed that enable detection and quantification of soil-borne inoculum for target pathogens such as *Colletotrichum coccodes* (black dot), *Rhizoctonia solani* AG3 (stem canker and black scurf) and *Spongospora subterranea* (powdery scab). For example, Budge *et al* (2009) developed a real-time PCR assay for *R. solani* AG3 and demonstrated that it could detect and quantify inoculum in soil. These tests should allow the relationship between levels of soil inoculum and disease to be determined. However, advances in linking molecular diagnostics to disease epidemiology have been slow to develop. Ophel-Keller *et al* (2008) recognised that issues such as sampling strategies and sample size can affect our ability to accurately interpret DNA-based soil tests. For diagnostic tools to be effective in predicting risk of disease, it is essential that the samples are 'fit for purpose'. That is, soil samples must adequately reflect what is in the field as a whole. To do this, we must understand how soil-borne pathogens are distributed in soils.

A soil-borne pathogen such as *R. solani*, if present, is likely to exhibit highly clustered or random distributions. Gilligan *et al* (1996) described how disease in potatoes resulting from soil-borne inoculum of *R. solani* occurred in patches. However, the work was done using bait plants laid out in linear transects of up to 25 m length. This is too small a scale of sampling to be useful agronomically. Currently, soil samples for potato cyst nematode (PCN) are collected over a 4 ha area per field. Consequently, members of the PCL-funded potato disease diagnostics project consortium (i.e. researchers at Fera, SAC and SCRI) have adopted the PCN sampling strategy to test for the presence of soil-borne fungal pathogens of potato.

The work presented in this paper was undertaken to describe the spatial distribution of *R. solani* AG3 in soil from an infested field. In addition, we set out to test whether the amount of inoculum detected in bulked soil cores from a sample area of 4 ha provides a robust mean value.

MATERIALS AND METHODS

Intensive sampling of fields to establish distribution pattern of *R. solani* AG3

A field in Portsoy, Aberdeenshire, in which potatoes were grown in 2007, and where progeny tubers exhibited black scurf, was selected for intensive sampling in April 2008. Within the field, a 4 ha (10 acre) area was identified and its location recorded. The corners of the square representing the 4 ha area were marked with canes. Within the 4 ha area, 100 points for sampling were identified on a 10 x 10 grid.

Soil samples (100) were taken to 15cm depth with a narrow blade trowel and the top 5cm of the sample discarded. Each sample was collected into a clean plastic bag and labelled. Upon receipt, samples were dried immediately or place in a cold room at 4^{o}C temporarily. Each sample was tested for DNA levels of *Rhizoctonia solani* AG3 as described in Brierley *et al* (2008).

Spatial analysis

The spatial distribution of pathogen inoculum was visualised by "kriging", a method originating in geostatistics to analyse data distributed in two dimensions. The kriging model analyses how successive data from sample points are correlated with each other. Kriged estimates of *R. solani* DNA levels sampled in a regular 10 x 10 grid within a 4 ha area were plotted on a contour map using Genstat v11 (VSN International Ltd). To measure the degree to which the pathogen was aggregated across a number of spatial scales from 20 to 200 m, Ripley's K-function was calculated for co-ordinates identifying the location of inoculum. To determine whether the pathogen distribution was uniform, clustered or random, the degree to which the K-function departs from complete spatial randomness (Kcsr) was assessed (Diggle, 2003)

RESULTS

Figure 1 shows the kriged estimates of variograms fitted to theoretical data simulating soil-borne pathogens with A, clustered and B, random distributions mapped on surface plots. The differences between a clustered and random distribution can be visualised but it is not possible to measure the degree to which pathogens are aggregated using this method. Figure 2 shows the same theoretical data analysed by plotting the Ripley's K-function against distance (m) between point samples. The K-function data representing the aggregated pathogen is clearly higher than the corresponding K-function for complete spatial randomness, Kcsr at scales of 100 m and below. Therefore, there is a strong degree of clustering at or below an area represented by approximately one quarter of the sampled area. The curve representing the randomly distributed pathogen is indistinguishable from the curve for Kcsr.

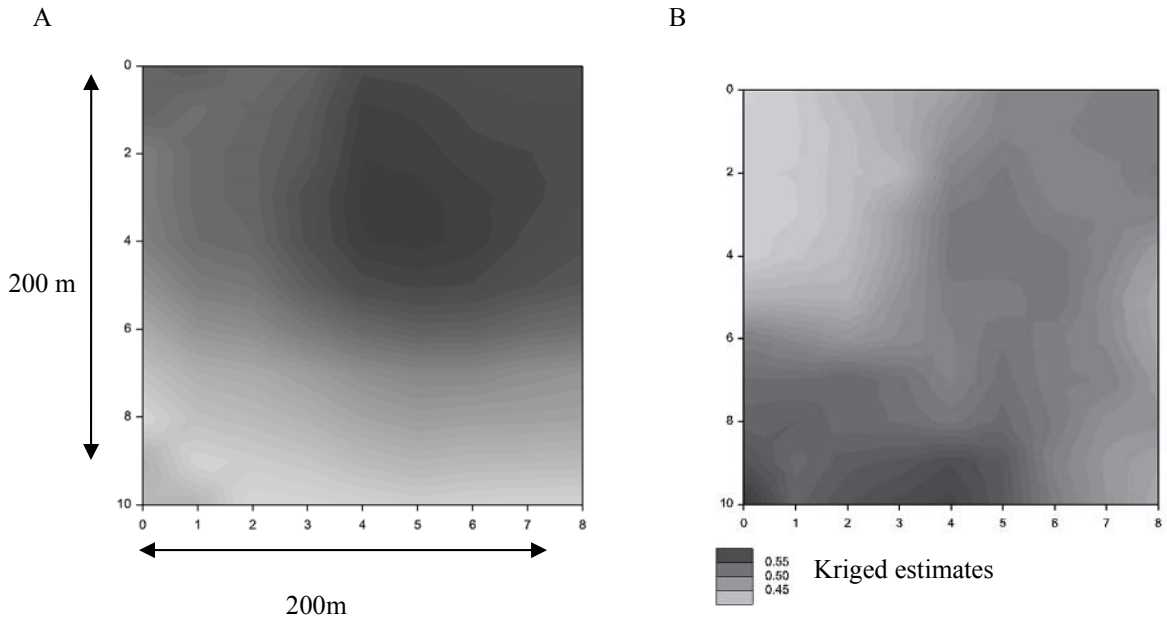

Figure 1. Theoretical data showing mapped kriged estimates of A, clustered and B, random distributions of a soil-borne pathogen in field soil. The darker regions represent higher inoculum levels.

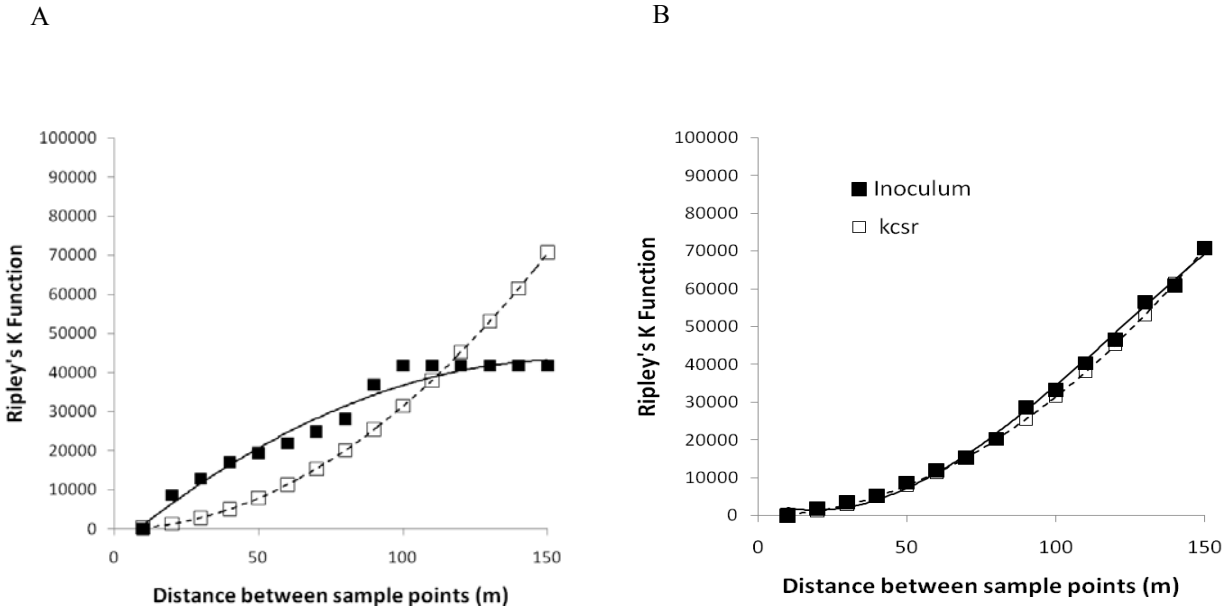

Figure 2. Theoretical data showing Ripley's K function for A, clustered and B, random distributions of a soil-borne pathogen in field soil plotted against distance from sampling points. Data are compared with K(csr) the measure of complete spatial randomness.

The same analyses were done on the field soil sampled in a regular 20 m x 20 m grid to determine the spatial distribution of *R. solani* AG3 inoculum. Mapped kriged estimates show the distribution of pathogen, as detected by quantitative PCR, in soil collected in December 2007 immediately following a potato crop (Figure 3). The inoculum appears to be distributed in one main cluster of between 50 to 120 m wide that stretches the whole length of the sampled area in a north-south orientation. A second, smaller cluster was found in the south westerly corner..

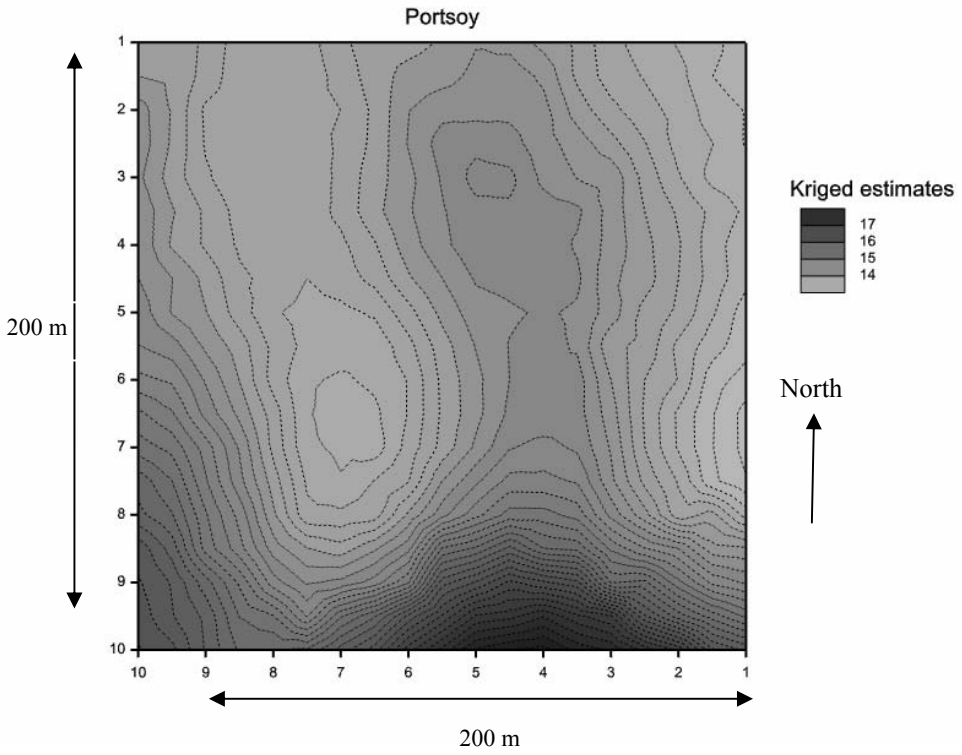

Figure 3. Kriged estimates (variance values) plotted as a two dimensional contour map showing the likelihood of finding *Rhizoctonia solani* AG3 DNA in soil sampled in a 10 x 10 regular grid within a 4 ha area (Aberdeenshire). Dark patches represent higher probabilities of finding pathogen inoculum than light patches.

An analysis was done to determine the degree to which the pathogen distribution deviated from randomness. Figure 4 shows the Ripley's K-function plotted against the distance (m) between samples containing inoculum. There was some degree of clustering from 90 to 130 m ($P<0.05$). At distances beyond 170 m, the pattern is uniform ($P<0.05$). Therefore, the pathogen displayed an aggregated distribution at approximately half of the area sampled.

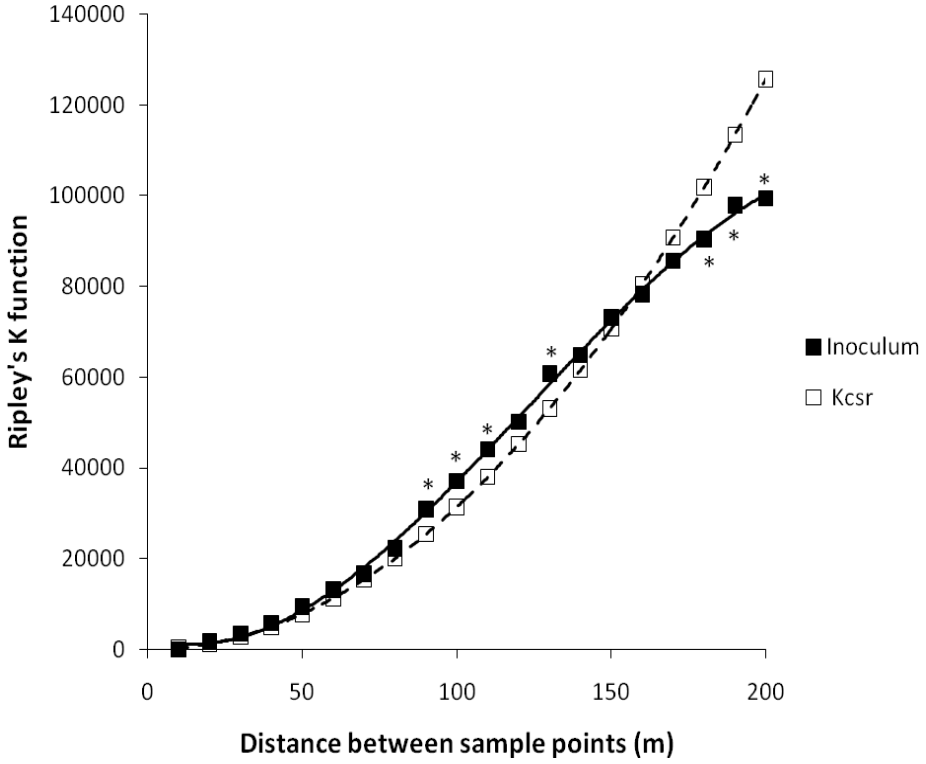

Figure 4. Distribution of *Rhizoctonia solani* AG3 in field soil sampled in December 2008 estimated by comparing Ripley's K function with K(csr) the measure of complete spatial randomness. * denotes inoculum K function that significantly deviates from complete spatial randomness (P<0.05). $LSD_{(P=0.05)}$=5110.

DISCUSSION

Gilligan *et al* (1996) showed that *R. solani* inoculum displayed an aggregated distribution within 24.7 m long rows. Their description of spatial distribution of inoculum is one-dimensional over distances that are fairly small when compared with sampling at the whole-field scale. This suggests that within the scale that approximates to the distance between sample points described in this paper, the distribution whilst aggregated was abundant in plots where rotations had been four years or less.

The distribution of inoculum of *R. solani* AG3 at one site in Aberdeenshire shortly after harvesting potatoes was aggregated at a scale of approximately 90 to 140 m wide for the length of the sampled area (i.e. 2 to 3 Ha). In practical terms, this means that testing the amount of inoculum across an area of less than 3 Ha would have been misleading. This is because the aggregate DNA level from samples collected over small areas are likely to grossly over- or under-estimate the amount of inoculum present in the whole field depending on where the samples are taken.
The data would support a minimum sampling area of 4 Ha for *R. solani* because at this scale,

the distribution of pathogen was uniform. An overall mean from 100 cores taken from 4 Ha is likely to represent a robust estimation of the true mean. This sampling strategy is currently the recommended sampling area for statutory PCN testing. However, the data presented is from one field and for one pathogen. Investigations are continuing to investigate the spatial distribution of other, predominantly, soil-borne potato pathogens such as *C. coccodes*, *R. solani* AG 2-1 and *S. subterranea*.

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THE APPLICATION OF MOLECULAR MARKERS WITHIN POTATO BREEDING PROGRAMMES

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Summary: The potato industry in Britain and Europe is trying to increase potato usage in an economically and environmentally sustainable way. New cultivars are required to have improved inherent resistance to pests and diseases, increased water and mineral use efficiency, and to meet consumer demands for healthy, flavoursome, convenience foods. As genetical knowledge accumulates, parents will be chosen to have desired genes which will be selected in their offspring. The application of robust molecular markers to select important heritable traits promises to make selection both more efficient and also potentially more cost effective by reducing or replacing expensive and expansive trialling and testing procedures which are currently required, often over many sites and/or seasons. The assessment of a range of diverse material for resistance to potato cyst nematode, to both *G. rostochiensis* and *G. pallida*, is discussed in respect of the deployment of molecular markers within breeding programmes and compared to the efficacy of conventional phenotypic tests.

INTRODUCTION

The European cultivated potato, *Solanum tuberosum* subsp. *tuberosum,* was derived from a narrow genetic base of a few introductions of subsp. *andigena* from South America in the late 16th century, and possibly further casual introductions in the 17th and 18th centuries. By the end of the 18th century, it had been adapted to long-day conditions through selection by its early cultivators for early-tuberisation and high yields. Given the narrow genetic base, the potato lacked sufficient genetical variation to afford adequate levels of resistance to a number of pests and pathogens which became a problem once it became widely grown as a staple food crop in many countries. The most notable pathogens have been late blight (*Phytophthora infestans*) since the middle of the 19th century and the golden and white potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in the 20th century. Hence, during the last 100 years, potato breeders have been introgressing genes for disease and pest resistance into Tuberosum potato varieties from the wild and cultivated species of Central and South America.

Following the discovery of new resistance genes within diverse related wild species that are effective against different pathogens, and their subsequent transfer into cultivated material, efforts are focused on developing acceptable cultivars for commercial use. The breeding of potato cultivars at the Scottish Crop Research Institute (SCRI) is commercially funded and success requires the selection of new cultivars with the qualities demanded by processors and supermarkets. We have developed an efficient potato breeding strategy at SCRI which avoids the ineffective practice of intense early-generation visual selection between seedlings in a

glasshouse and spaced plants at a seed site (Bradshaw *et al*., 1998, 2003), with emphasis placed on progeny tests (seedling tests for disease and pest resistance and visual assessment of tubers; and tuber tests for fry colour and further visual assessment) being used to discard whole progenies before starting conventional within-progeny selection at the unreplicated small-plot stage and to place selection pressure within the superior families for genotypes with improved quality traits and improved resistance levels to a number of important pathogens of the potato. However, potato breeding is now entering a new phase that promises much. Molecular markers have been developed to aid the selection of several important disease resistance and quality traits in potato. We are now starting to deploy such markers within the breeding programmes at SCRI. This is coupled to research at SCRI and elsewhere in a worldwide Potato Genome Sequencing Consortium (PGSC), aiming to elucidate the entire potato DNA sequence by the end of the year 2010. This will greatly facilitate gene isolation and allow molecular geneticists to use candidate gene approaches for trait gene discovery. This in turn will have radical effects on potato breeding.

One of the important pests affecting potato production is the potato cyst nematode (*Globodera pallida* and *G. rostochiensis*), one of the most significant soil borne pests of potatoes in the UK, Europe and worldwide. In Northern Europe, field populations of *G. pallida* comprising mixtures of pathotypes Pa2 and Pa3 are prevalent. Developing cultivars expressing high levels of resistance is a major objective of many European potato breeding programmes. The availability of DNA-based markers, which are easy to score, cost-effective and diagnostic for resistance to *G. pallida* Pa2/3 would greatly speed up the process of new variety development by allowing a marker-assisted selection (MAS)-based approach to develop lines in which multiple resistance sources were combined, preferably with each individual resistance locus present in high dosage states in the tetraploid potato genome. The application of molecular techniques as well as a fuller genetical knowledge of the important economic traits promises significant advances in the immediate future.

Parents

The choice of parents is all important as breeding can never simply be a numbers game. Crossing the 4000 cultivars listed in the World Catalogue of Potato Varieties (Hils & Pieterse, 2005) in all possible combinations would generate 7,998,000 progenies for evaluation, and raising 500 seedlings of each would give a staggering total of 3,999,000,000, compared with the 100,000 raised in a large modern breeding programme. In contrast, a trait assessment of 3200 cultivars is feasible, and so is a genotypic assessment of diversity and content with molecular markers. Hence breeders can now think in terms of capturing allelic diversity in a smaller core set of parents and of using association genetics to choose parents genotypically as well as phenotypically. They can also use genetic distance based on molecular markers to complement co-ancestry/pedigree analysis in order both to avoid closely related parents, and hence inbreeding depression, and to ensure genetical variation for continued progress.

As genetical knowledge accumulates, it should be possible to choose parents for use in pair crosses with complementary gene contents. Major genes have already been mapped for flesh, skin and flower colour, for tuber shape and eye depth, and for resistances to late blight, nematodes, viruses PVX, PVY and PVA, and wart. Quantitative Trait Loci (QTLs) with genes of large effect have also been mapped for maturity and resistances to late blight, potato cyst nematodes and virus PLRV. In contrast, many economically important traits still appear to be complex polygenic traits and these include dormancy, dry matter and starch content, fry colour,

resistance to *Erwinia*, tuberisation and yield. For these traits, breeders will still have to rely on trait data and use offspring-midparent regressions to determine heritability and crossing strategy. It is not clear at the present time how quickly information about expressed genes and gene sequence data will translate into genes for use in breeding programmes. But again, it can not simply be a numbers game. If the potato turns out to have 30,000 genes, as few as two variants per locus (one desirable, the other undesirable) would generate a large enough number of combinations to be infinite for all practical purposes. Hence, in the genomics age, gene discovery will need to be targeted at those genetic loci likely to have the biggest social and economic impact, and understanding the genetical control of key biochemical pathways could result in the fastest progress in the immediate future.

Molecular marker assisted selection

As knowledge increases about the number and chromosomal locations of genes affecting economically important traits, breeders should be able to design better breeding programmes. As well as selecting parents that complement one another genotypically, breeders will be able to determine the seedling population size required for certainty of finding the desired genotype, and more realistically, the number of cycles of crossing and selection required before this is achievable in practice in the size of population they can assess. A big impact on the efficiency and rate of progress would be the identification of superior clones genotypically as seedlings in the glasshouse and the use of modern methods of rapid multiplication to progress them to commercialisation, whilst also using them as parents in the next cycle of recurrent selection. This would require molecular-marker assisted selection or preferably direct recognition of the desired gene.

Significant progress is expected to be made and a number of possibilities can now be found in the literature. However, to date, the only good practical example from breeding programmes that we know of is the use of SCAR markers for the PVY resistance gene *Ry* (from Andigena, on chromosome 11). These markers should be powerful tools in marker-assisted selection as they showed high accuracy for detection of the *Ry* gene and one marker RYSC3 was generated only in genotypes carrying *Ry*, namely 14 out of 103 breeding lines and cultivars with diverse genetic backgrounds (Kasai *et al.*, 2000).

Research at SCRI has also sought to develop a range of parents with multiplex levels of desirable major genes. The H1 gene, the source of which is *S. tuberosum* ssp. *andigena* (CPC 1673), is extremely effective at reducing populations of the potato cyst nematode *Globodera rostochiensis* throughout the UK and Europe, providing hypersensitive resistance of potato to pathogens Ro 1 and Ro 4 of *G. rostochiensis*. An important breeding objective is to assess the copy number of the H1 gene in potato breeding lines, permitting clones possessing high dosages of the H1 gene to be identified and employed as breeding parents. Triplex and quadruplex parental material give rise to 100% H1 resistant progeny. The SCRI variety Spey is known to be triplex for the H1 gene and the recent SCRI variety Vales Sovereign is derived from the multiplex programme with both parents known to be duplex and is currently undergoing evaluation for H1 gene dosage. However, the identification of genotypes that are triplex and quadruplex for H1 is difficult and time consuming, requiring extensive resources during the lengthy process of phenotyping derived progenies from crosses between H1-bearing and susceptible parents and is illustrated in Figure 1.

Testcross to assess parental genotypes				Progeny
				Resistant:Susceptible ratio
Simplex	H1hhh	x	hhhh Nulliplex (susceptible)	1:1
Duplex	H1H1hh	x	hhhh	5:1
Triplex	H1H1H1	x	hhhh	All resistant
Quadruplex	H1H1H1H1	x	hhhh	All resistant

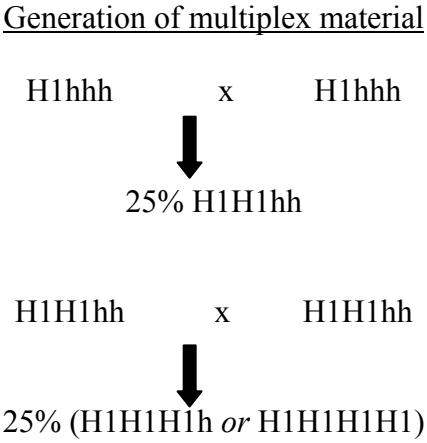

Figure 1. Procedure for identifying and producing multiplex H1 gene resistant parents.

The advantages of using triplex and quadruplex parental material is clearly illustrated within the 'Progeny' column in Figure 1, with all derived individuals having the H1 gene, eliminating the need to test for the resistance. We have employed a Pyrosequencing method to quantify relative levels of different sequence variants in a DNA sample, and applied this to a range of material with known dosages of the H1 gene. Currently these molecular markers distinguish between presence and absence of the H1 gene, and can be used to measure H1 gene dosage in some of the material assayed.

The H1 gene is located on the distal end of the long arm of chromosome V and is closely linked to RFLP markers *CP113* and *CD78*. SCRI has utilised markers from previous H1 mapping and cloning efforts to develop quantitative single nucleotide polymorphism (SNP) markers flanking the H1 gene that can be used indirectly to measure gene dosage (Gebhardt, 2001). PCR products sequencing from different H1 resistant and susceptible genotypes and comparison of sequence polymorphism data has been used to develop SNP markers whose dosage can be measured. A method was also developed for detecting gene *H1* in potato varieties and hybrids on the basis of RFLP marker *TG689*. A comparative analysis of nucleotide sequences of marker alleles revealed point mutations, on the basis of which specific primers have been selected for diagnosing gene *H1* by the PCR analysis method. The given method for the molecular marker TG 689 was made available by Walter S. DeJong.

With regard to resistance to *G. pallida*, research at SCRI has identified a major QTL for *G. pallida* resistance derived from *S. tuberosum* ssp. *andigena* CPC 2802 which is linked to a microsatellite marker (STM3016) located on linkage group 1V. A further 12 AFLP molecular markers tightly linked to the H3 locus have been identified and the results will be discussed.

The results presented demonstrate the potential of this approach for selecting nematode resistant genotypes within breeding programmes and also the potential to identify multiplex gene resistance status without lengthy backcross programmes and how these have direct application in potato breeding programmes. This method will be refined as more sequence information at the H1 locus becomes available and hence more closely associated, and therefore more accurate molecular markers are developed and deployed. The procedures used show promise for application to the major genes for disease and pest resistance common within conventional potato breeding programmes.

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THE IMPACT OF SOIL MIGRATORY NEMATODES ON POTATOES

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Summary: Glasshouse and field trials have evaluated the impact of soil migratory nematodes on the growth, development and yield of potatoes, resulting in quantitative assessments of the effects on root volume, root biomass, shoot growth and yield loss due to nematode feeding damage. Glasshouse tests using salad potatoes indicated that at relatively low nematode populations root biomass increases at the expense of shoot growth. However, at higher nematode populations, root biomass was reduced along with shoot growth. There was a significant relationship between salad potato yield and nematode population size. Field trials using several potato cultivars indicated a significant reduction in root volume and/or biomass and shoot biomass compared to nematicide treated potatoes with a consequent reduction in yield. Further work is necessary to develop robust treatment thresholds that can be used to determine whether nematicide use is justified in potato crops.

INTRODUCTION

Soil migratory nematodes are prominent pests in agricultural crops (Verschoor *et al*., 2001). Above ground symptoms of nematode infestation include reduced or abnormal growth and discolouration of foliage, and below-ground symptoms include a reduced root-system (Whitehead, 1998). Nematode populations in Scottish soils have increased significantly since the mid-1990's (Evans, 2006), as have reports of direct-feeding damage on potato crops, resulting in patchy emergence and subsequent reductions in yield.

This paper summarises a series of glasshouse and field trials aimed at quantifying the impact of nematodes on growth and yield of several potato cultivars, with a view to developing thresholds populations of nematodes to justify the use of a nematicide treatment to protect yield.

MATERIALS AND METHODS

Glasshouse trials

Soil samples from land used for ware potato production were used as a source of nematodes of varying population size and species composition. In addition 6 x 10 kg of soil was taken from a site in East Linton with very high nematode populations. Nematodes were extracted from soil using a Baermann funnel method, where nematode motility is used to separate them from soil samples washed through sieves (Hooper, 1986). Primarily *Trichodorus primitivus*, *Longidorus elongatus* and *Pratylenchus penetrans* were extracted from soil samples.

The salad potato cultivar Mimi was planted in pots containing soil with known nematode populations and species composition. In addition soil that had been oven dried for 48h to kill any nematodes was used as a standard control of zero nematodes in the soil.

Soil temperature was 16°C ± 3°C in a heated glasshouse during the months of December to April. Pots were watered twice weekly to maintain soil moisture. The majority of plants were harvested 35 days after planting in order to obtain assessments of shoot height and root biomass by drying in an oven for 24hrs at 100˚C. The rest were allowed to grow for 100 days before harvest and measurement of yield.

Field trials

A ware potato crop of King Edward was sown in April 2008 with nematicide (oxamyl) treated and untreated plots. The nematode populations for each plot were obtained through soil sampling prior to planting using the method of Hooper (1986). Four or five plants were removed from each plot 40 days after planting. An image of the roots of each plant was scanned and root volume was measured using the WinRhizo Pro root analysing system (Regent Intruments Inc.) The dry masses of shoots were obtained by drying in an oven for 24h at 100˚C.

Four months after the potatoes were planted, tubers were removed from each plot and separated into three size classes: <45 mm; 45-65 mm, and >65 mm. Those falling into the 45-65 mm and >65 mm were classified as 'marketable'.

Further field trials using similar methodology were undertaken in Ayrshire, East Lothian and Tayside in 2009, however full results were not available at the time of writing, but will be presented at the Conference. Partial results are given in the Results below.

RESULTS

Glasshouse trials

As there were varying populations of different nematode species in the soil samples used to grow potatoes in pots, an overall population count of *Trichodorus* + *Longidorus* + *Pratylenchus* is used in the results below.

There is a significant relationship ($P < 0.001$) between plant height after 35 days and nematode population (Fig. 1), with a decrease in plant height as nematode population increases.

At lower nematode populations, root biomass initially increased in response to nematode feeding on the roots (Fig. 2), but at higher nematode populations root biomass is reduced.

The yield of tubers (g per plant) was significantly decreased at high nematode populations (Fig. 3). Number of tubers per plant were also reduced by up to 50% (not shown).

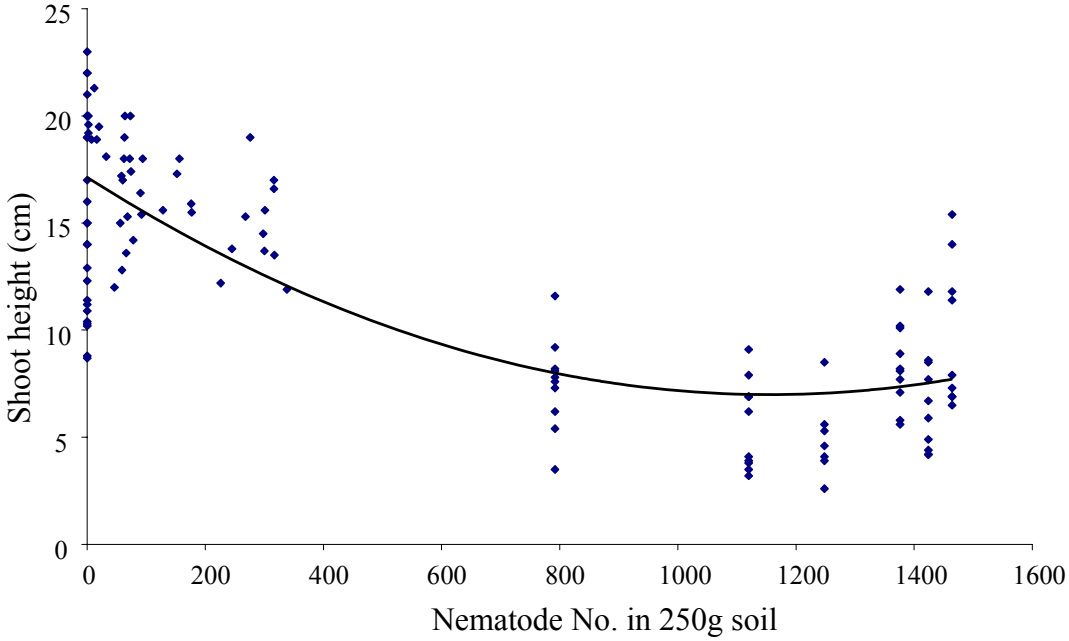

Figure 1. The shoot height of Mimi potatoes (n = 122) 35 days after planting in soil with varying populations of nematodes. $P < 0.001$ (polynomial regression, $R^2 = 0.646$).

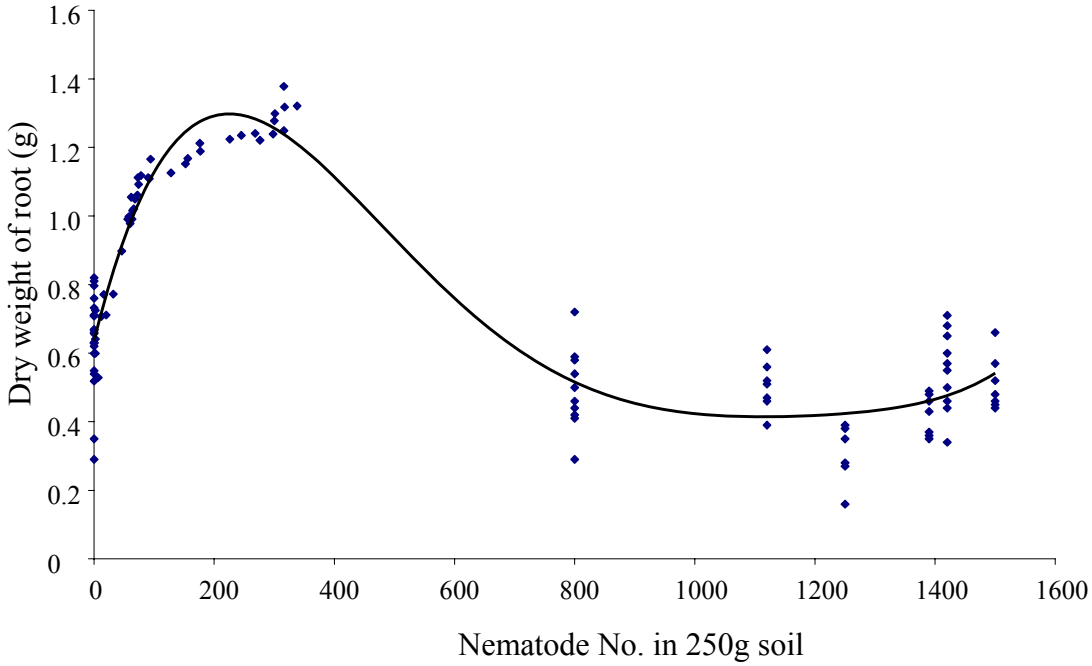

Figure 2. Dry root weight (g) of Mimi potatoes (n = 110) 35 days after planting in soil with varying populations of nematodes. $P < 0.001$ (polynomial regression, $R^2 = 0.781$).

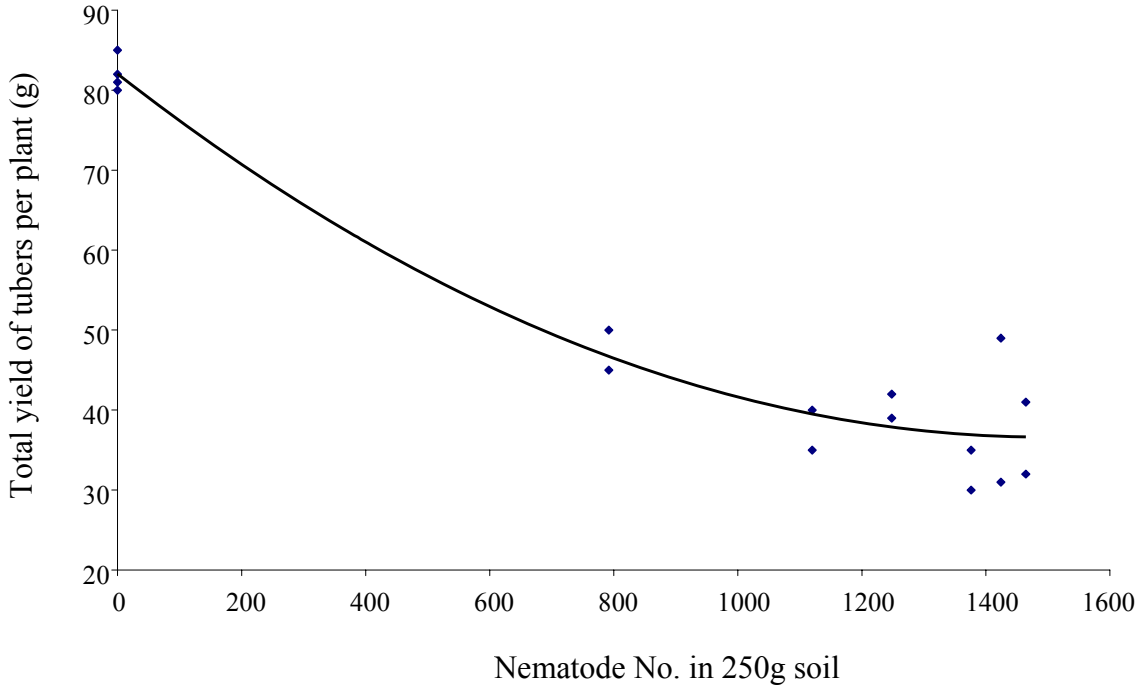

Figure 3. Yield of tubers/plant (g) of Mimi potatoes (n = 16) 100 days after planting in soil with varying populations of nematodes. $P < 0.001$ (polynomial regression, $R^2 = 0.94$).

Using the regression equation derived from Fig. 3, where:
Tuber yield = (82.05 – (0.06065 x Nematode No.)) + (0.000020 x Nematode No.^{2});
a nematode population of 200 nematodes/250g soil would decrease yield in Mimi potatoes by approx. 14%.

Field trials

Nematode populations in the untreated plots ranged from 405-1,094 nematodes/250g soil, and in the oxamyl plots from 715-822 nematodes/250g soil. There was a significant linear relationship between initial nematode population in the soil in the individual untreated plots and shoot biomass after 40 days ($P < 0.005$, Fig. 4).

There was a significant reduction in root volume in potatoes 40 days after planting compared to the nematicide (oxamyl) treatment (Table 1). Shoot biomass was greater on average in oxamyl treated potatoes but not significantly so (Table 1).

At harvest, the mean marketable yield of King Edward potatoes treated with oxamyl was significantly greater than those left untreated (Table 1).

Reductions in root and shoot biomass were observed in field trials in Ayrshire in 2009 in comparisons between untreated and oxamyl treated crops of Maris Piper and Carlingford. Total yield of Maris Piper was 9% greater in oxamyl treated plots with moderate FLN populations (approx. 260 nematodes/250g soil). In the crop of Carlingford (nematode populations approx. 200 nematodes/250g soil), an increase of 3 tonnes/ha was obtained through the use of oxamyl.

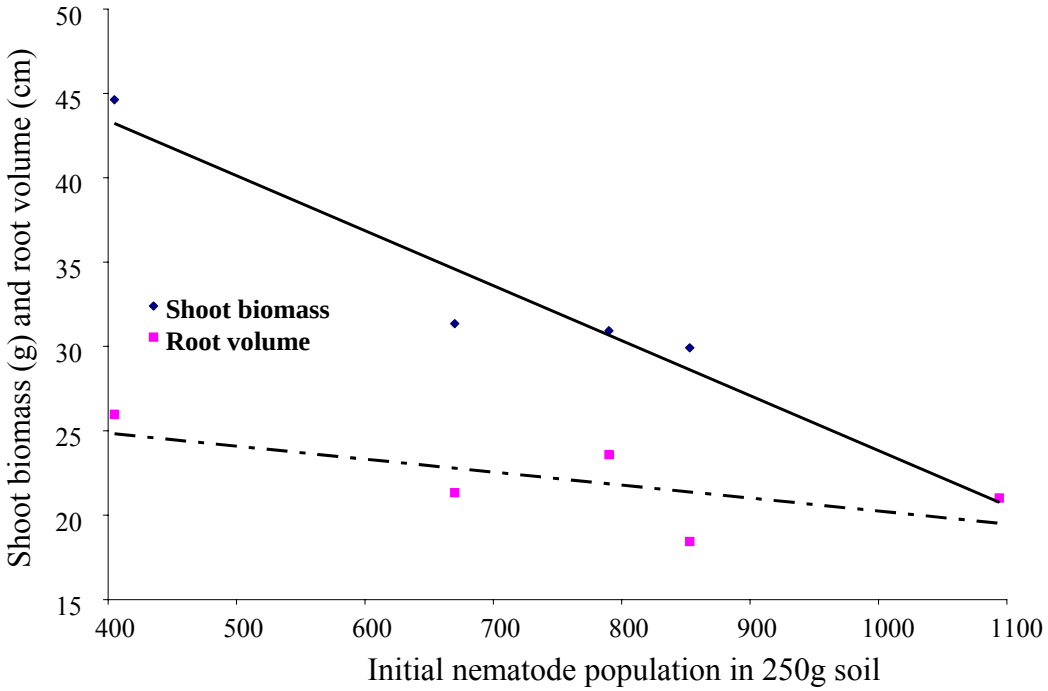

Figure 4. Shoot biomass (g) and root volume (cm^3) of King Edward potatoes (n = 5) 40 days after planting in soil with varying populations of nematodes. Shoot biomass - $P < 0.005$ (linear regression, $R^2 = 0.95$). Root volume -not significant ($R^2 = 0.47$)

Table 1. Mean root volume (cm^3) and shoot biomass (dry weight g) per plant 40 days after planting in untreated (n = 20 plants) and oxamyl treated plots (n = 16 plants). Mean marketable yield (tonnes/ha) from untreated (n = 5) and oxamyl treated (n = 4) plots.

Treatment	Mean root volume	Mean shoot biomass	Marketable yield tonnes/ha
Untreated	22.08 ± 1.27	31.57 ± 3.78	56.24 ± 5.2
Oxamyl	31.04 ± 1.84	37.45 ± 5.59	78.42 ± 12.5
	P < 0.005	ns	P < 0.05

DISCUSSION

There has been a lack of consensus on the relationship between nematode populations and their impact on potato yield (Olthof & Potter, 1973; Olthof, 1987; Mathias, 1990). Damage thresholds for *Trichodorus* tend to be at the 100 nematodes in 250 g soil level upwards (Mathias, 1990, Dale & Neilson, 2006), whereas *Pratylenchus* damage thresholds in potato have been stated from as low as 25 nematodes/250g soil (Olthof, 1987) up to 625 nematodes/250g soil (Dale & Neilson, 2006). *Longidorus* populations of 20-25 nematodes/250g soil have been reported as damaging to potatoes (Mathias, 1990; Dale & Neilson, 2006). Potato cultivars respond differently to nematode populations, and other factors such as soil temperature and moisture content which are important for nematode activity, damage and survival have a significant impact on whether yield loss occurs (Verschoor *et al.*, 2001; Dale & Neilson, 2006).

When conditions are particularly suited for nematodes, as in the glasshouse tests and field trial reported above, robust relationships between nematode populations and their effect on potato growth and yield can be determined. However, in practice environmental factors will have an impact on nematodes as well as on the crop, so any thresholds developed will have to assume nematodes have ideal conditions in which to cause damage.

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DEVELOPMENT OF A HIGH-THROUGHPUT METHOD FOR THE DETECTION AND SPECIATION OF PCN

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Summary: As a consequence of the new EU PCN directive it is expected that there will be a significant increase in the number of soil samples that need to be tested each year. A new diagnostic test based on the detection of PCN DNA has been developed that not only provides the level of accuracy required, but can also handle large numbers of samples at a cost per sample that compares with the current test. The new assay reliably detects both species of PCN (*Globodera pallida* and *G. rostochiensis*) from a range of sites and a single cyst of either species can be detected in a sample even when there a large number of the other species is present. This is essential if fields containing mixed populations are to be correctly identified.

INTRODUCTION

On the 1st July 2010 the new EU PCN directive (2007/33/EC) will come into force, with fields scheduled for planting from 2011 onwards being affected. One of the primary aims of this new legislation is the harmonisation of soil sampling rates across the EU. This will result in a significant increase in the amount of soil to be tested in the UK as the level currently adopted is the lowest across the EU. The new soil sampling rate is 1500ml of soil/ha, with a reduced rate of 400ml of soil/ha applying to fields where there is a documented test history showing freedom from PCN, or where potatoes are grown in a rotation of 1 in 7 years or longer. Further reductions can also apply for larger fields or where the field is situated in a pest free area. However, in spite of these relaxations, the reality of these changes is that it is expected that there will be a significant increase in the number of soil samples that need to be tested each year. Currently these samples are examined at SASA using well established methods. PCN cysts float and therefore they can be separated out from the soil by bubbling water through the soil, with any material that floats being collected on a sieve. A trained nematologist then has to sort through this material and identify any cysts. *Globodera pallida* and *G. rostochiensis* are subsequently separated on the basis of a range of morphological characteristics. However, with the expected increase in sample numbers it would be impracticable to continue using this method and expect to process sufficient samples prior to the start of planting.

Over recent years several tests for speciation of *G. pallida* and G. *rostochiensis* have been developed based on conventional (Bulman & Marshall 1997; Fullaondo *et al.* 1999; Mulholland *et al.* 1996; Pylypenko *et al.* 2005; Vejl *et al*. 2002) and real-time PCR (Bates *et al*. 2002; Madani et al. 2008; Quader *et al*. 2008). Whilst such tests have been well received,

only a limited number of samples can be processed at one time and the tests are often too expensive for routine use. Additionally, the previous tests did not have an appropriate level of accuracy and as a result did not discriminate between the full range of cyst nematodes found in Scottish soils. There was, therefore, a need to develop new testing methods that could process very high numbers of soil samples whilst maintaining or improving the accuracy of the current test.

MATERIALS AND METHODS

Soil sampling for PCN

To comply with the current PCN directive, agricultural staff of the Rural Payments and Inspections Directorate (RPID) take over 6000 soil samples from land intended for seed potato production. Each of these samples comprises at least 500ml soil from 100 cores of variable volume taken from an area of up to 4 ha. These soil samples are then processed in the Nematology Laboratory of Science and Advice for Scottish Agriculture (SASA). Several hundred samples were randomly selected for assay development from the samples submitted to represent both a cross section of soil types commonly occurring in Scotland and a range of sample sizes.

Cyst extraction from soil

The soil sample is air-dried at 37°C over 2 days. Cysts are extracted by flotation using a modified Fenwick can method (Fenwick 1940) and collected in 250 µm sieves. The residues are then dried prior to examination under a low power stereo microscope at a magnification of x10. Any *Globodera* cysts found are picked off resulting in a clean float sample. The "float" is then placed in a 2 ml centrifuge tube.

DNA Extraction

Prior to processing, single cysts of either one or both samples were added to the samples. Eight tungsten carbide beads (3 mm) (Qiagen) are added to the float prior to beating for 30 S at a frequency of 30 Hz on an MM300 mixer mill (Retsch) following which 1ml PBS is added and the mixture disrupted for a further 30 S at a frequency of 30 Hz. Samples are centrifuged for 5 min at 1500g and 200µl of the supernatant is transferred to a tube to which 200µl AP1 buffer and 2µl RNseA stock solution (BioSprint 96 DNA Plant Kit, Qiagen) is added. Samples are frozen at -20°C for a minimum of 1 hour. Samples are incubated for 15 min at 65°C after which 340µl of the lysate is loaded into an S block and processed in BioSprint96 (Qiagen) using BS 96 DNA plant program. Resulting DNA samples were eluted in a final volume of 200 µl.

Real-time PCR analysis

The primers and probes were designed in the ITS1 region. After aligning DNA sequences of 91 *Globodera* spp. and 3 *Punctodera* spp. using the ClustalW method the MegAlign program in Lasergene v7.0.0 (DNASTAR Inc.). The TaqMan *G. pallida*-specific MGB probe (CCGCTATGTTTGGGC labelled with 6-FAM) was designed based on the *G. pallida* accession FJ212165 using Primer Express v2.0.0 (Applied Biosystems). The *G. rostochiensis*-

specific probe (CCGCTGTGTATKGGC labelled with VIC) covers the same region as the *G. pallida* specific probe. The flanking primer sequences are (5' to 3') CGTTTGTTGTTGACGGACAYA and GGCGCTGTCCRTACATTGTTG and were synthesised by Applied Biosystems.

Real-time PCR reactions were set up using a Tecan GenesisWorkstation 150 (Tecan Inc.) in 384 well plates (Applied Biosystems). Reactions contained: 15.0 µL FAST BLUE qPCR MasterMix (Eurogentec Ltd), 1.25 µL each of the forward and reverse primers (at 5 pmol/µL), 0.25 µL of the *G. pallida*-specific probe (at 5 pmol/µL), 1.0 µL of the *G. rostochienesis*-specific probe (at 5 pmol/µL), 6.25 µL distilled water (Sigma) and 5.0 □L DNA. Triplicate 9.45 µL volumes of this reaction mixture were analysed. Control samples consisted of DNA extracted from purified cyst material of *G. pallida*, *G. rostochiensis*, and mixtures of both species at ratios of 1:1, 1:9 and 9:1. In addition, negative controls (water only) were included in triplicate in each run. Amplification was performed in an ABI 7900HT (Applied Biosystems) real-time machine run in 9600 emulation mode with the following cycling conditions, 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95° for 15 sec and 60°C for 1 min.

Detection of mixed populations

A $2m^2$ plot in a field on SASA's farm, which is known not to have grown potatoes for a period of at least 40 years, was intensively sampled to provide 70 samples, each containing 500ml soil. The float was prepared as described above prior to the artificial addition of ten *G. pallida* and *G. rostochiensis* cysts in the following proportions 0:10, 1:9, 3:7, 5:5, 7:3, 9:1 & 10:0. For each of the mixtures ten replicate samples were created. The samples were then "blind" tested using the PCR assay.

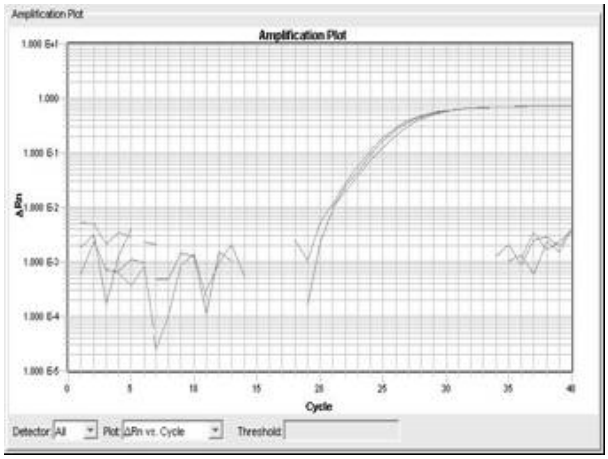
RESULTS

The *Globodera* species-specific primers and probes were found to detect only the desired species. Independent evaluation of the primers found them to correctly detect and differentiate a range of *G. pallida* and *G. rostochiensis* populations from Europe (Géraldine Anthoine pers. comm.). Utilisation of these primers in a multiplex reaction again found that they only detected the desired species with a Ct value in the region of 24.5. The DNA extraction method developed utilises a semi-automated system capable of processing 96 samples at a time reducing staff time associated with this activity leading to a lower unit cost per sample. Further automation of the PCR set-up using a Tecan GenesisWorkstation resulted in a total staff input of 2.2mins per sample.

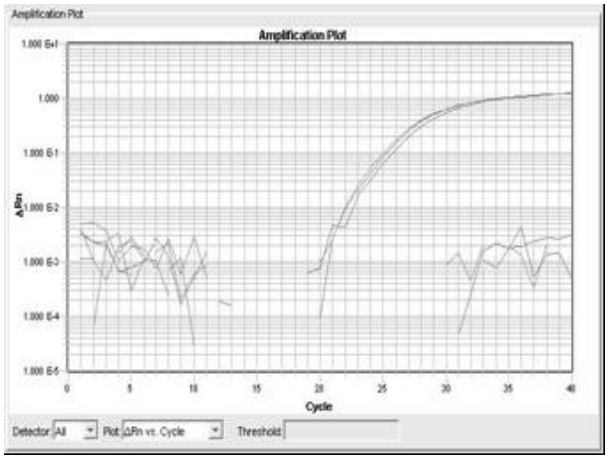
Analysis of approximately 400 floats which had been extracted from a random selection of soil samples representing Scottish seed tuber production areas artificially seeded with a single cyst of either *G. pallida* or *G. rostochiensis* or a cyst of both species found that on each occasion the real-time PCR assay correctly detected the species present.

Working with "float" samples seeded with artificial mixtures of PCN cysts the real-time PCR assay correctly detected the species present (Figure 1). For the 9:1 *G. pallida*:*G.rostochiensis* samples only seven of the ten replicates were correctly identified as containing *G. rostochiensis*. For the remaining combinations all ten replicates were successfully detected.

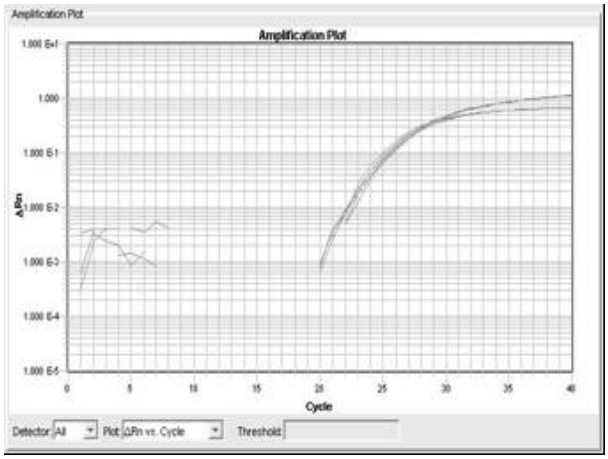
A



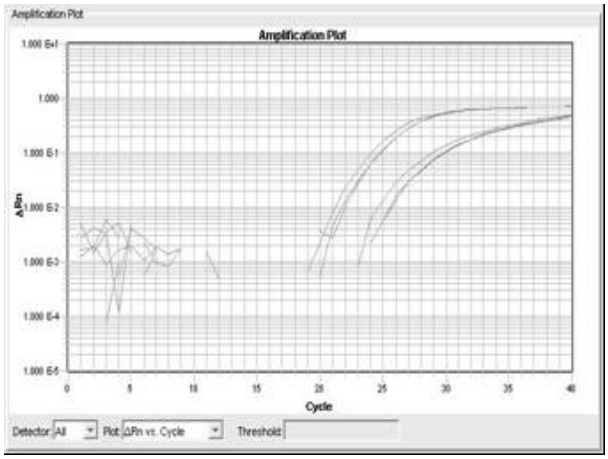
B



C



D



E

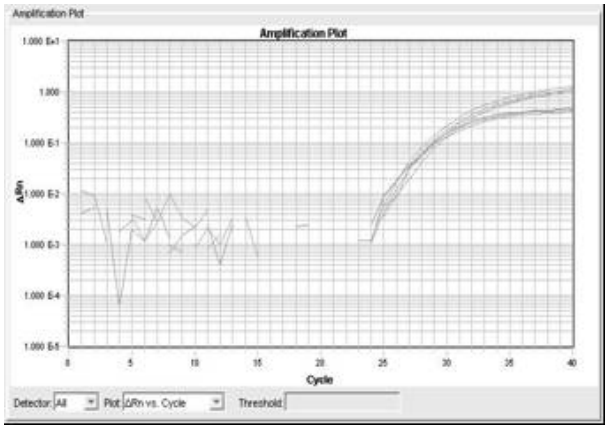


Figure 1. Detection of PCN in "floats" using the Taqman assay (A) *G. pallida* only, (B) *G. rostochiensis*, (C) 1:1 *G. pallida*:*G. rostochiensis* (D) 9:1 *G. pallida*:*G. rostochiensis* (E) 1:9 *G. pallida*:*G. rostochiensis.*

DISCUSSION

The new diagnostic assay based on the real-time detection and speciation of PCN not only provides the level of accuracy required but can also handle large numbers of samples at a cost per sample that compares with the current test. The new test still requires the cysts to be separated from the soil samples, but instead of visual examination the PCN present is detected and identified using a DNA test directly from the float. This assay has reliably detected both species of PCN from a range of sites even when only a single cyst is present. The specificity of the test is also important and whilst data from PCN statutory testing under the current directive suggests that mixed populations occur at relatively low levels in Scotland the new method reliably detects a single cyst of either species in a sample even when there is a number of the other species present. This is essential if fields containing mixed populations are to be correctly identified.

An important target in the development of the described assay was the capacity to process the very high numbers of soil samples expected and to ensure timely reporting of results. In recent years SASA has been investing in technology that automates many stages of DNA analysis and allows samples to be processed in batches of 96 with minimal 'hands on' time. The combination of rapid automated DNA extraction and PCR preparation using the BioSprint96 and Tecan Genesis workstation in combination with real-time PCR enables testing of hundreds of samples per day. An added benefit of using such technology is that it reduces the cost of the testing. As a result the new test is expected to be significantly cheaper than currently available DNA based tests.

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REPRODUCTION OF POTATO CYST NEMATODES: EFFECTS OF TEMPERATURE

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Summary In the UK both species of potato cysts nematodes, *Globodera rostochiensis* and *G. pallida* are widely dispersed in potato growing areas, with both species frequently occurring in the same field. The two species are difficult to distinguish based on morphological features, however, despite many similarities; the two species are reported to differ biologically in their rates of hatching, responses to temperature and reactions to different sources of resistance. These biological differences are likely to affect inter-specific competition. We are examining the effects of temperature on the life cycle of the two species and examining inter-specific competition using a recently developed qPCR assay.

INTRODUCTION

The potato cyst nematodes (PCN) *G. rostochiensis* (Woll.) and *G. pallida* (Stone) are the most economically important pests of potato (*Solanum tuberosum* L.). They are wide-spread in Europe and are found in many major potato growing areas world-wide. Despite legislative regulation in over 55 countries they continue to be found in new locations with recent detections in Idaho USA, Quebec Canada, Victoria Australia, Java Indonesia and the Ukraine. In the UK PCN are the most economically important nematode pests of agriculture and in a survey of England and Wales they were found in 65% of potato land (Minnis *et al*., 2002). They impose an annual cost in excess of £50 million on UK farmers and yield losses of ~9% have been estimated (Haydock & Evans, 1998).

The latest European Council Directive concerning PCN (2007/33/EC) comes into effect on July 1, 2010 and will introduce further measures to suppress PCN and prevent further spread and harmonize measures across the EU with common soil sampling regimes and official survey requirements. The withdrawal of the use of the aldicarb for use on potato crops in 2007 by the EU and the potential loss of other nematicides that are under review highlights the need for effective integrated pest management tools. The use of control measures to ensure that populations are suppressed and do not spread imposes costs and restrictions. Detection of PCN even at low levels will become even more important and have implications for growers. Control measures, other than nematicides, include long rotations, resistant cultivars and trap crops. These need to incorporate an understanding of the role of environmental factors including temperature on PCN population dynamics, inter-specific competition and selection. To this end we have been investigating how the two species respond to different temperatures, and because the two species frequently occur together in

the field, we have been assessing how the two species compete when present as mixtures using a recently developed quantitative PCR (qPCR) assay.

MATERIALS AND METHODS

Hatching in response to temperature

A temperature gradient table (Grant) was used to compare rates of hatching of laboratory propagated populations of *G. rostochiensis* and *G. pallida*. For each species 10 cysts were placed in 5mm petri dishes in 5 ml of water or potato root diffusate (PRD) and the dishes arranged on the temperature gradient table to achieve temperatures from 5º to 29ºC. Juveniles that had emerged were counted twice weekly for 5 weeks and the water or PRD replaced each time they were counted. Duplicate dishes were prepared for each treatment.

Male emergence

The effect of temperature on male emergence from potato cv Desirée was investigated. Plants were grown in root trainers in a 50:50 sand loam mixture and inoculated with 1500 juveniles of *G. pallida* or *G. rostochiensis* after 3 weeks. After one more week the plants were removed from the root trainers, soil washed from the roots and the plants suspended in funnels containing water. There were six replicated plants for each nematode potato combination and half of these were left in the glasshouse and the other half were placed in a growth cabinet with a daily cycle of 14h at 18ºC with light and 10h at 10ºC in the dark. Male emergence was monitored by draining 50ml from the funnel twice weekly and enumerating the number of males. The temperature of the water in a funnel in the glasshouse and growth cabinet was monitored using a USB-502-LCD data logger.

Reproduction of *G. pallida* and *G. rostochiensis* at different temperatures

The reproduction of *G. pallida* and *G. rostochiensis* on the susceptible cultivar Desirée was compared at three temperatures. 10 cysts for each species or a mixture of 5 each of the two species were placed in 10cm pots in a 1:1 steam sterilized sand:loam mix with a piece of sprouted Desirée tuber. Four replicates for each species or species mixture were placed in growth cabinets for 14h at 14, 18 or 20ºC with light and 10h at 10ºC in the dark in a random design. Pots were watered and fertilized as required. After 10 weeks half of the plants were removed from the growth cabinet and watering was stopped. The remaining plants were repotted into 20cm pots with 1:1 steam sterilized sand:loam. After a further 10 weeks the remaining pots were removed from the growth cabinets and watering stopped. Cysts were extracted from the soil by water flotation and counted.

Inter-specific competition of *G. pallida* and *G. rostochiensis*

DNA was extracted from cysts comprised of mixtures of the two species using a Qiagen DNeasy Blood & Tissue kit and used for qPCR analysis to determine the species composition. Primers and probes were designed for each species targeting the ribosomal internal transcribed spacer (ITS) (Blok *et al*., 2006).

RESULTS

Hatching in response to temperature

Little hatching by either species was observed in water over the entire temperature range though there was some hatching in the optimal temperature range from 13-25º. Both species were able to hatch in PRD over a wide range of temperatures but hatching in the 13-25º temperature range was more rapid during the first three weeks than at the lowest and highest temperatures. Hatching of *G. rostochiensis* was faster in the first 2 weeks than *G. pallida* at 13-15º whereas both species had similar hatching profiles at the higher temperatures from 23-29º. Hatching in PRD was still occurring after 5 weeks

Male emergence

Males of *G. rostochiensis* emerged approximately 7-10 days earlier than those of *G. pallida* in both the growth cabinet and glasshouse. Males of both species emerged ~ 1 week sooner in the glasshouse than the growth cabinets. The water temperatures in the funnel in the glasshouse were above 25ºC every day whereas those in the growth cabinet did not exceed 20ºC.

Reproduction of *G. pallida* and *G. rostochiensis* at different temperatures

Reproduction of *G. pallida*, *G. rostochiensis* or mixtures of the two species on the susceptible cultivar Desirée was compared after 10 and 20 weeks in three different temperature regimes. The differences in reproduction at the three temperatures after 20 weeks were much greater than after 10 weeks for both species with an increasing trend in reproduction with increasing temperature. After 10 weeks for *G. pallida* there was an increase in cyst numbers of ~50x at all three temperatures whereas after 20 weeks the increase from the initial cyst number was ~70, 250 and 800x at 14, 18 and 20º respectively. Similar results were obtained with *G. rostochiensis* with increasing numbers of cysts after 20 weeks with increasing temperature. The proportion of *G. pallida* in the mixtures after 20 weeks showed a slight increasing trend from 84.5% to 88% at 14 and 20º respectively.

DISCUSSION

There are reports that *G. rostochiensis* and *G. pallida* respond differently to temperature (McKenna & Winslow, 1972; Stone and Webley, 1975; Foot, 1978) and that this may affect how quickly they hatch and progress through their life cycles. These differences in temperature responses may also have implication for competition between the two species when present as mixtures and for responses of the host. We found that *G. rostochiensis* hatched more quickly than *G. pallida* at lower temperatures and this may give it a competitive advantage in host

invasion and feeding site establishment early in the UK growing season. However, these results differ from other reports including those of Foot (1978) who found a New Zealand population of *G. pallida* was more adapted to colder temperatures than *G. rostochiensis*. These observations may represent different adaptation of local populations and comparisons of more UK field populations are needed to assess whether *G. rostochiensis* and *G. pallida* show consistent differences in their responses to temperature. The two SCRI populations used in these experiments have been multiplied in glasshouse conditions for several generations and the temperature responses of UK field populations may differ. Differences in the temperature responses of populations within the same species can occur (Inagaki, 1977) and adaptation to temperatures in the field has been reported (Hominick, 1982) including faster reproduction at lower temperatures.

There was a difference of ~1 week between the two species in male emergence in both the glasshouse and growth cabinets with *G. rostochiensis* males appearing first in both environments. The emergence of females and timing of egg production by the two species was not monitored but it is reasonable to assume that *G. rostochiensis* would have completed its life cycle first.

After 20 weeks both species produced many more cysts compared to at 10 weeks. This increase at 20 weeks is assumed to be due to hatching from the cysts produced from the initial infection, and reinvasion by this second hatch to produce a second generation of cysts. This increase in cyst numbers after 20 weeks at the three temperatures tested implies that dormancy is not obligatory for PCN. Also the faster life cycle of *G. rostochiensis* indicated by the faster rate of hatch at lower temperatures and the faster emergence of males than *G. pallida* suggests that *G. rostochiensis* is more likely to complete 2 generations during the growing season. However, this requires further investigation with more populations of both species and a more detailed analysis of the life cycle in relation to temperature.

Temperature is a major factor that influences many aspects of the life-cycle of PCN; rate of hatch, development within the host and, significantly, the potential to initiate and complete a second generation in a single growing season. Potato cyst nematodes are found in many potato growing regions world-wide both in temperate and tropical countries (Brodie 1984). In temperate countries it has become generally accepted that PCN undergoes a single generation each year, with production of the eggs followed by an obligate dormant stage (diapause) which is not broken until the nematode has experienced a winter. In cold climates such as Finland PCN can complete its life cycle but early cropping can be effective in controlling PCN (Tiilikkala, 1987). However, selection of PCN populations which have adapted to local conditions can occur including adaptation to lower temperatures with a faster life cycle by repeated early cropping (Ellenby & Smith, 1975; Hominick, 1979, 1982).

Increasing soil temperatures in the future associated with climate change raises the prospect of a second generation of PCN occurring within a single growing season. The initiation and in some instances the completion of a second generation has been reported in UK (Jones, 1950; Evans 1969), France (Mugniery pers. comm.), Cyprus (Philis, 1980), Slovakia (Renčo, 2007) and Italy (Greco *et al*., 1988). The potential to select populations with more rapid life cycles that complete 2 generations of PCN in one growing season would exacerbate current problems with managing PCN. In temperate regions where there is a longer growing season the potential is greater for a second generation, especially associated with particular potato varieties such as

late maturing Cara (Dunnett, 2000). These factors should be considered with regard to regular development of a second generation of PCN in the UK.

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EXPLOITING THE *GLOBODERA PALLIDA* GENOME SEQUENCE FOR IMPROVED CONTROL

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Summary: A genome sequencing project for the potato cyst nematode *Globodera pallida* is currently in progress. This is a collaboration between SCRI, Leeds University, Rothamsted Research and the Wellcome Trust Sanger Institute. Work at SCRI is focused on identification and functional characterisation of secreted proteins that modulate interactions of *G. pallida* with the host – the effectors. A large family of effectors – the SPRYSEC proteins – has been identified in *G. pallida*. Some of these proteins localise to the cytoplasm when introduced into plant cells while others target the plant nucleus and nucleolus. The potential function and exploitation of this large gene family are discussed.

INTRODUCTION

Genome sequencing provides unparalleled insights into the basic biology of an organism. In the past, sequencing a eukaryotic genome was prohibitively expensive and the only such organisms for which genome sequences were available were model organisms such as *Caenorhabditis elegans* (The *C. elegans* sequencing consortium, 1998) and *Arabidopsis thaliana* (The *Arabidopsis* Genome Initiative, 2000) or organisms viewed of key importance to the development of medical science including mouse and human. By contrast, the small size of bacterial and viral genomes made sequencing a more realistic goal and genome sequences for a wide range of bacteria were rapidly sequenced, including plant pathogens (*e.g.* Bell *et al*., 2004). Information from these genome projects has assisted in the development of new management strategies for important crop pathogens and has allowed research into new control methods to be initiated.

The development and application of new, ultra high throughput sequencing technologies (*e.g.* Darby & Hall, 2008), along with a decrease in the costs of "traditional" sequencing methodologies has now led to a much wider application of genomics to eukaryotic organisms with large and complex genomes. In 2008 the first genome sequences of plant-parasitic nematodes were published (Abad *et al*., 2008; Opperman *et al.,* 2008).

Globodera pallida, the white potato cyst nematode (PCN), is the most economically important plant parasitic nematode in the United Kingdom and also causes problems for growers in many other parts of the world. Repeated use of cultivars containing the H1 gene, which provides complete control of the *G. rostochiensis* pathotypes present in the UK, has led to selection of *G. pallida* to such an extent that a recent survey found that it is present in 65% of fields used for growing potatoes in England and Wales (Minnis *et al*., 2002). A lack of major gene

resistance against *G. pallida,* coupled with legislative and consumer pressures for reduced inputs of effective nematicides, has led to problems in controlling this nematode. *G. pallida* is a biotrophic pathogen which induces complex changes in the roots of its host in order to produce a feeding site, on which it depends for all the nutrients required for its development. Like other biotrophic pathogens it needs to suppress host defences in order to keep the feeding site alive and complete its life cycle. The nematode factors that induce the formation of the feeding site and suppress host defences (the effectors) are produced in three gland cells (two subventral and one dorsal) and secreted into the host from a hollow protrusible stylet. Current work within the SCRI nematology group is focused on identification of *G. pallida* effectors and characterisation of their functions in the host parasite interaction with the overall aim of developing new control strategies against this nematode.

MATERIALS AND METHODS

The *G. pallida* sequencing project is a collaboration between SCRI, Leeds University, Rothamsted Research and the Wellcome Trust Sanger Institute. Details of the progress on the sequencing project can be found at http://www.sanger.ac.uk/sequencing/Globodera/pallida/. In addition to the genome sequence, a large Expressed Sequence Tag (EST) project for *G. pallida* has been undertaken (Jones *et al*., 2009).

Identification of effectors

Effectors were identified from *G. pallida* using two approaches. First, homologues of effectors identified in other plant parasitic nematodes (*e.g.* Elling *et al*., 2009) were sought. In order to identify novel or highly diverged effector sequences a bioinformatics pipeline (Jones *et al.,* 2009) was used that identified proteins with a signal peptide for secretion and lacked a transmembrane domain.

Subcellular localisation

The subcellular localisation of the nematode effectors in plant cells was investigated using translational fusions of the effectors with green fluorescent protein in Tobacco Rattle Virus vectors (Jones *et al*., 2009).

Analysis of the SPRYSEC gene family

One genome coverage of a fosmid library of *G. pallida* genomic DNA was spotted onto nylon filters and screened using probes generated from chorismate mutase (Jones *et al*., 2003) or SPRYSEC genes using standard protocols (Sambrook *et al*., 1989). For the *in silico* analysis, the amino acid sequence of the SPRYSEC protein RBP1 (accession number ACJ14490) was used to search the assembled supercontigs of the May 2009 *G. pallida* assembly through the project BLAST server (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/g_pallida).

RESULTS

A large number of candidate effectors have been identified from the *G. pallida* genome sequence and ESTs. These include homologues of a wide range of plant cell wall degrading or modifying enzymes (cellulases, pectate lyases, expansins) thought to have been acquired by horizontal gene transfer from bacteria (Jones *et al*., 2005). Effectors characterised from other plant parasitic nematode including chorismate mutase, CLE peptides and ubiquitin-like proteins were also identified. However, one of the most notable features of the *G. pallida* effector list was the presence of a large family of secreted proteins containing a SPRY domain - the SPRYSECs. This family has previously been identified in *G. rostochiensis* (Rehman *et al.,* 2009). Sixteen different SPRYSECs were identified in the EST dataset but subsequent analysis demonstrated that the family is considerably larger than this. Screening of the *G. pallida* fosmid library showed that 92 clusters of genes are present in *G. pallida* (Fig 1), although this figure may include some redundancy. Analysis of the currently available *G. pallida* genome data showed that 207 different genes similar to the SPRYSEC sequence RBP1 were present. Since less than half the genome has currently been assembled this suggests that the SPRYSEC gene family may consist of between 400 and 500 different sequences.

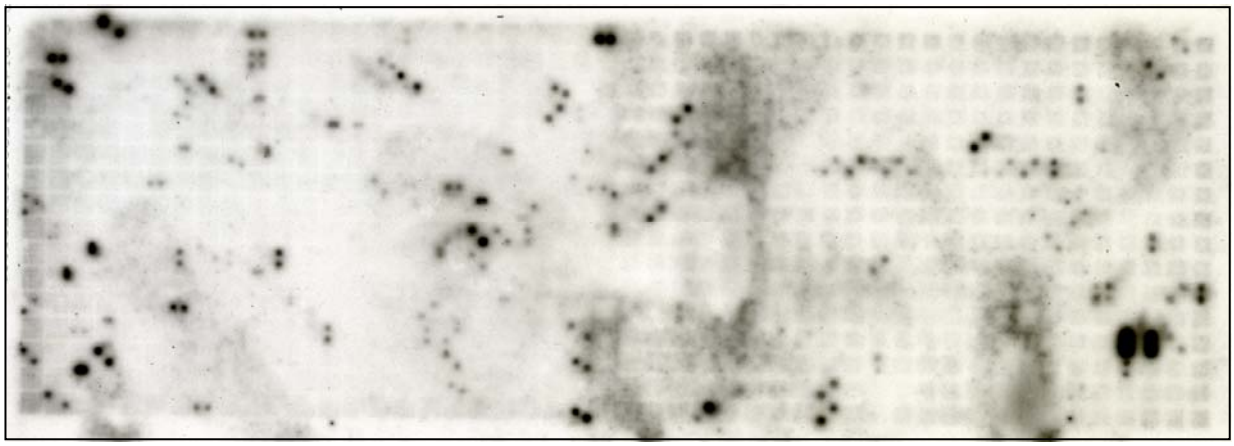

Figure 1. Hybridisation of a SPRYSEC probe to a fosmid library from *G. pallida*: 92 clusters of SPRYSEC genes are detected.

The subcellular localisation of five different *G. pallida* SPRYSEC genes was then examined. Some of the genes remained in the cytoplasm when introduced into plant cells (Fig 2a) while others were targeted to the nucleus and nucleolus (Fig 2b). The SPRYSEC proteins are therefore targeted to a range of plant subcellular structures.

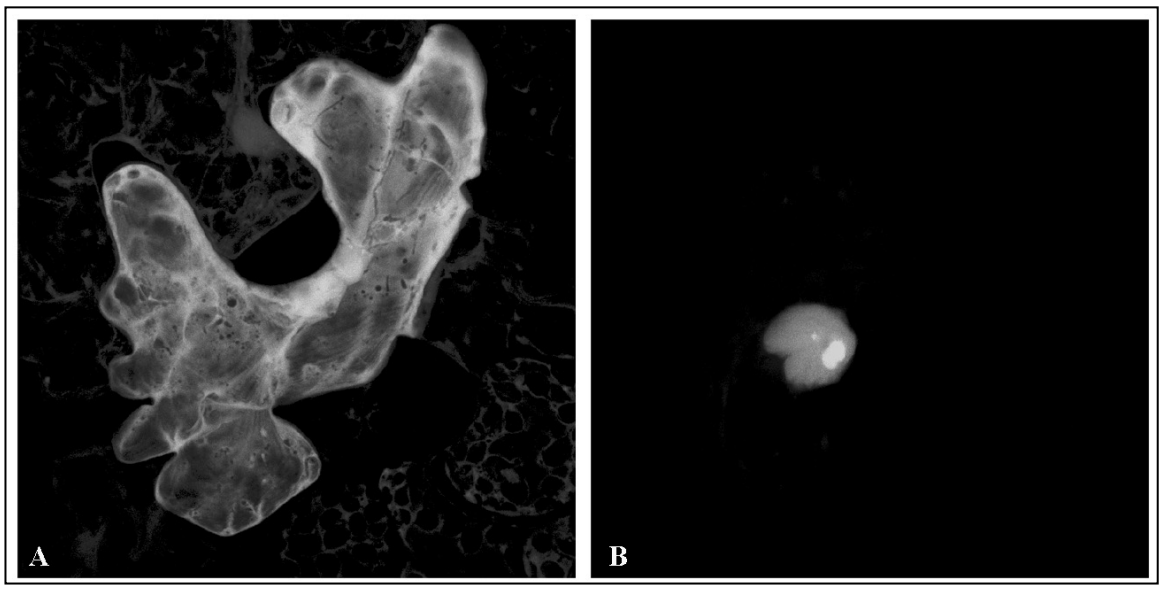

Figure 2. Subcellular localisation of SPRYSEC proteins: some localise in the cytoplasm (2A) while others are targeted to the nucleus (2B).

DISCUSSION

The *G. pallida* genome project will provide a range of scientific benefits including identification of the full suite of effector proteins secreted by this nematode during its interaction with the host. In addition, a wide range of practical benefits are likely to emerge from the project.

The SPRYSEC gene family represents the largest family of *G. pallida* effectors identified to date. It is therefore reasonable to assume that these genes are under strong diversifying selection pressure, possibly to evade recognition by the host. A recent study has identified one SPRYSEC protein as the first nematode avirulence factor –responsible for recognition by a host resistance gene (Gpa2) (Sacco *et al.,* 2009). It is possible that the SPRYSEC gene family harbours many other nematode avirulence genes; a large family of secreted effectors from the late blight pathogen *Phytophthora infestans* (the RxLRs) has been shown to contain a range of avirulence genes (Whisson *et al*., 2007). Identifying nematode avirulence genes has many important practical implications. A cloned avirulence gene can be used as a tool to identify potato breeding lines containing the cognate resistance gene without the need for nematode infections and screening. In addition, if SPRYSEC genes that are indispensable to the nematode can be identified these can be used to screen potato germplasm for novel resistance that should be durable in the field.

The project offers the opportunity for a change in our approach to management of *G. pallida* from the continued reliance on progressively failing control measures deployed for the last 30 years. The project will underpin the development of novel, benign nematicides by allowing identification of nematode specific or essential enzyme pathways that will be targets for these

chemicals. In addition, the identification of avirulence genes offers the prospect of improved breeding for resistance against *G. pallida*.

ACKNOWLEDGEMENTS

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POTATO VIRUSES PVA AND PVY – DO THEY HAVE THE SAME APHID VECTORS?

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Summary: Four years of field trials were carried out under Scottish conditions to assess the relative timing of transmission of PVY and PVA and to relate this to aphid activity. The variation observed between the different years indicates that the timing of transmission of both viruses is not constant. In all four seasons a measure of aphid vector pressure from the Gogarbank aphid suction trap provided a good explanation of the changes in the timings of the extent of PVY transmission. The epidemiology of PVA is less clear cut with the information from only two of the years (2008 and 2009) suggesting a good relationship between the aphid vector pressure and PVA transmission. As the patterns of changing incidence of PVY and PVA within the Scottish Seed Potato Classification Scheme have differed in recent years, it seems likely that there are some significant differences in the epidemiology of these two potyviruses and that these differences may lie in the species of aphids that play a significant role in their transmission.

INTRODUCTION

Potato virus Y (PVY) is a non-persistently transmitted potyvirus that is a major cause of crop loss in potatoes in many parts of the world, responsible for yield depression of up to 80% and total crop loss when in combination with other potato viruses. In Scotland, virus testing in support of the Seed Potato Classification Scheme (SPCS) over the period 1998-2009 has shown PVY to be responsible for, on average, 39% of all potato virus symptoms. Most natural spread of PVY can be attributed to aphids, with the Peach–potato aphid *Myzus persicae* recognised as the most efficient vector. Studies, mostly carried out under laboratory conditions using aphid and virus populations originating from north-western Europe, have demonstrated that another 25 aphid species are generally recognised as capable of vectoring PVY (Beemster & de Bokx, 1987). As some of these less efficient species can reach much higher field population levels in the UK than *M. persicae*, these species may be of greater significance in virus spread. In Scotland, the correlation between the incidence of mosaic symptoms and the suction trap catches of *M. persicae* is far weaker than it is for leafroll.

Like PVY, *Potato Virus A* (PVA) is a non-persistently transmitted potyvirus, but because it has traditionally been associated with mild symptoms, it has received very little attention in epidemiological studies. Most aphid species colonising potatoes are recognised as vectors, but the potential of non-colonising species to vector PVA is largely unknown although there has been at least one report of a non-colonising aphid, *Brachycaudus helichrysi,* vectoring PVA (Edwards, 1963). In many parts of the world where potatoes are grown, PVA is not one of the

more prevalent viruses. However, in the UK this virus has increased in incidence, particularly over the last 20 years. In Scotland, virus testing in support of the SPCS over the period 1998-2009 has shown PVA to be responsible for 20% of virus symptoms. Compared with PVY, PVA is prevalent in far fewer varieties, e.g. Desiree, Hermes and Estima, but as these are now some of the most popular varieties currently grown in the UK, the impact of PVA has become of increasing economic significance.

To develop aphid control measures that are appropriate for effective management of both of these non-persistent viruses, producers of healthy seed potatoes require a better understanding of which aphid species are responsible for the their spread. Key questions include whether both viruses are transmitted at the same time and are therefore likely to have the same aphid vector species. To obtain such information, SASA has conducted field trials at Gogarbank Farm to investigate how the timing of transmission of PVA and PVY (PVY^O) compares with aphid activity throughout four seasons (2006-2009). These trials involved the week by week exposure of tobacco bait plants within plots containing potato plants infected with PVA and PVY^O. The transmission of virus from potato to tobacco is supported by data covering transmission from potato to other potato plants, but on a much reduced scale. The basic design of the plot was adapted from a trial layout used at DARDNI, Northern Ireland (Roy Copeland, pers. comm.) to investigate timing of transmission of non-persistent viruses.

MATERIALS AND METHODS

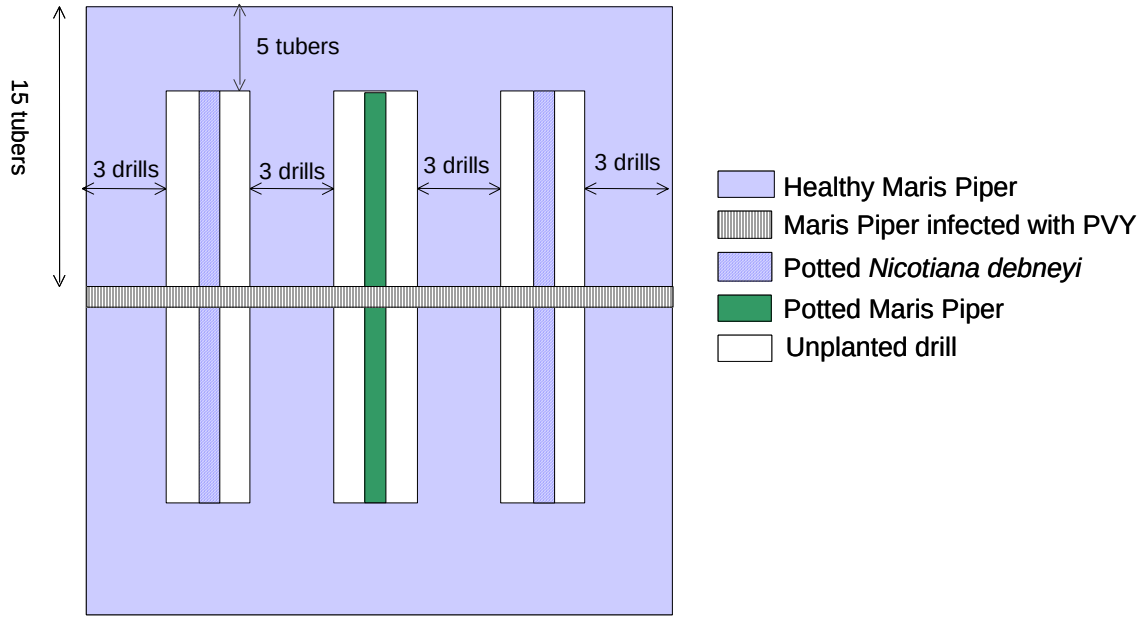

Figure 1. The trial design for investigation of the timing of transmission of PVY^O. A similar trial design using cv. Desiree was used for PVA.

The design of the trial plots (see Figure. 1) consisted of single rows of infected tubers planted across 21 drills. On each side of this row, healthy tubers were planted in a series of four blocks of three drills with ten tubers in each drill planted at 30cm intervals. Guard rows, five rows deep, were included at both ends of each drill across the entire width of the plot. Three sets of

three blank drills divided the blocks of healthy tubers. In the central set of blank drills on each side of the row of infected tubers, a total of 20 3-week old, glasshouse grown potted potato plants, were exposed for a period of one week. In each of the two lateral sets of blank drills, 12 pots of double potted, 3-week old *Nicotiana debneyi* bait plants were placed in the central drill of each set, on both sides of the row of infected tubers. Therefore a total of 96 tobacco bait plants were exposed to the virus source each week.

After exposure, the bait plants were removed from the field to the glasshouse and replaced with fresh 3- week old tobacco plants. In the glasshouse, the plants were immediately fumigated with nicotine shreds to prevent further aphid activity on the plants and subsequently grown in the glasshouse for a further three weeks. Leaves from each tobacco plant were then tested by ELISA, using monoclonal antibodies detecting either PVY^O (mAb raised against PVY^O strain, SASA, Edinburgh, UK) or PVA (mAbs raised against PVA strains 58/0 and 58/6, SASA, Edinburgh, UK) viruses. Negative controls were from healthy (non-exposed) *N. debneyi* plants. An ELISA test was deemed to be positive at an optical density value (A_{405nm}) greater than twice the mean of the negative control.

To investigate the transmission of PVY^O, both the infectors and the plot potatoes were cv. Maris Piper. Infected potatoes were known infected Maris Piper plants from the SASA Gogarbank collection. The rest of the plot was planted with commercial high-grade (Prebasic 2) seed potatoes. After 7 weeks of growth, leaves from all plants were sampled and tested for the presence of virus by ELISA. At this stage it can be confirmed that all plants grown from healthy tubers are free of any virus and that all plants grown from infected tubers were infected with PVY^O. For the PVA trial, the infectors and the plot potatoes were cv. Desiree and the trial was otherwise conducted in a similar manner.

Information on aphid activity was obtained from the collections of a 12.2 m tall suction trap (Macaulay *et al*., 1988) as used by the Rothamsted Insect Survey. This suction trap is located at Gogarbank and operated by SASA. All aphid species are identified and an index of aphid vector pressure can be calculated by summing the totals of each species known to vector non-persistent potato viruses multiplied by published relative efficiency factors for that species. The factors used are those adopted in the UK by the Potato Council (http://aphmon.csl.gov.uk) based on published studies that have assessed the efficiency of different aphid species to transmit a range of isolates of PVY (both PVY^O and PVY^N strains). No published efficiency factors are available for PVA.

RESULTS

Figure 2 illustrates the results of four years of field trials comparing the week-to-week variation in the rate of transmission of PVY from infected Maris Piper plants to *Nicotiana debneyi* plants with that of PVA from infected Desiree to *N. debneyi*. Although the data are not presented here, the results of transmission from infected potato plants to 3-week old, glasshouse grown potted potato plants closely reflects the pattern of transmission to *N. debneyi*. The data for transmission to *N. debneyi* are presented because they are sourced from a weekly sample size of 96 plants per trial, rather than the more limited data set of 20 plants per trial per week for the transmission to potato.

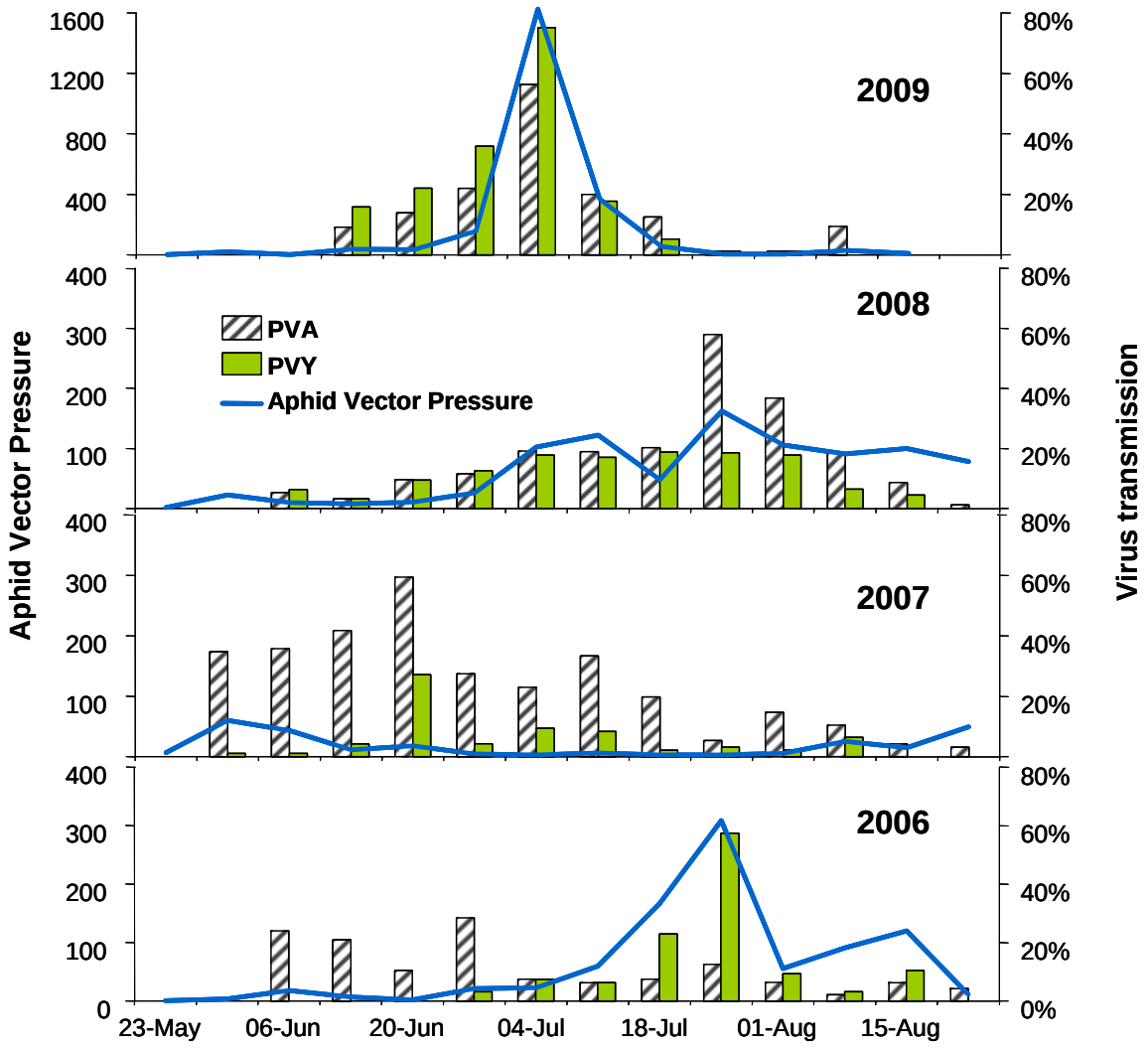

Figure 2. Week-to-week variation in the extent of transmission of *Potato Virus Y* (PVY^{O}) and *Potato Virus A* (PVA) in relation to variation in aphid vector pressure as measured by the Gogarbank aphid suction trap – 2006 to 2009 trial data.

2006

There was a marked peak in PVY transmission in late July that coincided with the peak in aphid vector pressure as recorded by the Gogarbank suction trap. No corresponding peak in the transmission of PVA was observed in 2006; instead a higher rate of transmission occurred earlier in the season, i.e. throughout June.

2007

With the exception of a brief peak in PVY transmission that was recorded for a single week in late June, transmission rates remained relatively low throughout the season. In contrast, PVA transmission rates were relatively high from the start of the trial in late May until mid July. The activity of known aphid vectors of potyviruses remained low throughout the season.

2008

The transmission rates of PVA and PVY remained very similar throughout the season, with the exception of a 4-week period at the end of the season when higher levels of PVA transmission were recorded. Aphid vector pressure was moderately high from late June through to early August, corresponding well with the amount of virus transmission

2009

There was a marked peak in the transmission of both PVY and PVA in late June and early July, coinciding with a peak in aphid vector pressure. The peak in aphid activity that occurred in early July was exceptionally high, so a different scale is used for 2009 as opposed to the other three years (peak at 1600 in 2009 as opposed to a maximum of 300 in 2006). Therefore, it appears as though the extent of virus transmission occurring in mid to late-June was greater than may be expected from the aphid vector pressure.

DISCUSSION

The first two years of this study show higher transmission rates of PVA in the earlier part of the growing season, when compared to the transmission of PVY. In contrast there was slightly greater transmission of PVA in the later part of the season in 2008. In 2009, the patterns of transmission of both viruses were similar. The variation observed between the different years indicates that the timing of transmission of both viruses is not constant. For the 2 years with clear peaks in PVY transmission, the peak was late in the season in 2006 (25 July) and three weeks earlier in 2009 (4 July). For a short-lived crop such as potatoes, such variation in the timing of transmission has significant implications for both the epidemiology and management strategies for these viruses. For the other two years, PVY transmission was either very low (2007) or moderate over a more prolonged period (2008).

In all four seasons the aphid vector pressure calculated using Gogarbank suction trap data and published measures of the efficiencies of individual aphid species as vectors of PVY provide a good explanation of the changes in the timings of the extent of PVY transmission. In 2006 and 2009, the aphid vector pressure reached peak levels at times coincident with the transmission of PVY. In 2007 the vector pressure was very low throughout the season and in 2008 it was moderately high from early July onwards. Therefore, it is reasonable to conclude that monitoring aphid activity using 12.2m suction traps (Macaulay *et al*., 1988) can provide accurate information with which to assess the risk of PVY transmission.

The epidemiology of PVA is less clear cut. The information from 2008 and 2009 suggests a good relationship between the aphid vector pressure and virus transmission. However the data from 2006 and 2007 are far less convincing. Firstly there is no evident correlation between periods of transmission of PVY and PVA, secondly both years showed high early season transmission of PVA during periods of low aphid activity and finally, in 2006 when PVY transmission responded to a peak in aphid vector pressure, there was no corresponding response for PVA transmission.

In recent years, SASA has requested leaf samples from plants seen to exhibit virus symptoms during inspections of Scottish seed potato crops. The results of diagnostic tests on these

samples can be used to monitor changes in the prevalence of different potato viruses. The patterns of changing incidence of PVY and PVA within the SPCS have differed in recent years (Figure 3). These changes cannot be simply explained by changes in the proportions of susceptible varieties under cultivation. Therefore, it seems reasonable to conclude from both the field trial and the SPCS data that there are at least some significant differences in the epidemiology of these two potyviruses. Ongoing work will hopefully elucidate whether these differences lie in the species of aphids responsible for their transmission, or elsewhere.

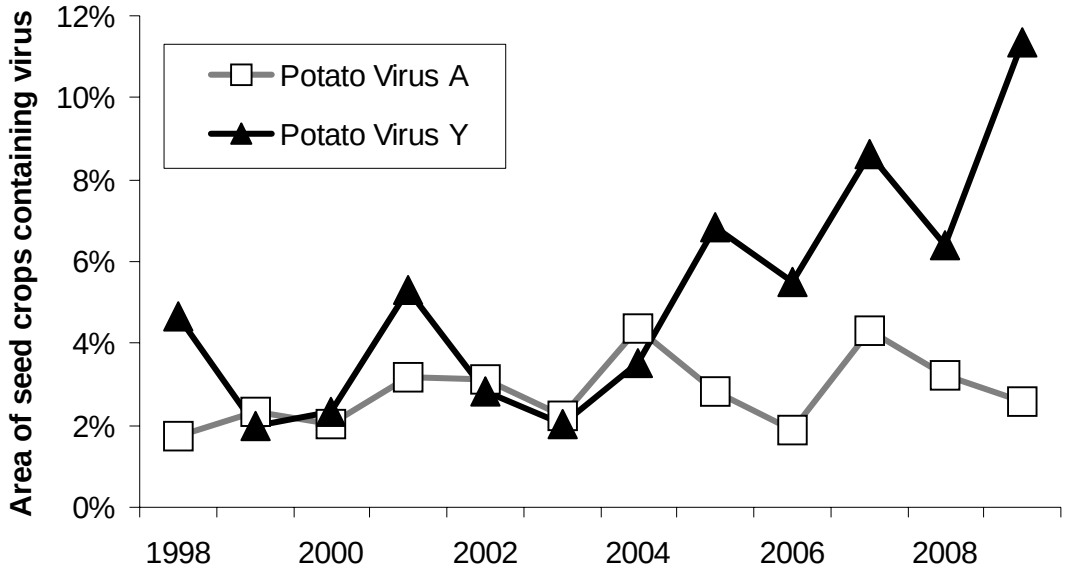

Figure 3. Estimated season-to-season variation in the incidence of Potato *Virus Y* (PVY) and *Potato Virus A* (PVA) in the Scottish Seed Potato Classification Scheme – 1998 to 2009.

ACKNOWLEDGEMENTS

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USING REAL-TIME PCR TO IDENTIFY POTATO STOCKS AT RISK OF SKIN SPOT

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Summary: Over 50 stocks of seed and ware tubers were tested at harvest for levels of *Polyscytalum pustulans* (skin spot) DNA using real-time PCR. These stocks were stored for 20 weeks under conditions that would encourage skin spot to develop. There were large differences in skin spot development, and pathogen DNA levels, between individual cultivars. Despite this, there was a general effect of harvest date, whereby crops harvested in November had higher levels of *P. pustulans* DNA at harvest, and skin spot symptoms after storage, than crops harvested in September and October. The ability of the molecular test to predict which stocks most at risk of developing skin spot is discussed.

INTRODUCTION

The fungus *Polyscytalum pustulans* is the causal agent of skin spot in potatoes. Symptoms of the disease includes lesions on roots, stolons and the below ground parts of stems. In storage, purplish-black, slightly raised spots with a diameter up to 2 mm develop on tubers following a period of at least 6 weeks (Allen, 1957). Infected seed tubers are considered the primary source of inoculum although the sclerotia of the fungus can remain viable in the soil (Hide & Ibrahim, 1994). The disease has been effectively controlled through a combination of cultivar resistance, fungicides and various cultural controls. Inclusion of the disease in UK seed potato classification schemes has also contributed to the control of skin spot. As a consequence of these measures, it was thought that the incidence and severity of the disease was in decline until recently (Allen & Scott, 2001; Lees *et al*., 2008).

The withdrawal of the seed treatment agrochemical, 2-aminobutane, in 2007 and the development of resistance to thiabendazole (Lees *et al*., 2008; Carnegie *et al*., 2008) may lead to an increase in the prevalence of the disease in UK potato crops. Recently, a real-time PCR assay for the pathogen was designed at the Food and Environment Research Agency (Budge *et al*., 2007). Real-time PCR is an established technique in plant diagnostic laboratories and can be used for the detection and quantification of the pathogen prior to the development of symptoms.

The aim of this study was to determine if a real-time PCR assay for *P. pustulans* could be used to quantify levels of the pathogen in potato stocks at harvest in order to determine the risk of skin spot developing in storage. The effect of cultivar and harvest date on disease incidence and pathogen DNA level was also investigated

MATERIALS AND METHODS

In 2008, samples of tubers of c. 25 kg were collected from the main potato-growing regions in Great Britain at harvest. Tubers were hand-graded to remove <35 mm and >85 mm sized tubers. Each sample was divided into two sub-samples: one sub-sample of 50 tubers was taken for immediate real-time PCR quantification in tuber peel at harvest; and the other stored at 6°C and 95% relative humidity with minimal curing to encourage skin spot development. After 20 weeks storage, these tubers were hand washed and then visually assessed for the disease. DNA was also extracted from tuber peel and the pathogen quantified by real-time PCR.

DNA was extracted from a strip of peel removed from each tuber (from rose to stolon end). Peel strips from each sub-sample of five tubers were weighed and placed into an extraction bag (Bioreba Ltd) containing 7.5 ml PB7 (2 ml tetrasodium pyrophosphate in 100 ml phosphate buffer pH7). The contents within each extraction bag were pulverised using a large sample grinder (Homex Ltd). The supernatant was then transferred into a labelled 5 ml sample tube. Sample tubes were spun at 1000 rpm for 10 minutes at 4°C (Sigma 4K15 centrifuge) and the supernatant transferred to a clean, labelled 5 ml tube and spun at 6200 rpm for 15 minutes at 4°C (Sigma 4K15 centrifuge) to pellet any sediment. DNA was extracted from each pellet using the Wizard® Magnetic DNA Purification System for Food kit (Promega) in conjunction with a Kingfisher ML magnetic particle processor (Thermo Electron Corporation). The extractions were completed using the gDNA program including the optional heating stage. Samples were eluted into 200 µl Tris-EDTA (TE) buffer and stored at –30°C until required.

All real-time PCR reactions were performed in 384-well reaction plates using TaqMan® Universal PCR MasterMix (Applied Biosystems). For each reaction, 10 µl DNA (diluted 1/5 with TE buffer) was added to 15 µl of master mix in the appropriate well. The PCR mix contained 200 µM dNTP mix, 0.3 µM primers, 0.1 µM fluorogenic probe and 0.125 U AmpliTaq Gold DNA polymerase in a 25 µl reaction volume. Reaction conditions were an initial 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Assays were performed using an Applied Biosystems 7900HT real-time PCR system.

RESULTS

Fifty-three samples representing ten different cultivars were obtained. The number of samples for each cultivar is shown in Table 1. After 20 weeks storage, all samples with skin spot had less than 1.3% mean surface area (i.e. severity) affected. The incidence of skin spot varied from 0 to 64% tubers affected. From these results there appeared to be wide differences between potato cultivar and levels of skin spot as determined visually post storage and by real-time PCR at harvest. Kerrs Pink and Lady Rosetta had higher incidence levels than the other varieties tested.

Overall, there is a clear relationship between DNA level on tubers at harvest and incidence of visual symptoms after storage. The amount of detectable pathogen DNA increased, with increasing incidence of skin spot detected at the end of a 20-week storage period with a goodness of fit for the exponential regressions, R^2, of 0.65 (Figure 1).

Table 1. Skin spot incidence (after 20 weeks storage) and DNA level (at harvest) in different cultivars.

Cultivar	Number of samples	Skin spot incidence (%)	Standard Error	DNA level at harvest (DNA pg μl^{-1})
Pentland Dell	2	2.9	6.08	132
Desiree	2	8.2	6.40	620
King Edward	19	7.0	2.06	285
Hermes	2	5.9	6.08	2229
Kerrs Pink	3	28.7	5.10	3307
Melody	1	4.3	8.82	37
Maris Piper	13	0.6	2.41	93
Lady Rosetta	4	21.1	4.23	181
Sante	2	0	6.06	63
Saturna	5	14.8	3.88	2260

Table 2. Effect of harvest date on skin spot incidence and DNA level at harvest.

Harvest date	Number of samples	Skin spot incidence (%) after 20 weeks storage at 6 °C	Standard error	DNA level at harvest (DNA pg μl^{-1})
September to early October	12	3.6	2.74	449
Mid to late October	27	6.5	1.72	218
November	14	14.7	2.55	692

Harvest date appeared to play a critical role in determining the levels of skin spot present. In general, the incidence of skin spot symptoms increased with later harvests (P=0.011; Table 2), although less DNA was detected. Crops that were harvested between 11 September to 16 October and 20 to 28 October had a mean skin spot incidence of 3.6% and 6.5%, respectively. Crops harvested in November had a mean skin spot incidence of 14.7%.

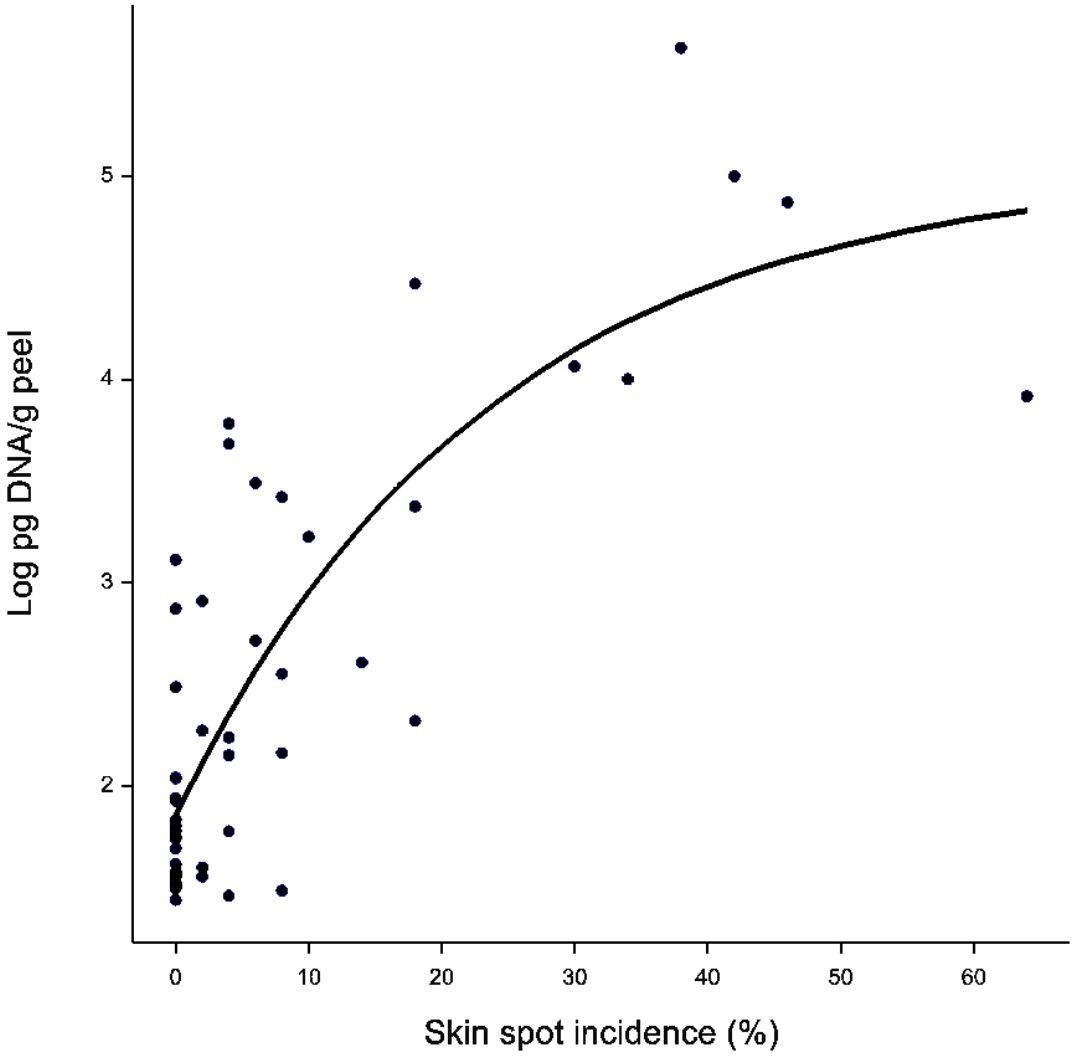

Figure 1. The relationship between *Polyscytalum pustulans* DNA level (log_{10} pg DNA/g peel) detected on progeny tubers at harvest and skin spot incidence on progeny tubers after storage ($R^2 = 0.65$).

DISCUSSION

Here we demonstrate the use of a real-time PCR assay to detect *P. pustulans* in 53 stocks of potatoes. The amount of *P. pustulans* DNA detected in tuber peel at harvest increased with disease development. The relationship between DNA levels in tuber samples at harvest and at the end of the subsequent storage period, and skin spot incidence on progeny tubers at the end of storage was best described by an exponential model or 'asymptotic regression'. The relatively good relationship could allow a prediction of the risk of skin spot developing from tubers tested previously at harvest. This could allow measures to manage the disease in store to be implemented.

However, this was an overall trend found in stocks of different varieties and cultivar. Large differences were observed between individual cultivars. In addition, harvest date appeared to play a critical role, with later harvest generally associated with increased risk of skin spot development. Further investigation of the specific influence of these factors could enhance the use of this assay to predict risk.

This work indicates that growing crop duration is a significant component of the disease epidemiology. This, as a factor, has been under-investigated to date. It is recommended that further work is done to refine the relationship between pathogen DNA levels in peel and skin spot levels by considering factors such as cultivar, crop duration and also geographical location of crop.

The data from the trial reported here supports the use of a quantitative PCR test in predicting which stocks are at risk of skin spot. Real-time PCR is a sensitive technique, whilst only 51% of tuber samples developed visible symptoms resembling skin spot after 20 weeks in store, 87% of samples had detectable levels of *P. pustulans* DNA. This may suggest that the pathogen is more widely prevalent than originally thought, albeit at levels too low to cause disease in many instances. Previous work determining the occurrence of *P. pustulans* in Scotland in the 1980s using baiting methods found *P. pustulans* in 32 out of 60 soil samples (Carnegie & Cameron, 1990). This is similar to the percentage of tuber samples with visible infection in this study.

This assay could also be used to quantify the level of pathogen in the soil. Lees *et al*. (2008) have separately developed a real-time PCR assay for *P. pustulans* and demonstrated its use on artificially infested soil. Real-time PCR assays are likely to be more sensitive than traditional methods to detect the pathogen in soil, such as direct isolation or the use of bait plant, and this would give new insights into the epidemiology of the disease.

ACKNOWLEDGEMENT

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FLUDIOXINIL – A NOVEL BROAD SPECTRUM POTATO SEED TREATMENT WITH ACTIVITY AGAINST COMMON SCAB AND SKIN SPOT

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Summary: A fludioxonil based formulation is currently undergoing assessment for approval as a treatment for potato seed tubers in the UK. Fludioxonil is known to have activity against a range of pathogens that can affect seed tubers. In a series of replicated trials, unreplicated plots in different locations in Northern Britain plus a glasshouse study, fludioxinil treatment consistently reduced the incidence and severity of skin spot and common scab, compared to an untreated control. Frequently the reduction observed was significant. It is unclear how a fungicide seed tuber treatment results in the reduction of common scab, a bacterial disease, but the glasshouse study suggests fludioxinil seed tuber treatment is effective against soil- and seed-borne inoculum.

INTRODUCTION

Potato seed tuber treatment with fungicide continues to be a significant element in the management of tuber pathogens down the production chain. Whilst there are several active ingredients available for control of *Rhizoctonia solani*, there are only two active ingredients (imazalil and thiabendazole) available for control of other tuber-borne pathogens. The reliance on these two fungicides increases the risk of resistance developing in pathogens. Thus the potential introduction of a novel fungicide seed treatment, especially one that embraces control of *R. solani* and tuber-borne diseases in a single product, is welcome.

Management of common scab (*Streptomyces spp*. including *S. scabiei*) currently relies on maintaining a low soil moisture deficit during and after tuber initiation. This is normally achieved by irrigation since, despite considerable testing; no consistent and effective chemical control treatments have previously been identified for this bacterial pathogen (Stead & Wale, 2004; Wale & Sutton, 2004).

This paper describes the results of a series of development trials conducted in Northern Britain with a potato seed tuber treatment, active ingredient fludioxinil, targeted at the reduction of common scab and skin spot. This seed treatment has been available for a numbers of years in non EU countries and has shown activity against a broad spectrum of potato pathogens including *Rhizoctonia solani*, *Helminthosporium solani* (silver scurf), and *Colletotrichum coccodes* (black dot). The product is currently under evaluation with the UK Regulatory Authorities and they will assess the efficacy data presented on these pathogens as well as *Streptomyces spp*. (common scab).

MATERIALS AND METHODS

Field trials were carried out by SAC from 2005 to 2008 where seed tubers were treated on a roller table with fludioxinil (25.0 g a.i./ tonne). The product was applied in c. 2 litres spray solution per tonne using two Delavan HC1.5 hollow cone nozzles at a pressure of 2 bars placed side by side across the roller table. Untreated tubers were always passed across the roller table prior to fludioxinil application. Treatments were carried out during early spring prior to planting (February to early April) and seed was usually unsprouted at the time of application. The manufacturer recommends application to dormant seed tubers. In replicated field trials, seed tubers were planted in a randomised block design. Fludioxinil treated tubers were compared to an untreated control (and other fungicide treatments – not reported here). Plot sizes were almost always four drills by 6.25m length (c. 100 seed tubers a plot) with assessments carried out on the inner two drills. Fertiliser and crop protection programmes were applied as per local practice. The trials were targeted at a range of pathogens but the data reported here concentrate on the results obtained with common scab or skin spot. The cultivar Maris Piper was mainly used in the common scab trials and Kerrs Pink in skin spot work. However, in one common scab trial varieties with a range of susceptibilities were used. All results reported here are from unirrigated field trials. The impact of treatment on tuber disease was made by assessing 50-100 tubers per plot sampled randomly after harvest.

Small unreplicated demonstration plots were planted from a stock of untreated and fludioxinil treated Maris Piper at a range of locations in northern Britain from Yorkshire northwards. At harvest 50-100 tuber samples were taken and assessed for common scab.

A glasshouse trial was carried out in 2008 to investigate the efficacy of fludioxinil against seed- and soil-borne *Streptomyces* spp. Commercial seed showing symptoms of common scab and symptomless mini-tubers of cv. Maris Piper were either treated with fludioxinil or untreated and planted into buckets containing field soil (sandy loam) or compost. The experiment was factorial with fludioxinil/untreated, growing medium and seed source as factors. There were six replicates. The crops were grown to maturity with water restricted around tuber initiation to encourage infection by *Streptomyces spp*. Tubers were harvested and assessed for incidence and severity of common scab.

RESULTS

a. Skin spot

In two trials investigating activity against skin spot, fludioxinil resulted in significant reductions in skin spot severity over the untreated control. The level of control was comparable to or better than that from imazalil (Table 1).

b. Common scab

In three replicated trials from 2005 to 2008, there were significant reductions in incidence and severity of common scab compared to the untreated control in two trials as a result of fludioxinil seed tuber treatment (Figure 1). These trials were carried out during 2005 and 2006 which were very dry seasons, particularly around tuber initiation. The third trial was carried out in 2008, a much wetter season. In 2008, fludioxonil did not significantly reduce incidence and severity but with fludioxinil treatment severity was under half that of the untreated control and this finding was in-line with the effects seen in the two previous studies.

Table 1. The incidence and severity of skin spot developing 5 months post harvest following pre-planting application of fungicides to seed tubers. Cv. Kerrs Pink

Treatment	% Incidence 2005/06		% Severity 2005/06		% Incidence 2006/07		% Severity 2006/07	
Untreated	77	a	7.6	a	90	a	8.05	a
imazalil (Fungazil 100SL)	61	ab	3	a	87	a	4.35	bc
fludioxinil	60	ab	2.2	b	78	ab	3.68	bcd
LSD (P=0.05)	16.2		2.82		14.9		1.724	

Source: Hilton & Wale (2006)

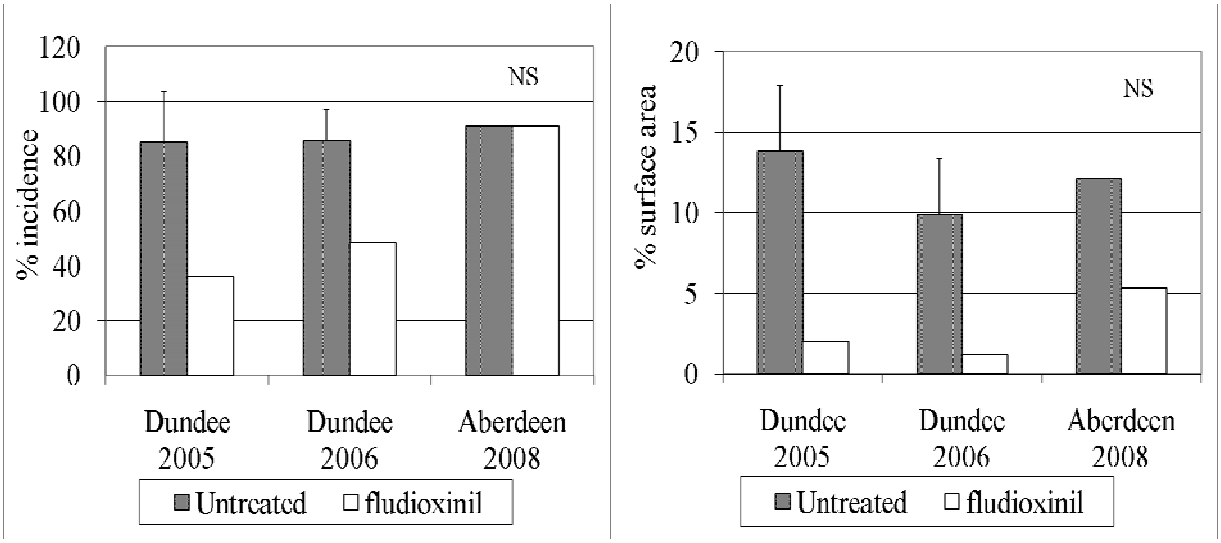

Figure 1. Incidence and severity of common scab on tubers grown from untreated and fludioxinil treated Maris Piper seed in three trials (bars are LSD)

In 2008, a second field trial evaluated the interaction of cultivar resistance to common scab (Anon., 2009) and fludioxinil seed tuber treatment. A range of varieties were tested with differing resistance to common scab ranging from very susceptible (Maris Piper) to moderately resistant (Orla). Reductions in incidence and severity as a result of fludioxinil seed treatment occurred with the susceptible cultivars Maris Piper, Spunta and Desiree but not with the cultivars Vales Everest, Marfona and Orla (Figure 2). Significant reductions were only recorded with incidence in the cultivars Maris Piper and Desiree.

The levels of common scab developing at ten demonstration sites, established in 2009 from a single stock of Maris Piper treated with fludioxinil or untreated varied substantially. Average common scab levels (incidence) for untreated and fludioxinil treated were 86% and 73%. When the tubers were assessed for common scab severity the averages were 10.5% and 6.3% respectively. To ascertain the impact of fludioxinil treatment on marketability, a threshold of

5% surface area was adopted above which tubers were considered unmarketable. Increases in tubers achieving marketability as a result of fludioxinil treatment ranged from 0% to 52% with an average of 17% (Figure 3).

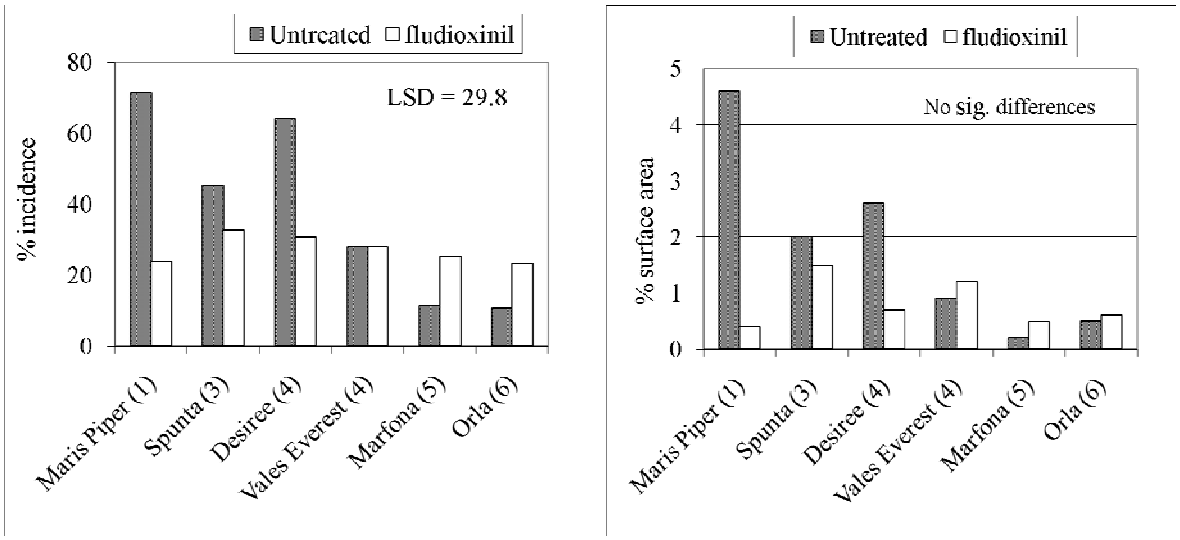

Figure 2. Incidence and severity of common scab on tubers grown from untreated and fludioxinil treated seed of six varieties with different resistance ratings (in brackets; Anon., 2009) to common scab at Dundee in 2008

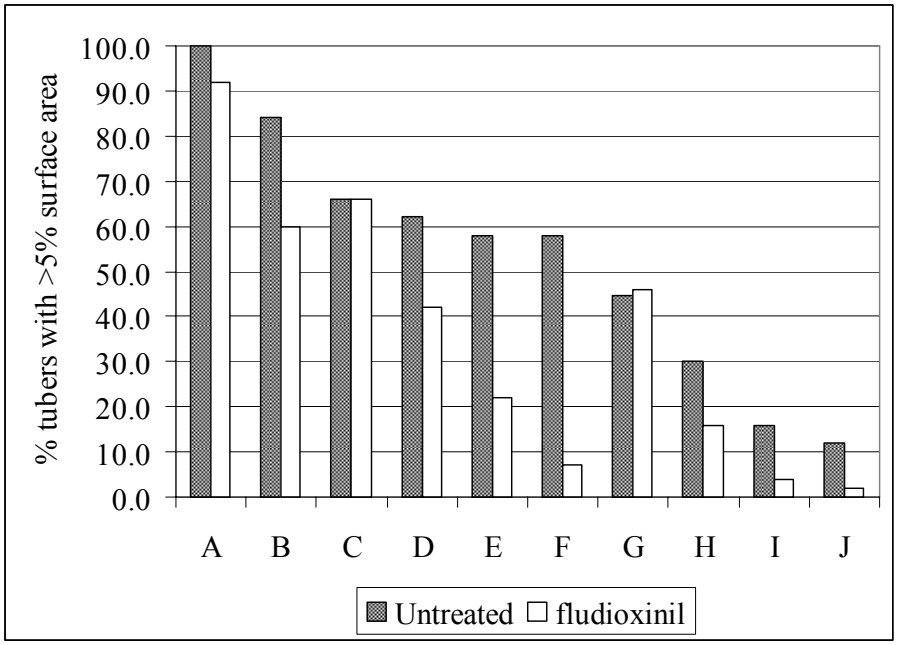

Figure 3. Percentage of tubers with greater than 5% surface area of common scab on tubers grown from untreated and fludioxinil treated seed of a stock of Maris Piper grown in unreplicated blocks at 11 locations in northern Britain in 2009

In the glasshouse study, fludioxinil treatment significantly reduced the incidence and severity of common scab overall (Table 2). There was also a significant effect of growing medium and seed source on incidence and of seed source on severity of common scab. Commercial seed (with symptoms of common scab) planted into soil resulted in the highest incidence of common scab, although this was not significantly greater than the incidence for commercial seed planted in compost. Mini-tubers planted in soil resulted in a significant reduction in incidence whilst the combination of mini-tubers and compost resulted in the lowest incidence of common scab.

Severity of common scab followed the same pattern as incidence. However, the greatest severity was the combination of commercial seed and compost, significantly more than any other combination. Fludioxinil treatment reduced incidence and severity across every combination of seed source and growing medium, mostly significantly.

Table 2. Interaction of seed treatment, seed source and growing medium on percent incidence (a) and severity (b) of common scab in a factorial glasshouse experiment. Cv. Maris Piper

a)

	Commercial Seed		Mini-tubers		
Treatment	Compost	Soil	Compost	Soil	Average
Untreated	71.9	84.5	17.6	51.8	56.4
fludioxinil	47.2	53.4	12.3	44.9	39.4
LSD's	Treatment 15.72 *, Medium 15.72 ***, Seed source 15.72 *** Treatment x Medium 22.2 ns, Tuber type x Medium 22.2 ns, Treatment x seed source 22.2 ns, Treatment x Tuber type x Medium 31.4 ns				

b)

	Commercial Seed		Mini-tubers		
Treatment	Compost	Soil	Compost	Soil	Average
Untreated	24.4	9.4	0.3	6.3	10.0
Fludioxinil	3.6	2.8	0.1	1.0	1.9
LSD's	Treatment 4.27***, Medium 4.27 ns, Tuber type 4.27*** Treatment x Tuber type 6.04 * Treatment x Medium 6.04 ns, Tuber type x Medium 6.04 *, Treatment x Tuber type x Medium 8.54 *				

DISCUSSION

With the withdrawal of the fumigant 2-aminobutane as the most effective fungicide treatment for the control of skin spot, reliance has fallen on imazalil or imazalil + thiabendazole fungicide seed tuber treatments alone. These are spray-applied tuber treatments. The introduction of another spray-applied option for the reduction of skin spot which is equal to or more effective than imazalil may help to limit development of fungicide resistance.

Reduction or control of common scab can be effectively achieved by soil moisture management at and after tuber initiation. However, the option to manipulate soil moisture by

irrigation is not always available and sufficient irrigation to sustain a low soil moisture deficit is not always achievable. Where a highly susceptible variety is grown, the availability of an additional management tool for reducing common scab would be valuable.

Fludioxinil seed treatment has shown consistency in reduction of common scab across a series of trials. The level of reduction of common scab has varied but on occasions has been substantial. Understanding what influences the level of activity will be important for its effective use. However, the ability of a seed tuber fungicide treatment to provide a reduction in common scab may provide support to irrigation where it is applied in controlling common scab especially if irrigation is not as effective as required. Additionally, where a cultivar is susceptible or highly susceptible to common scab and irrigation is limited, fludioxinil can provide some protection if dry conditions persist.

The glasshouse study showed that both seed tuber- and soil-borne inoculum contributed to the incidence and severity of powdery scab. However, fludioxinil seed treatment appeared to be effective against both sources of inoculum. The mechanism of this activity is not understood.

From one trial, it would appear that a cultivar / seed treatment interaction exists for the reduction of common scab. Reduction of common scab as a result of fludioxinil seed treatment was greatest in susceptible or highly susceptible cultivars. Thus, whilst the seed treatment is broad spectrum and has benefits for all cultivars, where control/reduction of common scab is specifically required treatment will be of most value to susceptible cultivars.

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POTENTIAL MANAGEMENT OF FUNGAL POTATO DISEASES USING INORGANIC SALTS

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Summary: Proposed restrictions on synthetic fungicide use illustrate the importance of intensifying research into and development of effective alternative methods of disease control. Our literature survey demonstrated that inorganic salts have potential to partially replace some of the synthetic fungicide used on potato (*Solanum tuberosum* L.) crops in the UK. Foliar sprays (preventive) or dip treatments (preventive and curative) of inorganic salts (phosphites, bicarbonates, chlorides, phosphates or carbonates) were found to significantly reduce the severity of foliar and tuber late blight (*Phytophthora infestans*), silver scurf (*Helminthosporium solani*), dry rot (*Fusarium sambucinum*) and pink rot (*Phytophthora erythroseptica*). Tuber late blight was the most effectively controlled disease with 91% decrease in severity following foliar application of a phosphite salts product. Therefore, the use of inorganic salts in potato integrated disease management strategies may be useful and merits further investigation. Regulatory innovation will be needed to register such compounds as biopesticides in the UK.

INTRODUCTION

There is increasing pressure for potato growers to reduce reliance on conventional fungicides and adhere to the principles of integrated disease management. Contributing factors have been the public health and environmental concern about pesticide (including fungicides) use (Mandal *et al*., 2009), the rising financial costs (Mann *et al*., 2004) and the risk of loss of important fungicides from 2011 onwards due to the proposed revision of EU Directive 91/414/EEC (Richardson, 2009). There is now a considerable risk that important active substances, such as mancozeb, may be withdrawn, leading to resistance management problems and substantial yield reductions (PSD, 2009). In order to keep the UK potato (*Solanum tuberosum* L.) industry competitive, it is therefore necessary that research efforts are channelled towards the development and utilisation of alternative, environmentally benign, cost effective and efficient methods of fungal disease control.

Past research at Harper Adams University College and a preliminary search of the scientific literature have shown that foliar applications of inorganic salts suppressed important fungal diseases of cereal (e.g. septoria leaf blotch of wheat, *Septoria tritici*; Mann *et al*., 2004) and cucurbit (e.g. powdery mildew of cucumber, *Sphaerotheca fuliginea*; Ziv & Zitter, 1992) crops.

The above findings prompted further investigations on the potential of inorganic salts as fungal control agents (Deliopoulos *et al*., 2008a,b; Deliopoulos *et al*., 2009a,b). Deliopoulos *et al*. (2008b) have reviewed the global scientific and technical literature on fungal disease management with inorganic salts and assessed the scope for their potential use in the UK on crops with intractable disease problems.

The present article summarises information on the efficacy of inorganic salt treatments against potato diseases and assesses whether these compounds have potential for reducing conventional fungicide input.

MATERIALS AND METHODS

Data were collected from peer-reviewed publications retrieved by performing keyword searches in bibliographic databases including Web of Science, ScienceDirect and Google Scholar. Information on formulation, other technical aspects and current commercial use of inorganic salt-based fungicides was obtained from manufacturers' websites as well as through direct communication with companies selling these products.

RESULTS

Evidence for antifungal activity on potatoes was found for 15 different inorganic salts, which included six phosphites (e.g. KH_2PO_3; Cooke & Little, 2002), three bicarbonates (e.g. NH_4CO_3; Olivier *et al*., 1998), two chlorides (e.g. $AlCl_3$; Hervieux *et al*., 2002), two phosphates (e.g. K_2HPO_4; Strömberg & Brishammar, 1991) and two carbonates (e.g. Na_2CO_3; Olivier *et al*., 1999).

Potato diseases for which published evidence was found for reduced severity in response to treatment with inorganic salts (percentages in brackets indicate maximum efficacy) included late blight (*Phytophthora infestans*, 91%; Johnson *et al*., 2004), silver scurf (*Helminthosporium solani*, 90%; Hervieux *et al*., 2002), dry rot (*Fusarium sambucinum*, 49%; Mecteau *et al*., 2002) and pink rot (*Phytophthora erythroseptica*, 54%; Johnson *et al*., 2004). Of these salts, only phosphites appeared to be target specific, controlling *Phytophthora* spp. only in the case of potato (Cooke & Little, 2002). All other salts were found to possess broader spectrum of activity.

The percentage reduction in disease severity following inorganic salt treatment varied with the experiment, fungal pathogen, concentration of salt and timing of application and variety tested. In the case of *P. infestans* for example, control by inorganic salts ranged between 33-91%, relative to the untreated, with maximum protection obtained 91% (Johnson *et al*., 2004) for tuber and 70% (Strömberg & Brishammar, 1991) for foliar blight.

Some of these inorganic salts, which are commonly applied as foliar sprays (repeated applications at 10-14 day intervals are necessary; Cooke & Little, 2002; Johnson *et al*., 2004) or post-harvest dip treatments (Olivier *et al*., 1998), have been commercially formulated in the USA and have approved uses for potato fungal disease control. An example of such a formulated product is Fosphite® Fungicide (JH Biotech, Inc., Ventura, CA). Further details with respect to the commercial use of inorganic salts are available in Deliopoulos *et al*. (2008b).

DISCUSSION

The best salts with the most potential for each of the four potato diseases were as follows: 1) for *P. infestans*: potassium phosphate for foliar (Strömberg & Brishammar, 1991) and various phosphite salts for tuber late blight (Johnson *et al*., 2004), 2) for *H. solani*: aluminium chloride (Hervieux *et al*., 2002), 3) for *F. sambucinum*: aluminium chloride (Mecteau *et al*., 2002) and 4) for *P. erythroseptica*: various phosphite salts (Johnson *et al*., 2004).

Consequently, the existing evidence for the suppression of potato diseases by inorganic salts suggests that these compounds may be a very useful tool in potato integrated disease management strategies. More specifically, incorporation of inorganic salt treatments in the spray programme may enable a reduction in the number of fungicide applications needed to control certain potato diseases of economic significance. The prospective use of inorganic salts against potato diseases has also some other positive aspects, such as the generally low cost and a favourable safety profile.

Based on the USA experience, a prerequisite for their effective exploitation in the UK is greater regulatory innovation (as with other potential biopesticides; Chandler *et al*., 2008), in order for inorganic salts to receive approval for use against fungal pathogens. Nevertheless, it must be emphasised that since control levels by inorganic salts are generally below those achieved by conventional products, these substances should be in no way considered as full replacements of synthetic fungicides.

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OUTPUTS FROM RERAD FUNDED POTATO PATHOLOGY WORK 2006-2010

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Summary: With rising costs and demand for food worldwide, as well as a requirement to protect our natural resources for future generations, we have an increasingly difficult task to produce food sustainably. This is made more difficult by the recent introduction of legislation by the EU to reduce the use of pesticides, by peoples' worries about the use of biotechnology, and by the unpredictability of climate change. As part of the current funding from the Scottish Government Rural and Environment Research and Analysis Directorate (RERAD) scientists from SCRI and SAC are developing ways to protect the potato industry from the effects of pests and diseases. This presentation describes our research progress over the last 4 years within the Potato Pathology Work package 1.5 and our plans for the future.

INTRODUCTION

Food security is, and will remain, high on government agendas world-wide, with the UN predicting that food production will need to increase by 50% by 2030 and to double by 2050 to meet rising demands. Since land is a finite resource and bringing new land into crop production is unsustainable, we must increase crop yields on existing farm land. A major way to contribute to this increase is by improving control of pests and diseases through better surveillance, optimising chemical inputs and developing resistant crops.

Potato is the world's fourth largest crop (and the third largest food crop) and is second only to barley in Scotland. Here, over a million tonnes of potatoes are produced each year at a value to the economy of £150-200 million. Due to the high health status of potatoes grown in Scotland, approximately 25% of production is high value seed, most of which is exported. Unfortunately, pests and diseases are responsible for major losses to potato in Scotland, as they are in other parts of the world. It has been estimated that (across the UK as a whole) as much as 15% of seed and ware crops are lost to pests and diseases each year, despite the use of pesticides costing over £100 million. To add to this problem, under certain circumstances some older pesticides may be harmful to the health of people and the environment, so recent EU legislation (Directive 91/414/EEC) is aiming to remove them. In the long-term, this is undoubtedly a good thing, but it must be recognised that pesticides play a vital role in controlling pests and diseases (Fig. 1). For example, it was recently estimated that without chemical control, yearly losses to potato in the UK due to just one disease, late blight, would increase from £55 million to £363 million (almost half the UK's potato production) (Twining *et al.* 2009).

Figure 1. Reductions in the use of pesticides could have serious implications for protecting potatoes from pests and pathogens, including potato cyst nematode (picture shows two strips in the centre treated with nematicide).

Our challenge is to find ways to reduce crop wastage (in this case potato) caused by pests and diseases in a way that is sustainable; by optimising the use of pesticides, whilst protecting people and the environment both now and into the future, yet still supporting a profitable potato industry.

OUR RESEARCH

SCRI, in association with SAC, is carrying out research on methods of disease control that will help to reduce or eliminate the need for pesticides. We have developed improved surveillance techniques, a better understanding of the environmental survival and spread of pests and pathogens, as well as resistant potato varieties. Durable host resistance is seen as the ideal and most 'environmentally friendly' way to control diseases because agrochemicals do not need to be deployed.

New diagnostics have been developed that are being used for both statutory and commercial testing, as well as providing tools for our research. These include a new molecular test for potato cyst nematode used in conjunction with the Scottish Government through SASA (Science and Advice for Scottish Agriculture) to allow increased capacity in testing as part of new EU legislation (2007/33/EC).

As with statutory testing, avoiding contamination by pests and pathogens will reduce the need for pesticides. We are achieving this through a better understanding of the survival and spread of pests and pathogens in the environment. For example, we are investigating the role of wild plants in the survival and spread of the bacterial potato pathogens *Pectobacterium* and *Dickeya* (formerly *Erwinia*) and applying this knowledge to reducing pathogen spread to the potato crop.

Resistant cultivars are a major weapon against pests and diseases that, due to the ready availability of pesticides, have so far been under-exploited. At SCRI, we aim to combine different types of resistance to develop cultivars that are resistant to a wide range of pests and pathogens and which remain so over a long period of time (durable resistance). To enable us to do this we are examining the interactions between pests / pathogens and their potato hosts to identify the genes and pathways involved in resistance. For example, we have recently shown that the late blight pathogen, *Phytophthora infestans*, injects up to 500 proteins into the plant cell, some of which target major defence pathways. Using similar knowledge in *Pectobacterium* we have shown in a model experiment that it is possible to develop resistant potato plants using a transgenic approach. Important defence pathways against viruses have also been identified and are currently being investigated. Knowledge from this research is being used by crop geneticists in SCRI's breeding programmes.

The extent of variation in a pest or pathogen population, and the ability of a population to change under different conditions, e.g. climate change, have important consequences for their survival, spread, pesticide resistance, aggressiveness and ability to overcome crop resistance. Understanding pest and pathogen populations is therefore an important part of our fight against disease. Recently, in association with a Potato Council study, we discovered that a new genotype (13_A2) of *P. infestans* is dominating much of the European crop. This genotype is more aggressive than previous isolates, is resistant to some chemical agents, and has overcome some forms of potato resistance that have been used successfully for over 40 years (Fig. 2).

Figure 2. Potato variety Stirling remains resistant to *P. infestans* genotype 10_A2 (left) but this resistance is overcome by the new and dominant genotype 13_A2 (right).

We are now investigating the biology of this genotype, using it to screen commercial varieties, and to identify new sources of resistance in the Commonwealth Potato Collection (which has over 1500 accessions of both wild and cultivated potato) housed at SCRI. Fingerprinting populations of the peach-potato aphid, *Myzus persicae*, has recently discovered a new genotype exhibiting a combination of pesticide resistance and environmental resilience that will render it a problem for the foreseeable future. Our recent review on the "Impact of climate change on pests and diseases of potato in Scotland" has highlighted key targets for our work on sustainable crop production in the future, and includes increased threats from existing pathogens, such as *Myzus persicae* and the virus pathogens it carries, and new threats such as *Dickeya* species.

To ensure that the outputs from our research are available to relevant end-users, we have worked hard to ensure that information is transferred to industrial partners, other stakeholders and our funders – The Scottish Government. For example, over the previous 4 years SAC has held a series of events called "Potatoes in Partnership", where practical outputs from our work have been demonstrated and discussed. Our continued input into other events (including Potatoes in Practice) committees, grower reports and information sheets, as well as national and industry publications, will ensure that our research outputs continue to be disseminated to relevant end-users and that feedback from them can be incorporated back into our research.

ACKNOWLEDGEMENTS

We acknowledge the support of the Scottish Government Rural and Environment Research and Analysis Directorate (RERAD).

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PESTICIDE USE IN SCOTTISH POTATO CULTIVATION 1998-2008: TRENDS AND PREDICTIONS

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Summary: There was little change in the proportion of Scottish potato crops treated with pesticides between 1998 and 2008. Minor variations in quantity and application rate reflected temporal differences in pest pressure and in the composition of the active ingredients used. The comparative environmental impact of pesticide use, measured by EIQ analysis, decreased greatly over the period. This was primarily due to reduced use of sulphuric acid and, to a lesser extent, the withdrawal of several active ingredients in 2003. The current revision of 91/414/EEC will significantly reduce the number of active ingredients available to the Scottish potato industry.

INTRODUCTION

Almost 30,000 ha of potatoes, with a total market value of £193 million, were grown in Scotland in 2008 (Anon, 2009a). This economically important crop receives considerably greater pesticide input than any other Scottish crop, with each hectare grown receiving approximately 9 kg pesticides (excluding sulphuric acid use, which has been applied at up to 200 kg/ha of crop in the past). In comparison, cereals receive *ca* 3 kg pesticides/ha, vegetables *ca* 4 kg/ha and soft fruit *ca* 7 kg/ha.

The aim of this poster is to highlight trends of pesticide application to potato crops over the last 10 years, to estimate their comparative environmental impact and to discuss factors that may influence future pesticide use.

METHODS

Pesticide Use

Pesticide application data for the period from 1998 to 2008 were obtained from the dataset collected by SASA during their biennial arable pesticide use surveys (Snowden & Thomas, 1999; Kerr & Snowdon, 2001; Snowden & Thomas, 2003; Snowden *et al.*, 2005; Struthers, 2007; Reay, 2009). These surveys are conducted by collecting pesticide data from a random sample of holdings classified by farm size and geographic region. National estimates of pesticide use are produced from the collected sample data by applying raising factors based on actual crop areas obtained from the annual agricultural census.

Environmental Impact of Pesticide Use

The comparative environmental impact of the pesticides applied to potato crops was estimated using the Environmental Impact Quotient (EIQ) method (Kovach *et al.*, 1992). This approach uses a quotient to represent the intrinsic hazard of an active ingredient based on hazard ratings for farm workers, consumers and the environment:

$$EIQ_{pesticide} = (EIQ_{farm\ worker} + EIQ_{consumer} + EIQ_{environment}) / 3$$

$$EIQ_{farm\ worker} = C \times [(DT \times 5) + (DT \times P)]$$

$$EIQ_{consumer} = [C \times ((S + P) / 2) \times SY) + (L)]$$

$$EIQ_{environment} = [(F \times R) + (D \times ((S + P)/2) \times 3) + (Z \times P \times 3) + (B \times P \times 5)]$$

C = chronic toxicity, DT = dermal toxicity, P = plant surface half life, S = soil half life, SY = systemicity, L = leaching potential, F = Fish toxicity, R = run off potential, D= bird toxicity, Z = bee toxicity B = beneficial arthropod toxicity.

EIQ values were calculated for the 20 most commonly used active ingredients, categorised both by spray area and weight, for each survey year i.e. the pesticides which were most abundantly applied in each year. These were dominated by fungicides, accounting for 50% of those included in the analysis, with the remainder split evenly between herbicides and insecticides/nematicides. The quotients were multiplied by the quantity of the active ingredient applied to give an estimate of risk and then divided by the area onto which they were applied to give an indication of environmental impact (EI) per unit area.

Predictions of Future Use

The potential influence of both the current revision to the Plant Protection Products Directive (91/414/EEC) and the implementation of the Water Framework Directive (2000/60/EC) on future pesticide use on Scottish potato crops were considered.

RESULTS AND DISCUSSION

Pesticide Use 1998-2008

There has been little change in Scottish potato cultivation area from 1998 to 2008 (Table 1) although the proportion of the crop grown for seed production has decreased from 50% to 40% during that period.

Table 1. The area of potato crops cultivated and proportion of crops receiving pesticide applications 1998-2008.

	1998	2000	2002	2004	2006	2008
Area of potato crops (ha)	28,762	29,689	30,204	29,353	28,123	29,836
	Proportion of crop receiving a pesticide application (%)					
Fungicides	98	99	99	100	98	99
Herbicides	98	93	96	100	97	96
Insecticides/Nematicides	81	72	70	70	74	72

The proportion of crops treated has also shown little variation over the last 10 years (Table 1), with an average of 99, 97 and 73% of crops treated with fungicides, herbicides and insecticides/nematicides respectively. The total quantity of pesticides applied and the average dose rate of pesticide applications (both excluding sulphuric acid) are presented in Figure 1.

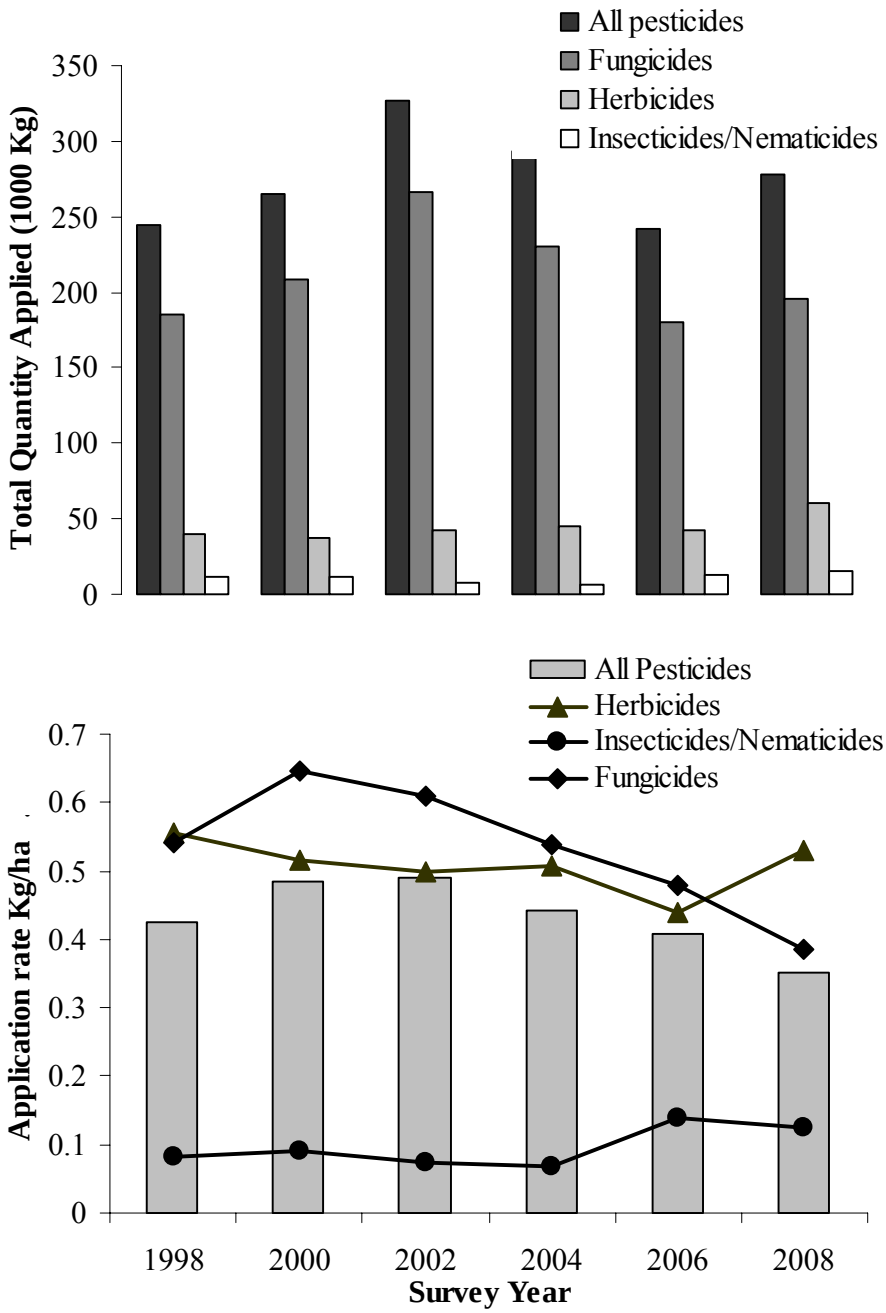

Figure 1. Quantity and mean application rate of pesticides applied to potato crops 1998-2008.

The quantity of fungicides applied far outweighs that of any other pesticide group and dominates the application pattern shown by pesticides as a whole. The increase in fungicide quantity from 1998 to 2002 was a function of both increased disease pressure and greater use of actives such as mancozeb and copper oxychloride, which are applied at high application

rates. The subsequent decrease in quantity and dose rate from 2002 to 2006 was primarily due to a decline in use of the aforementioned actives coupled with an increasing use of cymoxanil, which is applied at less then a tenth of the rate of mancozeb. The slight increase in quantity applied in 2008 was mainly due to high blight incidence, resulting in both increased precautionary sprays and applications to crops with active blight. The latter are treated with more than one active ingredient to reduce the risk of resistance development.

Herbicide applications show very slight variation in relation to both total quantities and dose rate. The increase in quantity and application rate in 2006 and 2008 (Figure 1) is an artefact of the fact that sulphuric acid is not included in these data. Sulphuric acid applications for haulm destruction have declined from over 6 million kg in 1998 to 800,000 kg in 2008. With the decline of sulphuric acid, which has a final use date of 2010, applications of other herbicides for desiccation, primarily diquat, have greatly increased. Pre-harvest applications of diquat were recorded on *ca* 3,500 ha in 1998 and 43,000 ha in 2008.

Insecticide use has also been fairly static over the reported period. The main trend was an increase in dose rate and quantity in 2006/08. This was due to a combination of increased pest pressure and a rise in the use of organophosphate and carbamate formulations, both of which are applied at relatively high rates, whilst pyrethroids, which are applied at a tenth of the rate, have shown little change in application pattern. Neonicotinoid use was first recorded in potatoes in 2006 (3,400 ha thiacloprid) and increased significantly in 2008 (2,344 ha thiamethoxam and 15,088 ha thiacloprid).

Environmental Impact Analysis

Whilst the pesticide use data details what quantities are applied, it doesn't indicate the relative toxicity or potential impact of their use. Figure 2 presents the results of the EIQ analysis of potato pesticide application, which attempts to address this issue.

Environmental impact (EI) is linked to the quantity of active ingredient; therefore the overall pattern is similar to that of quantity applied (Figure 1). As previously discussed, the pattern is influenced by the increased use of actives that are applied at lower rates regardless of their intrinsic hazard. However, the magnitude of the decrease in EI from 2002 to 2004 (40%) is far greater than the corresponding decrease in quantity applied (10%). This is mainly due to the withdrawal of several commonly used active ingredients in 2003 which were not supported during the revaluation of existing active substances under Directive 91/414/EEC. In particular, the removal of fentin acetate, trietazine and oxadixyl greatly contributed to the decline in overall EI. These actives all have particularly high impact values in relation to the environment component of the index and were also applied at relatively high dose rates. The increase shown in 2008 is mostly due to increased rates of fungicide application as discussed in the previous section, but is also a function of increased diquat use, as diquat also has a high environmental index score.

Sulphuric acid EI is reported separately from the overall analysis as its application rate is almost twenty times the weight of the next most abundantly applied active ingredient, and this, coupled with a very high EIQ, dominates all patterns in comparative analyses. However, the decline in this active ingredient has the greatest influence in the decrease of the environmental impact of potato chemical use. When sulphuric acid is considered in the analyses, the overall environmental impact decreased by almost 90% over the ten year period.

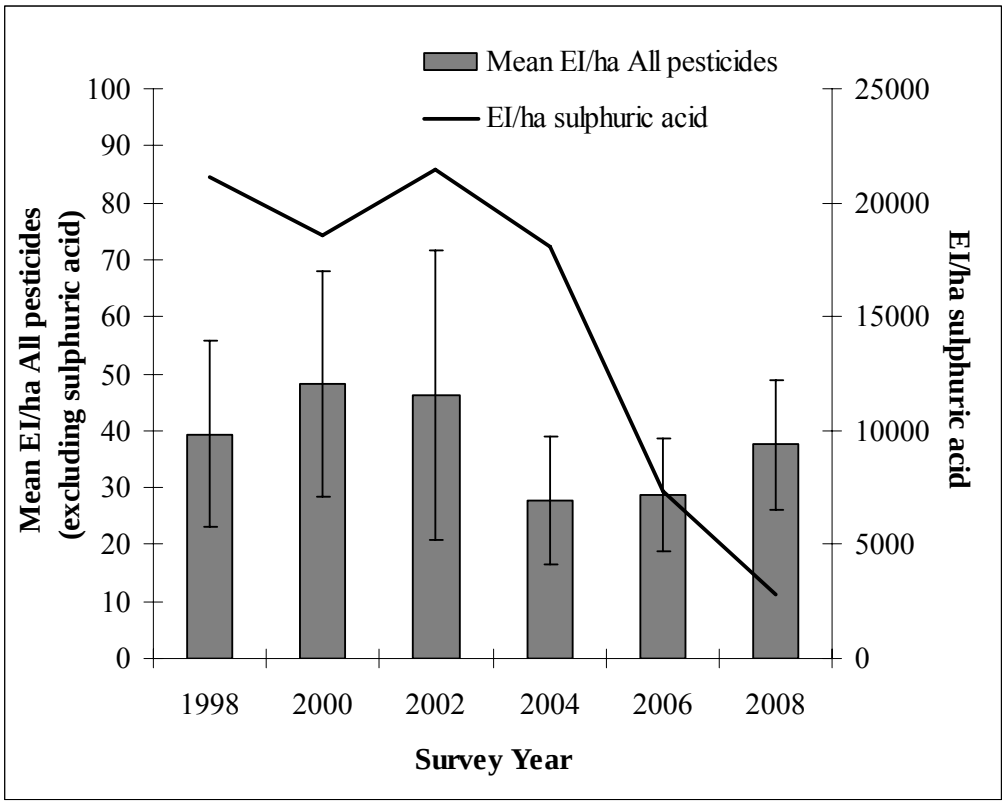

Figure 2. Environmental impact per hectare 1998-2008. Error bars indicate the standard error of the mean.

Factors influencing future pesticide use

The main factor that is likely to influence future pesticide use on Scottish potato crops is the replacement of Directive 91/414/EEC as part of the new EU Thematic Strategy for Pesticides. The directive is being replaced with a regulation which has an approval process that is hazard rather than risk based. This will result in a number of active ingredients that are currently on Annex I being withdrawn, either immediately or being substituted over time.

One of the main impacts of this process is the expected loss of mancozeb, which forms the basis of the current blight control programme in both seed and ware crops. Mancozeb is applied to approximately 100,000 ha of Scottish potato crops annually. In addition, chlorothalonil and famoxodone are classified as candidates for substitution under the new regulation. The potential loss of these fungicides will have repercussions for both disease control and resistance management strategies.

In relation to herbicides, the expected loss of linuron (applied to 13,000 ha in 2008) and the potential loss of diquat, the two main herbicides in potato crops will severely reduce weed control options, particularly in relation to post-emergence applications.

The availability of insecticides and nematicides are also likely to be severely affected by the changes to the directive. The expected loss of thiacloprid (applied to 66% of the seed crops in 2008) in conjunction with lambda-cyhalothrin and pirimicarb (applied to 56,000 and 36,000 ha in 2008 respectively) being candidates for substitution would severely reduce control options.

In addition, all 3 nematicides currently on market in the UK (fozthiazate, oxamyl and ethoprophos) are also all candidates for substitution. This makes effective insect and nematode control extremely difficult, particularly in the wake of the loss of aldicarb in 2007. These losses will have a major impact on control of PCN and aphid vectors of viruses and will particularly affect Scotland which grows the bulk of the UK's seed potato crop (82% in 2006 Garthwaite *et al*., 2007).

In addition to the changes associated with 91/414/EEC there is also a secondary threat of losses due to the implementation of the Water Framework Directive, which must ensure that pesticide levels in drinking water are below 1 ppb by 2015. Metaldehyde, applied to 20,060 ha of potatoes in 2008, is already under scrutiny and several key cereal herbicides are also occurring above the 1 ppb limit. However, none of those currently classified as high priority substances are widely applied to potato crops.

In conclusion, high pesticide input is integral to potato production, particularly for seed crops. The proposed changes to pesticide availability will dramatically change the current pesticide application regimes. It's unclear whether reduced availability will reduce overall use of agrochemicals through adoption of agronomic techniques such as use of resistant varieties, trap cropping or biological control, or whether use of the remaining pesticides will increase to replace those that have been lost. What is evident is that the potato industry faces a major challenge over the next 10 years.

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CHARACTERISATION OF RECENTLY ISOLATED *DICKEYA SPP.* AND THEIR POTENTIAL THREAT TO THE SCOTTISH POTATO INDUSTRY

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Summary: A new bacterial pathogen of potato, provisionally named "*Dickeya solani*", has recently emerged in Northern Europe. The principal mechanism of spread is through the movement of infected seed but it is likely that infested irrigation water also has a role. A number of *Dickeya* isolates recovered in Scotland from trial material of non-Scottish origin, a ware crop grown from non-Scottish origin seed, imported ware potatoes and irrigation waters, were characterised by *16S rDNA* and *recA* gene sequencing, alongside reference strains from the genera *Dickeya* and *Pectobacterium.* Both approaches produced essentially similar results, in that all isolates from potato and one from irrigation water were identified as *"D. solani"*. This work confirms the presence of "*D. solani"* in Scotland, albeit in a limited capacity, and highlights the variety of routes this pathogen enters the country by and a means by which it could persist here.

INTRODUCTION

Many species within the genus *Dickeya*, previously *Erwinia chrysanthemi*, cause disease on potato. Symptoms include blackleg, soft rot and wilt. Until relatively recently, *D. dianthicola* was recognised as the principal pathogen of potato in Northern Europe (Samson *et al.*, 2005). It is now evident that new strains of *Dickeya* have emerged that are spreading rapidly in potato crops in continental Europe and Israel (Laurila *et al.*, 2008; Tsror *et al.*, 2009; Sławiak *et al.*, 2009a, b). These new strains appear to be more aggressive than *D. dianthicola* and *Pectobacterium atrosepticum*, previously *Erwinia carotovora* subsp *atroseptica*, the usual cause of potato blackleg in Northern Europe. Observations from the Netherlands indicate that once established, these strains will rapidly displace others and take over as the principal cause of wilting and backleg-like symptoms in potato crops. Production losses experienced by the Dutch seed potato industry were valued at €30M annually (Sławiak *et al.*, 2009a), due to downgrading and rejection, almost entirely due to this group. Although it is recognised that the primary mechanism of spread is through the movement of infected seed (Tsror *et al.*, 2009; Sławiak *et al.*, 2009a,b), studies in Finland have confirmed the presence of these strains in irrigation water (Laurila *et al.*, 2008).

A range of approaches have been used to circumscribe diversity within the genus *Dickeya*; biochemical tests (Palacio-Bielsa *et al.*, 2006), rep-PCR fingerprinting (Tsror *et al.*, 2009; Sławiak *et al*, 2009b) and sequencing of *16S rDNA* (Samson *et al.*, 2005; Laurila *et al.*, 2008;

Sławiak *et al*, 2009b), *16S-23S rDNA* intergenic spacer region (Laurila *et al.*, 2008), *dnaX* (Sławiak *et al.*, 2009b), *recA* (Parkinson *et al.*, 2009) and a range of other house keeping genes (Ma *et al.*, 2007). The most comprehensive of these analyses were performed by Sławiak *et al.* (2009b) and Parkinson *et al.* (2009); in the former study, 65 strains of *Dickeya* isolated from potato were characterised using a polyphasic approach encompassing rep-PCR fingerprinting, *16S rDNA* and *dnaX* sequence analysis. A group of potato strains, recovered between 2005-2007 in Finland, Israel, the Netherlands and Poland, were found to be identical, clearly different from the six *Dickeya* species previously described and distinct from all other potato strains isolated in Europe between 1979 and 1994, which were all identified as *D. dianthicola* (Sławiak *et al.,* 2009b). Parkinson and co-workers (2009), in a bigger study based on *recA* sequence analysis of 188 *Dickeya* strains, were also able to identify previously undescribed diversity within the genus and could distinguish a group of potato pathogens which could not be assigned to any previously described species. More recent, unpublished work has confirmed that these previously undescribed groups identified independently in both studies are the same (van der Wolf & Elphinstone, personal communication), and although yet to be formally named, the name *"D. solani"* has been proposed.

At present, control of *D. dianthicola* and *Pectobacterium atrosepticum* is achieved through seed classification schemes in many European countries. Early indications from the Netherlands suggest that such an approach, which is based on visual inspection for blackleg and wilt in the field, may be insufficient to control the spread and impact of *"D. solani"*. The Scottish Government is therefore consulting on proposed amendments to its Seed Potato Certification Scheme (SPCS) and Plant Health Order designed to minimise the impact of *Dickeya* on the Scottish potato industry. As a prerequisite to any change in legislation, it is vital that an accurate picture is obtained as to whether *Dickeya* spp. are already present in Scotland and to determine the nature and frequency of potential introduction routes. To this end a number of *Dickeya* isolates recovered in Scotland from a National List candidate variety, a ware crop grown from non-Scottish origin seed, imported ware potatoes and irrigation waters (see Cahill *et al.,* 2010 for details) were characterised by *16S rDNA* and *recA* gene sequencing, alongside reference strains from the genera *Dickeya* and *Pectobacterium.*

MATERIALS AND METHODS

Strains and DNA Extraction

Details of 21 strains, comprising a range of *Dickeya* strains isolated from infected potatoes and irrigation waters (see Cahill *et al.*, 2010), and reference strains from the genera *Dickeya* and *Pectobacterium* are given in Table 1. Strains were maintained at -80˚C in 20% glycerol (aq. v/v) and grown on Nutrient Agar plates at 36˚C or 25˚C for 48 hours prior to extracting DNA using a Wizard Genomic DNA Purification Kit (Promega Corporation, USA), according to the manufacturer protocol.

DNA Sequencing

The *16S rDNA* and *recA* genes were sequenced using primers described by Lane (1991) and Waleron *et al.* (2007), respectively. PCR products were visualized on agarose gels, then purified using NucleoSpin Extract II kits (Macherey-Nagel GmbH & co. KG, Germany). Sequencing reactions were performed using Big Dye Ver. 3.1 sequencing kits (Applied

Biosystems Inc., Foster City, CA, USA) then visualised on an ABI3130xl Genetic Analyser (Applied Biosystems), as per the manufacturer's protocols. Results were analyzed using Sequencing Analysis Software (Ver. 5.2; Applied Biosystems) and alignments performed with Lasergene-SeqMan 7.0.0 (***DNASTAR*** Inc., Madison, WI, USA). The consensus sequences were saved as contig files and then analysed further by MEGA4 (Center for Evolutionary Functional Genomics, AZ, USA) to produce neighbor-joining trees using single linkage clustering. Bootstrap values were based on 500 calculations.

Table 1. Details of *Dickeya* strains isolated from irrigation waters and infected potatoes and reference strains from the genera *Dickeya* and*Pectobacterium.*

Strain Number	Name	Host/Source
Potato isolates:		
MK11	*Dickeya* sp.	*Solanum tuberosum* tubers (Israeli ware import; Scotland 2006)
B1	*Dickeya* sp.	*Solanum tuberosum* tubers (Spanish ware import; Scotland 2009)
B2744	*Dickeya* sp.	*Solanum tuberosum* trial material (Scotland, 2009)
*CSL20710504	*Dickeya* sp.	*Solanum tuberosum* cv. Markies (England 2007)
CSL20610923	*Dickeya* sp.	*Solanum tuberosum* cv. Markies (England 2006)
A101-9	*Dickeya* sp.	*Solanum tuberosum* (Poland)
DM157	*Dickeya* sp.	*Solanum tuberosum* ware (Scotland, 2009)
Water isolates:		
MK1	*Dickeya* sp.	River 1, Central Scotland
MK8	*Dickeya* sp.	River 2, South East Scotland
MK20	*Dickeya* sp.	River 3, North East Scotland
RW 192-1	*Dickeya zeae*	River water (England)
RW 240-1	*Dickeya* sp.	River water (England)
Reference strains:		
‡NCPPB312	*Pectobacterium carotovorum*	*Solanum tuberosum* (Denmark, 1952)
NCPPB402	*D. chrysanthemi*	*Chrysanthemum morifolium* (USA, 1958)
NCPB453	*D. dianthicola*	*Dianthus caryophyllus* (England, 1956)
NCPPB454	*D. dadanti*	*Philodendron* sp. (USA 1957)
NCPPB516	*D. chrysanthemi*	*Chrysanthemum* sp. (1957)
NCPPB549	*P. atrosepticum*	*Solanum tuberosum* (England, 1958)
CSL2264 (A5305)	*D. dianthicola*	*Solanum tuberosum* (England, 1995)
NCPPB3532	*D. zeae*	*Solanum tuberosum* (Australia, 1980)
NCPPB3533	*D. chrysanthemi*	*Solanum tuberosum* (USA, 1987)

* CSL – Research collection of the Central Science Laboratory, (now Fera), York, UK.
‡ NCPPB – National Collection of Plant Pathogenic Bacteria, Fera, York, UK.

RESULTS

Analysis of the *16S rDNA* sequence data revealed that all of the recently isolated potato strains (from 2006 onwards) were recovered in one major cluster, distinct from all of the *Dickey*a and *Pectobacterium* reference strains (Fig. 1a). Although the reference strains included potato pathogens recovered between 1952 and 1995 (Table. 1), none clustered with these more recent isolates. This cluster contained B2744 isolated from a Dutch candidate variety undergoing National List trials in Scotland, and DM157, isolated from a Scottish ware crop grown from once-grown English seed of Dutch origin. These strains were identical to isolates previously obtained from infected potatoes grown in Poland (A101-9) and England (CSL20610923 & 20710504), and also from Spanish (B1) and Israeli (MK11) ware imports into Scotland. MK8, isolated from a river in South-East Scotland, was also found to be identical to the other members of this cluster. This strain was clearly distinct from MK1 and MK20, which were also recovered from Scottish rivers but which were shown to be only distantly related to the bulk of potato isolates studied here. MK1 appeared distinct from all other strains, whilst MK20 clustered with *Dickeya zeae* NCPPB 3532. Analysis of the *recA* sequence data generated an essentially similar tree topology to that revealed by the *16S rDNA* analysis, namely that the majority of potato isolates were recovered in a major cluster that contained the Scottish isolates B2744, DM157 and MK8 alongside strains isolated from infected potatoes grown in Poland and England and recovered from Spanish and Israeli ware imports to Scotland. MK1 and MK20, isolated from Scottish irrigation sources, did not cluster with MK8 and were distinct from all other strains studied.

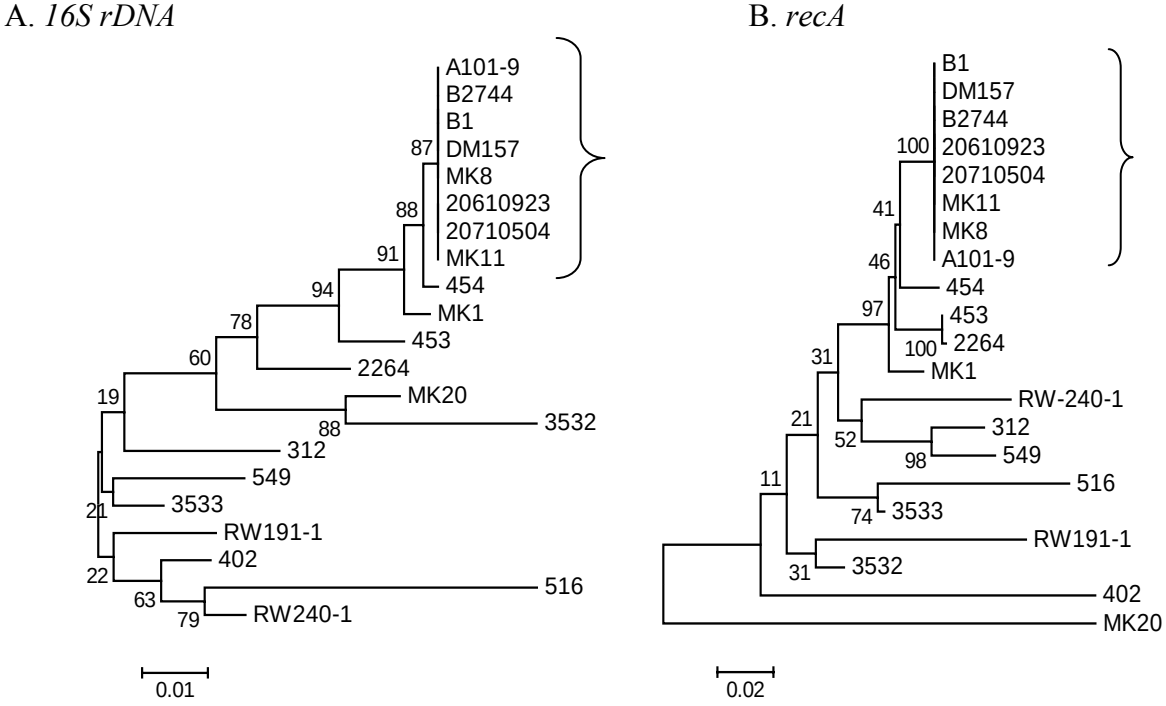

Figure 1. Neighbour-joining trees of river and potato isolates, with *Dickeya* and *Pectobacterium* reference strains. Bootstrap values at branch points are expressed as percentages of 500 replications. Cluster of potato and river isolates is highlighted. A. *16S rDNA*. Bar, 1 substitution per 100nt. B. *recA*. Bar 2 substitutions per 100 nt.

DISCUSSION

The sequence analysis of *16S rDNA* and *recA* genes produced similar results in that all potato isolates were found to be identical and distinct from all known members of the genus *Dickeya*, including *D. dianthicola*, hitherto considered the primary potato pathogen from this genus in Northern Europe. This finding is entirely in keeping with the earlier work of Sławiak *et al.* (2009b) and Parkinson *et al.* (2009) in which recent potato isolates were found to be distinct from isolates recovered prior to 2005 and the existence of "*D. solani*" was first tentatively recognised. The inclusion of Polish strain A101-9, a member of "Clade IV", as described by Slawiak *et al.* (2009b; Lojkowska and Slawiak, personal communication) and CSL20610923 & 20710504, members of "DUC-1", as described by Parkinson *et al.* (2009; Elphinstone and Parkinson, personal communication) identifies the more recent potato isolates recovered from Scottish grown potatoes as "*D. solani"* and confirms for the first time the presence of this pathogen in Scotland, albeit in trial material and a ware crop grown from non-Scottish seed. In addition, this study provides evidence that *"D. solani"* is entering Scotland in ware imports and has infested at least one source of irrigation water, specifically a river in South East Scotland. This is the first finding of this pathogen in irrigation waters outside of Finland (Laurila *et al.*, 2008) and contrasts with findings from surveys of English irrigation sources where no evidence of *"D. solani"* in water was found (Elphinstone, personal communication). The finding of a bacterial pathogen in irrigation water is not unusual and parallels can be drawn with the epidemiology of *Ralstonia solanacearum* in Northern Europe where a clear link between infested irrigation waters and infection has been demonstrated (Janse, 1996). It is not clear at this stage however, how *"D. solani"* was introduced nor whether the weed, *Solanum dulcamara*, also plays a role as a disease reservoir for *"D. solani"* in the same way it does for *R. solanacearum.* It is clear from some preliminary work that *"D. solani"* can establish an infection when irrigation water spiked with low cell densities are applied to growing plants (Cahill, unpublished work), raising the very real prospect that should naturally infested waters be used to irrigate potato crops, infection is likely to follow.

It is clear that sequencing of *16S rDNA* and *recA* genes provide an accurate means of identifying *"D. solani"*, however this is an inefficient approach in terms of time and cost. Further work is now required to establish more efficient diagnostics, almost certainly based on PCR, that can facilitate the rapid and unequivocal identification of this pathogen in future.

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THE EFFECT OF LATE BLIGHT POPULATION CHANGES ON HOST RESISTANCE RATINGS

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Summary: Trials to assess foliar late blight resistance of various cultivars of potato were carried out at several sites over recent years. This work was carried out to ascertain whether the published resistance ratings of cultivars were still reflective of a cultivar's performance to current populations of *Phytophthora infestans*, particularly in GB where one genotype (13_A2) has become dominant. Results showed that, for some cultivars, genotype 13_A2 did appear to overcome their resistance. Compiled data for a range of cultivars will be discussed, where two or more independent trials have been carried out and proposed changes to resistance ratings will be communicated to the industry.

INTRODUCTION

Control of potato late blight caused by *Phytophthora infestans* is likely to become more difficult in the UK in future because of the impact of environmental changes and evolution in the *P. infestans* population towards increased virulence, aggressiveness and fungicide insensitivity.

Pesticide usage survey data show that potato crops in the UK receive, on average, thirteen blight sprays at close to the full label recommended dose. Continuing to rely heavily on repeated applications of fungicide for control may be less acceptable to multiple retailers and consumers of produce in the future, and in that case greater use may need to be made of cultivar resistance as part of an integrated control strategy. However, the consequences of poor blight control are severe, so the potato industry is very risk-averse about reducing fungicide inputs, even where research demonstrates that combining better cultivar resistance with more appropriate fungicide inputs does not compromise control and offers economic benefits.

According to potato variety database information there is a wide range of foliar late blight resistance ratings among potato cultivars in GB. However, many of the most widely grown cultivars are susceptible to foliar blight (Table 1). Preliminary research has shown that an increase in cultivar resistance of one rating point can make a substantial contribution to blight control (Bradshaw & Bain, 2007). In spite of this, commercial fungicide programmes are generally similar for both resistant and susceptible varieties (Bradshaw & Bain, 2005). This is a reflection of growers' concerns about the potential effects of blight on both yield and quality. The principle of reducing fungicide inputs on potato cultivars with good resistance to foliar

blight has been established for many years (Fry, 1978; Gans *et al.,* 1995; Clayton & Shattock, 1995; Kirk, 2005), but only recently have attempts been made to provide sufficient data to allow agronomists to recommend fewer fungicide inputs on cultivars with a higher disease rating. Robust information on cultivar resistance is a pre-requisite for successful integrated control.

In recent years, significant changes to the *P. infestans* population have been detected. Monitoring of approximately 1000 isolates of *P. infestans* each year between 2006 and 2008 as part of the Potato Council funded 'Fight Against Blight' campaign showed that the proportion of A2 mating type isolates in the GB population had increased dramatically in comparison with previous years. The presence of A2 isolates is not in itself a problem; however, their presence provides the opportunity for greater isolate diversity through sexual recombination and for oospore production and deposition leading to infested soils. Sexual recombination may lead to increased diversity in the population and a risk of accelerated host resistance breakdown and fungicide insensitivity. Currently, there is no evidence for increasing levels of recombination in the UK and the *P. infestans* population is made up of relatively few clones.

This recent increase in A2 mating type isolates has been associated with an increase in just one genotype of *P. infestans* (the arbitrarily named 13_A2 genotype, sometimes referred to as 'blue 13'), characterised using microsatellite markers (Lees *et al.,* 2006) and first noted in the UK in 2005. This genotype has represented approximately 70-80% of the population in recent years. In addition, isolates of this genotype have a complex race structure, i.e. are able to overcome most of the 11 known host resistance (R) genes.

The published cultivar resistance ratings for foliar blight need to be re-evaluated because of concerns in the UK and elsewhere in Europe, that the current ratings for some cultivars are no longer appropriate (Wander *et al.,* 2005; Cooke *et al.,* 2006; Wale *et al.,* 2006). Similarly, sources of late blight resistance in wild species and breeding material must be re-tested in light of the new population. This paper seeks to combine information from several sources to inform the GB situation on host resistance.

Table 1. Foliar late blight resistance ratings (1-9 scale of increasing resistance) according to the British Potato Variety Database of the 10 most widely grown cultivars in 2009 (Area data taken from The Potato Council Table of Plantings by Cultivar for England, Scotland and Wales).

Cultivar	% of total planting area 2009	Late blight foliar resistance rating
Maris Piper	18.6%	4
Estima	7.5%	4
Lady Rosetta	5.3%	4
Maris Peer	4.5%	4
Markies	4.5%	7
Marfona	3.9%	4
Saxon	3.4%	3
Saturna	3.1%	4
Harmony	3.1%	3
Hermes	2.9%	3

MATERIALS AND METHODS

Foliar Late Blight Resistance Tests

Tests to assess cultivar resistance to late blight were conducted in the field over several years at sites in GB. All trials in 2008 and 2009 were inoculated using an isolate of 13_A2 genotype. In general, a method similar to the agreed European protocol (www.eucablight.org) was used and common reference varieties were used in all trials. For example, the SASA and SCRI experiments consisted of a randomised complete block design with three replicates, each consisting of two plants of the test cultivar. Each block was surrounded by plants of the late blight susceptible cultivar King Edward. Greenhouse-grown plants of cv. King Edward artificially inoculated with a genotype 13_A2 isolate of *P. infestans*, were placed at 1-2m intervals along the drills of cv. King Edward and the percentage area of foliage of each test plant affected by late blight was assessed on at least four occasions during the epidemic. Scores for two plants within a plot were averaged and a 1-9 score calculated. Cultivars and clones tested included commonly grown cultivars and those undergoing Independent Variety Trialling along with breeding and genetic resources material.

RESULTS

Resistance ratings

There is evidence that some published foliar resistance ratings are inaccurate and these ratings fall into 2 categories**.** Trials from across Europe that have been conducted using a range of isolates (including 13_A2 in the UK trials and possibly others) indicate that the original ratings for some cultivars may over-estimate their level of resistance. An example is shown in Fig. 1 for Eucablight reference cv. Robijn where its resistance rating of 8 was recorded in only one test out of 29 (data taken from www.eucablight.org) and its average resistance rating was c. 6 on the 1-9 scale of increasing resistance. However, the resistance rating did not vary over the years, suggesting that assessments were not being affected by a changing late blight population.

In contrast, some cultivars that were originally determined to be resistant were found now much more susceptible. An example is given in Fig. 2, where the breakdown in resistance of cv. Stirling from an original published rating of 7 to the current rating of c. 3 can be seen to correspond to a change in the isolate used to inoculate the plants. In addition, there were cultivars such as Sarpo Mira for which resistance ratings did not change, even if challenged by the new GB isolate (Figure 3.)

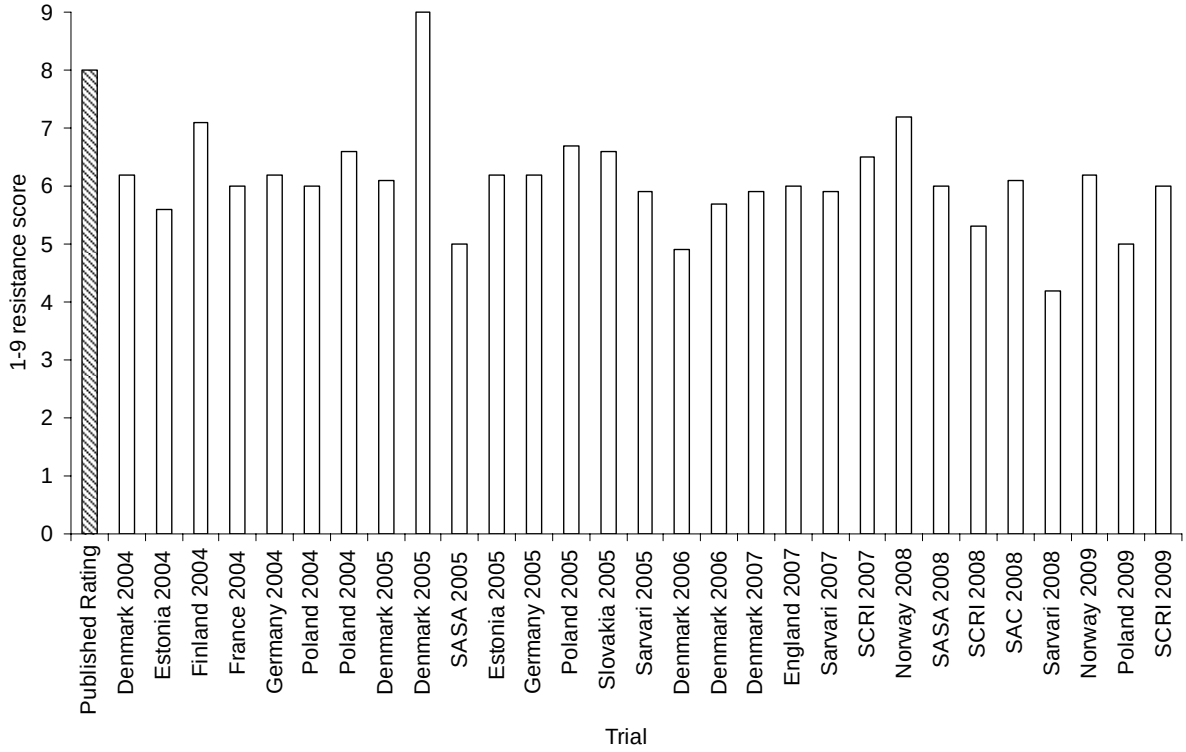

Figure 1. Foliar late blight resistance rating (1-9 scale of increasing resistance) of Eucablight reference cv. Robijn included in 29 trials across Europe between 2004 and 2009 compared with published resistance rating. Data obtained from Eucablight website.

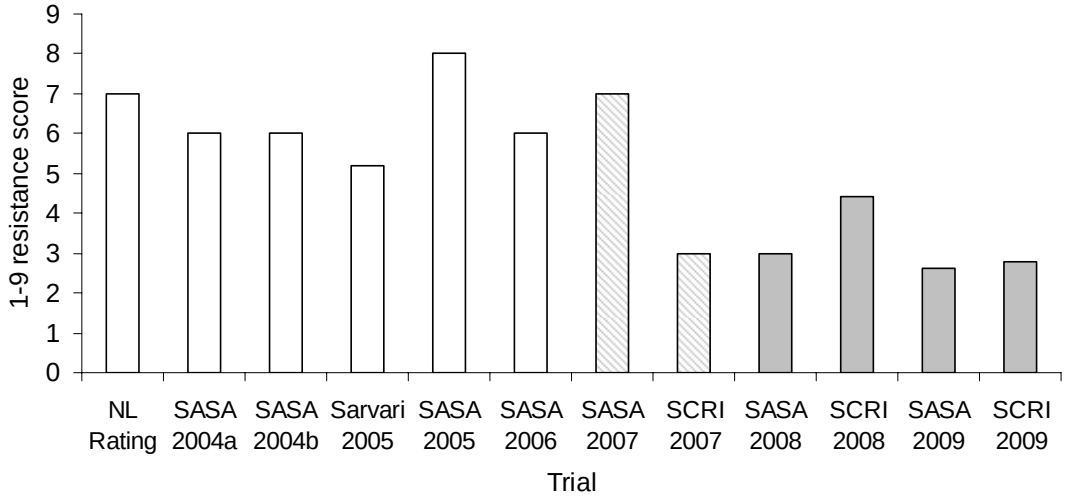

Figure 2. Foliage late blight resistance ratings for cv. Stirling as assessed in various trials 2004 – 2009. NL = National List Rating. ☐ = assessed with a non-13_A2 population of *P. infestans*, ▩ = assessed using isolates of 13_A2, ▨ = 13_A2 genotype present in trial site.

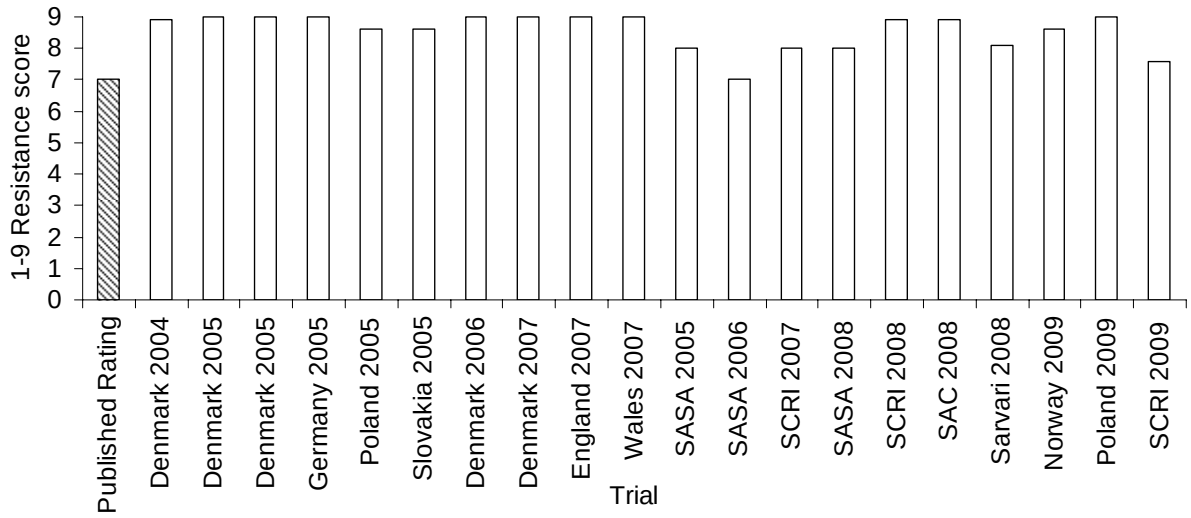

Figure 3. Resistance score (1-9 scale of increasing resistance) of cv. Sarpo Mira as assessed in various trials 2004 – 2009.

DISCUSSION

Although the resistance rating of a cultivar to foliar late blight is presented as a single figure, the reaction of a cultivar will vary over tests and years. The degree of variation is likely to be greater for cultivars in the middle of the resistance scale i.e. 4, 5 and 7, than at either end of score scale. In addition, the initial determination is likely to be based on a very limited number of tests and, as more data becomes available, the original rating may need some revision. Changes in test isolate used, test equipment, rating of reference cultivars and method of calculating rating may influence a cultivar's rating over time. Our results also confirmed that the historic ratings of late blight resistance for some existing cultivars were not accurate when these cultivars were challenged by new genotypes such as 13_A2. In general, the susceptibility rating of most cultivars purported to be resistant was lower, to varying degrees, than the historic rating. Nevertheless, it is clear that useful resistance to the new genotype was present in some cultivars, both old and new. The virulence phenotype of the 13_A2 genotype may, however, be fairly static. SASA screened two isolates of 13_A2 taken from cv. Pentland Dell in 2008 and both were of the same virulence as the 2007 isolate used in field testing. Testing of cultivars with isolates with only one virulence profile may run the risk that cultivars with high race specific resistance but low non-race specific resistance may identified as being field resistant in testing. For this reason, it may be worthwhile considering whether, in future, commonly grown cultivars should also be screened with isolates belonging to several other of the most common genotypes and, in particular, isolates of the 2nd most prevalent GB genotype 6_A1.

A full description of the resistance ratings of cultivars tested with genotype 13_A2 in two or more independent trials will be given, along with information on how changes to cultivar ratings based on these findings will be communicated to growers by the Potato Council.

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INVESTIGATING FACTORS AFFECTING INFECTION OF POTATO BY *SPONGOSPORA SUBTERRANEA*, THE CAUSE OF POWDERY SCAB

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Summary: Trials are being carried out in several countries worldwide to gain understanding of the environmental factors affecting the development of root gall and powdery scab development. Results of the first year's trial carried out at SCRI are reported. In addition, real-time PCR has been used to identify the timing of root and tuber infection by *S. subterranea*. In subsequent years infection by PMTV will also be monitored. Datasets from contributing partners will allow an analysis of factors affecting infection and disease development under different environmental and agronomic conditions.

INTRODUCTION

A diagnostic assay for the detection and quantification of *Spongospora subterranea* in plant tissue and soil was previously developed (van de Graaf *et al.,* 2003) and work showed that soil-borne inoculum is of greater importance in causing disease than seed-borne inoculum. However, the relationship between soil inoculum concentration and resulting disease on progeny tubers has been difficult to establish conclusively (van de Graaf *et al.,* 2005, Nakayama *et al.,* 2007). It is thought that this discrepancy occurs due to multiplication of the pathogen in the roots, with the result that low levels of inoculum can cause significant disease under suitable environmental conditions.

Powdery scab symptoms are particularly prevalent at temperatures between 12-15°C and root galls at approximately 17°C (van de Graaf *et al*., 2005; 2007). However, some basic knowledge of the factors that govern survival, infection and disease development, is lacking. In order to understand the epidemiology of the disease and therefore progress risk assessment and decision support for powdery scab control, work is needed to determine the conditions required for infection (e.g. temperature, water and window of infection). This work was identified as the next important research task by international researchers at the 2nd powdery scab workshop held in Switzerland in 2007 (http://www.spongospora.ethz.ch/EUworkshop07/). This type of data cannot be gathered in isolation and it was therefore proposed that similar trials were conducted by experienced powdery scab researchers in as many countries and at as many sites as possible that were pre-disposed to powdery scab epidemics (Scotland being amongst the most important of these).

This study, carried out at two sites in Scotland in 2008 and 2009 (SCRI and Scottish Agricultural College, SAC), followed the standard trial protocol agreed by the partners. In addition, diagnostic assays were used as an additional tool to determine the time and extent of infection by *Spongospora subterranea* and, in 2009, also Potato Mop Top Virus (PMTV), the virus vectored by *S. subterranea* and responsible for causing spraing symptoms. Preliminary results from the trial carried out at SCRI in 2008 are presented here and 2009 results will also be reported in the poster presentation

MATERIALS AND METHODS

Field Trial

The trial was planted at SCRI on 14th May 2008, according to the EU standard protocol and consisted of four replicated plots each of cultivars Agria and Nicola. Plots were artificially infested with *S. subterranea* by spreading ground peelings taken from tubers showing severe powdery scab symptoms across the plot. No seed treatments were applied and irrigation was applied from emergence until 4 weeks after tuber initiation (25mm was applied when the soil moisture deficit reached 18mm). Fertiliser, herbicides, late blight and aphid control were applied as per standard practice.

Environmental conditions were monitored using in-field monitoring equipment and meteorological station data. The following parameters were measured at least once per day:

- air temperature
- air humidity
- precipitation
- soil temperature
- soil moisture content

Plant Sampling

Four plants from each of four replicate plots were taken from each cultivar at seven sampling dates commencing on 1/7/08 at tuber initiation (3 weeks after 50% emergence), and weekly samples thereafter. Plants were dug carefully in order to catch as much of the root system as possible and roots were carefully separated from tubers, stems and stolons, and washed to remove soil but without damaging root tissue. If necessary, the root ball was soaked in water to loosen the soil. Roots were then dried, weighed and assessed for root galls. Tubers from each plant were washed carefully to remove all soil and all tubers in each sample were assessed for powdery scab. Disease scores were made using agreed root and tuber scoring tables (http://www.spongospora.ethz.ch/LaFretaz/scoringtable.htm).

Real-time PCR assessment of samples for *S. subterranea*

At each sampling date a root and tuber sample from one plant from each plot was also tested for the presence of *S. subterranea*. The whole root system from each plant and tuber peelings

taken from symptomless tubers from the same plant were collected. The fresh weight of each root sample was recorded after defrosting and the whole root sample was chopped up and placed in a 50 ml tube and freeze dried, then ground in liquid nitrogen. The dried weight of each sample was recorded and extraction buffer was added to each sample to a total volume of 30 ml. After thoroughly mixing the sample, three 1.5 ml aliquots were taken from each sample for DNA extraction. Each aliquot was centrifuged (Eppendorf centrifuge 5415 D) at 6000rpm (2600 g) for 5mins after which the supernatant was removed and kept.

Each sample of tuber peel (weighing between 7 and 70 g) was placed into a Bioreba grinding bag (Long Special Universal bags: 15 x 28.5 cm Cat. No. 470100b) and the weight noted prior to the addition of 15 ml SPCB extraction buffer. The sample was then pulverised to give an oat-meal consistency using a Homex grinder. Three 1.0 ml aliquots were taken for DNA extraction. DNA extractions on root and tuber samples were carried out according to the method of Cullen *et al*. (2001).

RESULTS

Effect of environmental conditions on root galling and powdery scab disease development:

Root galling was first observed on 22/7/08 and the severity of root galling increased over subsequent weekly sampling dates. At each date, cultivar Agria showed a significantly greater root gall severity than cultivar Nicola (Figure 1). Powdery scab symptoms were first observed on 29/7/08 (Figure 2) and were significantly more severe on cultivar Agria than Nicola, as would be expected according to disease resistance rating.

S.subterranea detection in roots and tubers using real-time PCR

DNA of *S. subterranea* was detected in both cultivars from the earliest sampling date (1/7/08) and there was a general trend for increasing detection of *S. subterranea* DNA in roots over time. Significantly more DNA of *S. subterranea* was detected in roots of cultivar Nicola compared with cultivar Agria at all sampling dates except the first. This is in contrast with the results for root galling symptoms and tuber symptoms where in each case Agria had significantly more disease. DNA of *S.subterranea* was detectable in symptomless tubers from the time of tuber formation (8/7/08). Significantly more DNA was detected in symptomless tubers of cultivar Agria compared with Nicola. A summary of sampling dates and disease and DNA observations is given in Table 1.

DISCUSSION

Preliminary results from one year's trials showed that DNA of *S. subterranea* was detected in roots of both cultivars at the earliest sampling date. Significantly more DNA of *S. subterranea* was detected in roots of cultivar Nicola compared with cultivar Agria at all sampling dates

except the first. This is in contrast with the results for root galling symptoms and tuber symptoms, where in each case Agria had significantly more disease. This may indicate that environmental conditions or host resistance mechanisms affect symptom development, not host infection. Root galls were not visualized until 5 weeks or more after root infection had occurred. This shows the importance of studying the conditions occurring at the very earliest stages of infection and during symptom development, in terms of understanding the epidemiology of the disease.

DNA of *S. subterranea* was detectable in symptomless tubers from the time of tuber formation, showing that infection takes place at a very early stage of development. Significantly more DNA was detected in symptomless tubers of cultivar Agria (which also had more severe disease) compared with Nicola suggesting that symptomless infections went on to cause disease symptoms in cultivar Agria. Tuber symptoms were first seen 3 weeks after infection was detected.

Environmental conditions were conducive to disease development. The mean daily temperature was approximately 15°C. Results from international trials will add to the interpretation with regard to weather conditions.

The final analysis will incorporate additional datasets from Scotland (SCRI and SAC), including data on PMTV detection from 2009 onwards. Additionally, data from European and International partners carrying out similar trials will be analysed, allowing an interpretation of the factors affecting infection and disease development in crops grown under different environmental and agronomic situations. This combined knowledge could lead to improved integrated disease control through interpretation of the *S. subterranea* soil tests and timing of agronomic factors such as irrigation.

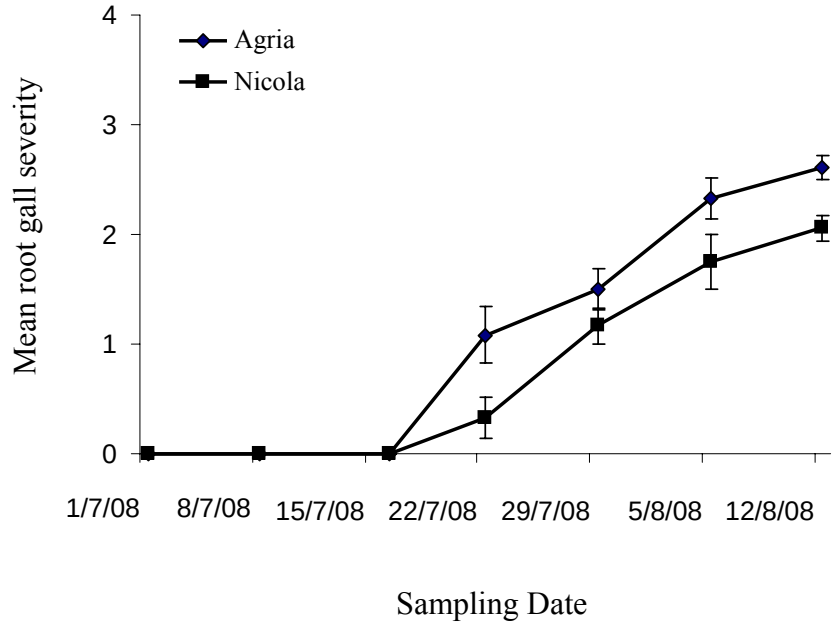

Figure. 1. Mean root gall disease severity on a 0-4 scale of increasing severity measured at weekly intervals on a) cultivars Agria and Nicola. Standard errors of means are shown**.**

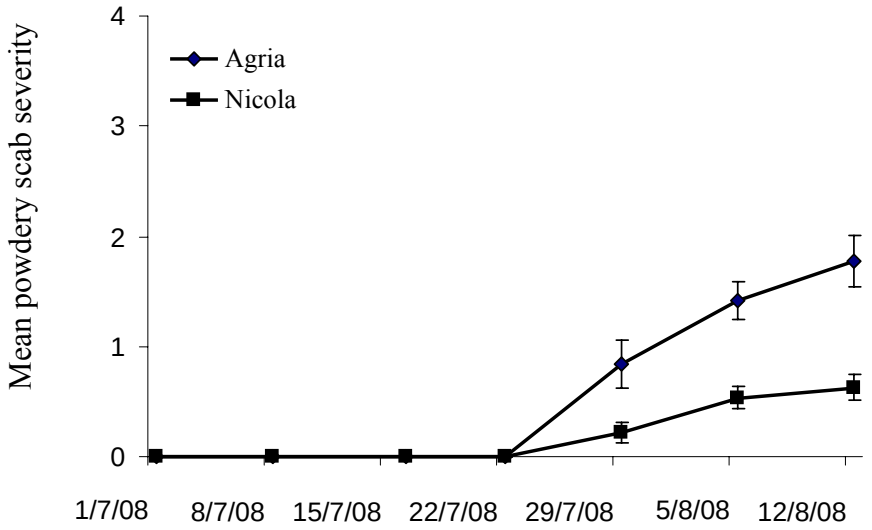

Figure 2. Mean powdery scab severity on a 0-6 scale of increasing disease measured at weekly intervals on cultivars Agria and Nicola. Standard errors of means are shown.

Table 1. Summary of first observations of disease and detection of *S. subterranea* DNA in roots and tubers of cultivars Agria and Nicola in 2008 n/a = no tubers formed, √ = detected, x = not detected.

Sampling Date	Root infection detected (DNA)		Root Gall symptoms observed		DNA detected in symptomless tubers		Tuber symptoms observed	
	Agria	Nicola	Agria	Nicola	Agria	Nicola	Agria	Nicola
1/7/08	√	√	x	x	n/a	n/a	n/a	n/a
8/7/08	√	√	x	x	√	√	x	x
16/7/08	√	√	x	x	√	√	x	x
22/7/08	√	√	√	√	√	√	x	x
29/07/08	√	√	√	√	√	√	√	√
5/8/08	√	√	√	√	√	√	√	√
12/8/08	√	√	√	√	√	√	√	√

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ABBREVIATIONS

The following abbreviations may be present, without definition, in the papers in this and previous editions of the Proceedings of the CPNB Conferences.

acid equivalent	a.e.	litres per hectare	litres/ha
active ingredient	a.i.	logarithm, common, base 10	log
approximately	c.	logarithm, natural	ln
body weight	b.w.	low volume	LV
boiling point	b.p.	maximum	max
centimetre(s)	cm	maximum residue level	MRL
coefficient of variation	CV	metre(s)	m
colony-forming unit(s)	cfu	metres per second	m/s
compare	cf	milligram(s)	mg
concentration x time product	ct	milligrams per kg	mg/kg
concentration required to kill 50% of test organisms	LC_{50}	millilitres(s)	ml
		millimetre(s)	mm
correlation coefficient	*r*	Minimum	min
cultivar	cv.	minimum harvest interval	MHI.
cultivars	cvs.	minute (time unit)	min
day(s)	d	moisture content	M.C.
days after treatment	DAT	molar concentration	M
degrees Celsius (centigrade)	DC	more than	>
degrees of freedom	df	no significant difference	NSD
dose required to kill 50% of test organisms	LD_{50}	not less than	|<
		not more than	|>
emulsifiable concentrate	EC	page	p.
enzyme-linked immuno-sorbant assay	ELISA	pages	pp.
		parts per billion	ppb
fast-protein liquid chromatography	FPLC	parts per million	ppm
for example	e.g.	parts per trillion	ppt
freezing point	f.p.	pascal	Pa
gas chromatography-mass spectrometry	gc-ms	percentage	%
		polyacrylamide gel electrophoresis	PAGE
gas-liquid chromatography	glc		
genetically modified	GM	polymerase chain reaction	PCR
genetically modified organism	GMO	post-emergence	post-em.
gram(s)	g	pre-emergence	pre-em.
growth stage	GS	pre-plant incorporated	ppi
hectare(s)	ha	probability (statistical)	*p*
high performance (or pressure) liquid chromatography	hplc	relative humidity	r.h.
		revolutions per minute	rev/min
high volume	HV	second (time unit)	S
hour	h	standard error	SE
integrated crop management	ICM	standard error of the difference	SED
integrated pest management	IPM	standard error of the mean	SEM
kilogram(s)	kg	soluble powder	SP
kilogram(s) per hectare	kg/ha	species (singular)	sp.
kilometres per hour	km/h	species (plural)	spp.
least significant difference	LSD	square metre	m^2
less than	<	subspecies	ssp.
litre(s)	litre(s)	suspension concentrate	SC
systemic acquired resistance	SAR	mega (x 10^6)	M

tandem mass spectrometry	MS-MS	kilo	($x10^3$)	k
technical grade	tech.	milli	($x10^{-3}$)	m
temperature	temp.	micro	($x10^{-6}$)	μ
thin-layer chromatography	tlc	nano	($x10^{-9}$)	n
time for 50% loss; half life	DT_{50}	pico	($x10^{-12}$)	p
tonne(s)	t			
tonne(s) per hectare	t/ha			
ultralow volume	ULV			
vapour pressure	v.p.			
variety (wild plant use)	var.			
volume	**V**			
water dispersible granule	WG			
weight	*wt*			
weight by volume	*wt/v*			
weight by weight	*wt/wt*			
wettable powder	WP			