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SCIENCE SUPPORT FOR RURAL AFFAIRS AND THE ENVIRONMENT – STRATEGIC PRIORITIES FOR SCOTLAND

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Scotland has a long and exemplary record in sustained investment in land-based science. This investment of around £57m per year pays dividends in the reputational quality of Scotland's agricultural and environmental research, which in turn underpins the science and delivery of, for example, plant health policy.

This presentation explores the future needs and priorities for the Scottish Government rural affairs and environment portfolio of research and the developing strategy that, through consultation, will provide the framework to commission research for the period 2016-21. Examples broadly pertinent to crop science are used to illustrate how the strategy needs to draw from integrating the science to address gaps in our knowledge. By means of illustration, it is acknowledged at a strategic level that food, water, and energy security are inextricably linked[1] yet our understanding of the soil system that sustains these functional linkages has significant knowledge gaps at decision-making scales.[2] An effective strategy will help address such knowledge gaps to ensure that the way we manage the land surface in the future does not promulgate actions that in the past that have led to unintended consequences such as the degradation of soils, with associated loss of soil organic matter, biodiversity and crop productive capacity. A step in the right direction is to ensure the strategy integrates appropriately the many pillars of research activity using a systems approach. So for soils, bringing together our understanding of the many essential ecosystem services the soil system provides including, sustaining nutrient cycling and crop production, supporting freshwater quality through natural filtration, supporting a bio-based economy and future bioenergy demand (i.e. bio-based energy, industrial chemicals and feed-stocks)[3], and mitigating climate change through carbon sequestration.

[1] World Economic Forum 2012. Global Risks 2012: Seventh Edition. (available at: http://www.weforum.org/reports/global-risks-2012-seventh-edition). 64pp.
[2] Investing in Soils 2010. Nature Geoscience 3, 295. Robinson et al. 2012. Natural capital, ecosystem services and soil change. Vadose Zone J. doi:10.2136/vzj2011.0051
[3] Reay et al. 2012. Nature Climate Change 2, 410-16.

COMPOSTS AND DIGESTATES - OPPORTUNITIES AND BENEFITS FOR FARMERS AND GROWERS

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Summary: European and UK legislation now encourages the recycling of organic wastes into composts and digestates, thereby reducing waste and carbon emissions, and saving finite resources. Compost is a valuable soil conditioner and fertiliser: it contains stable organic matter, valuable plant available nutrients including low concentrations of readily available nitrogen (RAN) and it often has a liming value. Digestate is primarily a valuable fertiliser, which contains high concentrations of RAN. An understanding of the relevant legislation and best practice guidance is essential in order to use composts and digestates safely and to best agronomic and economic effect. Key legislation and guidance is outlined in this paper.

INTRODUCTION

Compost can be defined as a stable, sanitised, soil-like material, which has been made from park and garden wastes and sometimes food wastes through mixing, self-generated heating and aeration. It is used as a soil conditioner and a source of nutrients for crops. Compost produced in accordance with British Standards Institution Publicly Available Specification 100 – BSI PAS100 conforms to strict safety standards (BSI, 2011). In England, Wales and Northern Ireland, the Compost Quality Protocol (CQP) (WRAP and Environment Agency, 2012) builds on BSI PAS100 by clarifying which waste materials can be used in compost production and by ensuring accurate record keeping where BSI PAS 100 certified compost is used in agriculture, field horticulture, landscaping and land restoration. CQP-compliant compost is classed as a product, not a waste, and therefore does not require an environmental permit/license for transport or application to agricultural land. The CQP does not apply in Scotland, where BSI PAS100 compost (without blending with other wastes, materials, composts, products or additives) can be used without further recovery, where there is a market for the material. Non BSI PAS100 compost is regarded as waste in the UK and is subject to waste regulations.

Around 2.8 million tonnes of compost are produced annually in the UK and of that, approximately 67% is used in agriculture (WRAP, 2012). Compost is typically made on large, centralised sites under an Environmental Permit (England and Wales), a Waste Management Licence (Scotland) or a Pollution Prevention and Control (PPC) Permit. Most sites (around 65% of input tonnage) treat only garden wastes in outdoor windrow systems to produce “green compost”. A further 33% include food wastes in their input materials and produce “green/food compost”; these operators are therefore subject to the strict requirements of the Animal By-Products Regulations (Scottish Government, 2013a).

Anaerobic digestate can be defined as a liquid (or fibre) produced through the controlled biological decomposition of biodegradable materials in the absence of oxygen. Digestates are valuable fertilisers (and in the case of fibre digestates, also soil conditioners). Digestates certified under the UK Biofertiliser Certification Scheme are also sometimes called "biofertilisers". The Biofertiliser Certification Scheme (BCS) provides a baseline quality standard for digestate, ensuring that it is consistent, safe and reliable to use (BCS, 2013). Only digestates certified under this scheme should be called biofertilisers. Non BCS-compliant digestates are regarded as wastes in the UK and are subject to waste regulations. The majority of digestates produced in the UK at present are whole digestates (~3 to 10% dry matter), although a number of anaerobic digestion (AD) plants are investigating options for separating the whole digestate into liquid (~ 1 to 3% dry matter), and fibre fractions (~ 18 to 42% dry matter), in order to facilitate more cost-effective distribution.

Around 1 million tonnes of digestate (largely food-based) were produced in the UK in 2010 (WRAP, 2012), although this tonnage is set to rise considerably in future. Almost all of the digestate produced was used in agriculture. Digestates are typically made on large waste management sites under a Pollution Prevention and Control (PPC) Permit. Most of the large AD plants include food and food processing wastes in their input materials, and are therefore subject to the requirements of the Animal By-Products Regulations (Scottish Government, 2013a). However, some AD plants are based on farms and these typically digest animal manures and/or purpose-grown crops.

THE PROPERTIES OF COMPOSTS AND DIGESTATES

Compost has several properties that make it useful in agriculture and field horticulture. For example, it contains stable organic matter, valuable plant available nutrients including low concentrations of readily available nitrogen (RAN). It often has a liming value. The key properties of green and green/food compost are summarised in Table 1.

Compost produced in accordance with BSI PAS 100 must conform to strict safety limits on human and animal pathogens, potentially toxic element (PTE) content, stability/maturity and physical contaminant levels (BSI, 2011). The properties of waste composts may differ and users should consider having samples analysed for BSI PAS 100-required parameters prior to use to establish that they are safe. Users of PAS 100 accredited and non-accredited composts are also advised to obtain information on the nutrient content of products used.

Digestate is a valuable source of major plant nutrients and organic matter. Whole and separated liquid digestates are primarily a source of nutrients (particularly RAN), whilst separated fibre digestates also supplies useful amounts of organic matter and can have a liming value. The typical nutrient contents of food-based and manure-based whole digestates are summarised in Table 2. As the nutrient content of whole digestates will vary between AD plants, it is advisable to obtain recent digestate analysis data for use in nutrient management planning.

Digestates produced in accordance with the BCS must conform to strict safety limits in terms of their human and animal pathogen content, PTE content, stability/maturity and physical contaminant levels which are set out in BSI PAS 110 (BSI, 2010). The properties of waste digestates may differ and users should consider having samples analysed to establish that they

are safe. For example, they could be tested for the parameters required under BSI PAS 110 along with nutrient content prior to use.

Table 1. Typical properties of green and green/food compost.

		Green compost	Green/food compost
Nitrogen (N)	kg/fresh tonne	7.5	11
Dry matter content	%	60	60
Phosphate (P_2O_5)	kg/fresh tonne	3.0	3.8
Potash (K_2O)	kg/fresh tonne	5.5	8.0
Magnesium (MgO)	kg/fresh tonne	3.4	3.4
Sulphur (SO_3)	kg/fresh tonne	2.6	3.4
Organic matter content	% dry matter (dm)	30	35
Carbon (C):nitrogen (N) ratio	-	12	10
Neutralising value (NV)	% CaO in dm	3	3

Table 2. Typical nutrient contents of food-based and cattle slurry-based whole digestate.

		Food-based digestate	Cattle slurry-based digestate
Dry matter content	%	4.0	4.0
Nitrogen (N)	kg/m^3	5.0	2.6
Phosphate (P_2O_5)	kg/m^3	0.5	1.2
Potash (K_2O)	kg/m^3	2.0	3.2
Magnesium (MgO)	kg/m^3	0.1	0.6
Sulphur (SO_3)	kg/m^3	0.4	0.7

THE BENEFITS OF USING COMPOSTS AND DIGESTATES

As compost and digestate contain valuable amounts of plant available nutrients, farmers can save on the cost of purchased fertilisers if their use is integrated into crop nutrient plans. Whole digestates are primarily fertilisers and most of the nitrogen is readily available (usually around 80% of the N present in food-based digestate is readily available). Composts and fibre digestates as well as being a source of nutrients, also act as soil conditioners, since they contain useful amounts of organic matter and often have a liming value. Their organic matter content is particularly important when these materials are used on arable land, as many arable soils have become depleted in organic matter over the past few decades.

Organic matter is a vital component of fertile soils. Composts and digestates can form part of a long-term strategy to maintain and enhance soil organic matter levels, and thereby help to maintain soils in good agricultural and environmental condition. A ‘typical’ application of 30t/ha of green compost supplies around 5t/ha of organic matter and 20t/ha of green/food compost around 4t/ha of organic matter. Compared to other organic materials that are commonly applied to agricultural land, compost supplies organic matter in a much more long-lasting form (due to its relatively high lignin content).

Soils that have optimal organic matter levels tend to perform much better than soils which are depleted in organic matter. Compost helps to improve soil structure, structural stability and water holding capacity. Increases in soil organic matter levels positively affect a range of soil physical, chemical and biological properties. The benefits of compost tend to be greatest where regular additions (typically 20-30 t/ha) are made over several years to low organic matter content (<5%) soils. Crops often grow and yield better where compost has been used over several years.

LEGISLATION AND GUIDANCE

Land managers using compost and digestate must be aware of Nitrate Vulnerable Zones (NVZ) regulations, and those using materials based partly on food waste must also understand the implications of the Animal By-Products Regulations.

If food waste or certain types of food processing waste are included in the feedstocks from which composts or digestates are made, then the Animal By-Products Regulations will apply during the production and use of these materials. The main aim of these regulations is to prevent animal by-products presenting a risk to animal or public health through the transmission of disease. Although the regulations differ slightly between Scotland, England and Wales, the rules regarding the safe manufacture and use of green/food compost and food-based digestate are similar in all three countries. Compost or digestate destined for use in agriculture must be clearly identified as being derived from animal by-products material. When compost or digestate containing animal by-products is applied to pasture land, that land cannot be used for livestock grazing (or harvested for forage) within 3 weeks (or 2 months for pigs) of application.

The NVZ rules in Scotland are similar to those in England and Wales (Scottish Government, 2013b). As compost contains very little readily available N (RAN), it is not subject to closed spreading period rules in NVZs. The small amount of crop available N supplied by green/food compost must be taken account of when calculating manufactured fertiliser recommendations, as part of the Nmax rule. As the RAN content of whole/separated liquid digestate exceeds 30% of the total N content, digestates (like cattle and pig slurry) applications are subject to mandatory closed spreading periods during autumn/winter in NVZs. Digestate (in common with other high RAN organic materials) must not be applied prior to legume crops, as these crops have no N requirement.

In NVZs, compost and digestate can be applied at rates which provide up to 250 kg/ha total N in any 12 month period, or for BSI PAS100 certified compost up to 500 kg/ha total N in any two year period when applied as a mulch or soil conditioner. The “Prevention of Environment Pollution from Agricultural Activity” (PEPFAA) Code for farmers in Scotland describes best practice (some of which is mandatory in NVZs) for using organic manures (including compost and digestate) in such a way that the environment is protected and nutrient losses are minimised (Scottish Government, 2013c). Similar codes of Good Agricultural Practice exist in England and Wales.

LOGISTICAL AND AGRONOMIC CONSIDERATIONS

In order to calculate the fertiliser replacement value of composts and digestates, total nitrogen, phosphate, potash, magnesium and sulphur contents should be known. Average nutrient contents are shown in this paper (Tables 1 and 2) and published in SRUC Technical Note 650 (SRUC, 2013). However, it is better to analyse the products being used. Composts and fibre digestates are typically applied at rates between 20 and 60 t/ha, and whole and liquid digestates between 20 and 40 m^3/ha.

When using green compost, farmers should not alter manufactured fertiliser N applications as field experimental data have indicated that green compost supplies negligible amounts of crop available nitrogen. Green/food compost does supply small amounts of crop-available N and farmers should subtract 5% of the total N content (irrespective of application timing) from the manufactured fertiliser N requirement. Following the regular use of green and green/food compost, long-term soil N supply will be increased.

Most of the nitrogen in whole/separated liquid digestate will become available to the crop in the year of application, as it is mainly present as RAN (i.e. ammonium-N). However, the RAN content of digestate can easily be lost to the environment by two main routes: ammonia volatilisation to air and, following the conversion of ammonium-N to nitrate-N in the soil, though nitrate leaching to surface and ground waters. These losses represent a reduction in nutrient value to the farmer as well as a source of pollution. Two key recommendations can dramatically improve the efficiency with which N is taken up by the crop. Firstly, digestate should only be applied when there is crop demand, during spring and summer. Secondly, precision equipment such as bandspreaders (trailing hose/trailing shoe) or shallow injectors should be used. Bandspreading equipment is now available that allows accurate topdressing across full tramline widths, without causing crop damage and contamination, and as a result increases the number of spreading days. When applied to land prior to cultivations and sowing a crop, rapid soil incorporation following application (i.e. within 6 hours) will also reduce ammonia losses and odour nuisance, and increase N use efficiency.

Around 50% of the total phosphate content of compost/digestate and 80% of the total potash content are estimated to be crop available in the year of application; with the total amount of P and K being available in the longer-term.

Further details on how to calculate crop nutrient requirements following compost/digestate application are contained in SRUC Technical Note TN650 “Optimising the Application of Bulky Organic Fertilisers” (SRUC, 2013) and in the Defra Fertiliser Manual (Defra, 2010). In addition, nutrient management planning can easily be undertaken using decision support systems such as MANNER-*NPK* (MANure Nutrient Evaluation Routine) or PLANET/PLANET Scotland. These systems predict the fertiliser N replacement value of field applied organic materials, including composts and digestates, taking into account manure analysis (total N, ammonium N, nitrate N), soil type, application timing and technique, ammonia volatilisation, denitrification and nitrate leaching losses, and the mineralisation of organic N.

It is important to maintain soils at their target pH values in order to ensure the optimal availability of nutrients and trace elements. Compost (typical neutralising value 3%) and some fibre digestates have a small liming value that can help increase the pH of soils below the

target value. Products with a 'high' neutralising value (>6%) should only be applied to soils which require lime, as over application can induce trace element deficiency (e.g. manganese in arable crops).

The majority of farm assurance schemes and produce buyers support the use of compost and digestate where they are used in accordance with current legislation and best practice. However, some have rules restricting use, hence, users are advised to consult both their assurance scheme(s) and their crop/produce buyer(s) about any possible restrictions on compost or digestate use.

ACKNOWLEDGEMENTS

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TOOLS FOR IPDM AND ANALYSIS OF "SUSTAINABLE" CROP MANAGEMENT IN CEREALS

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Summary: Integrated pest and disease management for cereal-based cropping systems comprises many potential components. These include fungicides, resistance elicitors, cultivars and their deployment strategy in mixtures, integration of resistance genetics with crop protection treatments, soil tillage and other agronomy strategies including crop sequence. In future non-pathogenic microbe interactions, such as beneficial endophytes, may be important components of the system too. Some of these options will interact detrimentally with yield or profitability, so management decisions must select and implement those that give additive or synergistic interactions for achieving the desired benefits, be they economic, environmental or social. A system assessment tool called DEXiPM is being evaluated to assist this process and report overall and component sustainability.

INTRODUCTION

Environmental regulation, withdrawal of pesticides and the cost of inputs are all encouraging progress towards more Integrated Pest and Disease Management (IPDM) approaches. IPDM requires more management input and understanding of the crop system complexity. It also requires new tools both for its implementation and assessment of its success at achieving more sustainable crop production. Many approaches where we and others are carrying out active research contribute to crop protection and sustainability. However, whether combining them together is additive, synergistic or detrimental is more difficult to assess. The aim of this paper is to highlight the resources and tools that are being developed and a method of determining how they affect sustainability.

MATERIALS AND METHODS

Some approaches, their attributes and potential utility, are briefly listed below.

Fungicides and their timing

Some pathogens such as *Rhynchosporium commune* grow without symptoms on some cultivars or some environments, but still carry a yield penalty. Other non-pathogenic microbes may have positive or negative effects on yield such as through induction of defence mechanisms. Early season (T0) fungicide sprays can be cost-effective depending on the cultivar and inoculum load. Other appropriate dose decisions also need tailoring to cultivar-related risk.

Resistance elicitors

Research and development of resistance elicitors has increased considerably and is likely to give new products on the market soon. They act by priming the plant's own resistance mechanism to work more effectively when challenged by pathogens whether fungal, bacterial or viral. They also interact with cultivars differentially and both formulation and plant physiological state, influenced particularly by nutrition, affect efficacy and must be managed appropriately. Most have no direct toxic effect on any organisms and therefore must be treated differently from fungicides (Walters *et al*., 2005).

Cultivar and their deployment

Disease resistant cultivars are a valuable component of any crop management approach, but both choice and their deployment strategy will affect their efficacy and longevity. Mixing several cultivars together, preferably in a "patchy" way, can not only reduce disease but also enhance yield through greater resource utilisation such a nutrients and light (Newton & Guy, 2009; Newton *et al*., 2009). A small proportion of a resistant cultivar can have a disproportionately large effect on disease reduction (unpublished data). Overall crop resilience is increased or risk reduced, which may facilitate reductions in other pesticide inputs. Individual cultivars may have extended useful life when deployed in mixtures.

Resistance genetics

Durable resistance that does not carry a yield penalty is an important component of breeding new cultivars and minimum resistance standards are required for Recommended Listing. New approaches combining different, complementary modes of action as well as controlling asymptomatic infection are likely to improve not only durability, but also achieve high yield in the absence of response to fungicides (Looseley *et al*., 2012; 2014 (in press)). Response to fungicides, may be better correlated with asymptomatic infection than observed disease.

Soil tillage

Reduced tillage is becoming increasingly common. Weed problems can be exacerbated whilst some diseases are increased, others decreased. However, over time soil structure can improve and microbial interactions can reduce disease risk, affecting crop yield directly and indirectly. There are also cultivar interactions with some cultivars adapting to reduced tillage more than others (Newton *et al*., 2012).

Beneficial endophytes

Endophytes are microbes that live within the plant but confer no outward symptoms. They may be fungal, bacterial or viral. The beneficial effects of one in barley, *Piriformaspora indica*, are known, particularly enhanced biomass (yield), resistance to pathogens and to abiotic stress such as salt (Newton *et al*., 2010a; 2010b). However, some have host genotype preferences so deployment strategies may require special consideration. More persistent beneficial endophytes adapted to field conditions could be developed in the context of reduced pesticide inputs.

Crop sequence

Rotations are largely driven by the need for maintaining soil health or fertility and controlling disease risk, constrained by demands of needing to grow profitable crops and the cost of inputs, i.e. profit margins. They can also be influenced by policy and sustainability drivers. Therefore considerations about incorporating fertility-building crops such as legumes must be balanced against immediate crop profit, value to subsequent crop production and regulatory targets.

DEXiPM

To help make decisions on which of these and other methods to choose we are investigating the use of a sustainability assessment tool. DEXiPM is a qualitative multi-criteria assessment tool incorporating integrated pest (and disease) management built on a decision support system called DEXi (Pelzer *et al*., 2012). It has 75 basic indicators describing the cropping system and the context of the assessment, and 86 aggregated indicators, assessing the usual three dimensions or criteria of sustainability in terms of social, environmental and economic issues. These three criteria can be expressed graphically and used to assess the consequences of cropping practice decisions before they are implemented and subsequently validated against real crop data.

Systems trial

The use of DEXiPM is being tested on a wheat-based rotation experiment being carried out on Balruddery Farm at the James Hutton Institute, Dundee in conjunction with five other similar experiments in France, Denmark, Germany and Poland within the EU IPM project PURE. Three different rotations were compared: Current - winter wheat, winter wheat, oilseed rape; Intermediate - winter wheat, spring barley, oilseed rape; and Advanced - winter wheat, peas, oilseed rape. In addition, the intermediate and advanced treatments included some of the approaches listed above: reduced dose and more effectively targeted fungicides, resistance elicitors, cultivar choice and mixtures, reduced herbicides and fertiliser, and all treatments had minimum tillage.

RESULTS

Not all the methods noted above will be fully compatible. If a resistance source stops asymptomatic infection by a pathogen as well as symptoms (disease) then response to T0 sprays is likely to be considerably reduced. Some cultivars may perform better with particular microbial communities or soil conditions resulting from continuous cultivation of the same crop or minimum tillage where more debris is retained (unpublished data). The rhizosphere and phylloplane microbial community can be strongly influenced by cultivar and therefore development of pathogen infections will be indirectly influenced this way (Birch *et al*., 2014). Resistance elicitors may work in some combinations but not others due to cross-talk between resistance induction pathways, and their efficacy is currently somewhat variable (Birch *et al*., 2014). However, they may be compatible with some endophytes whereas systemic fungicides would not be compatible with fungal endophytes. Cultivar mixtures may be less effective in conjunction with resistance elicitors as part of their mode of action is resistance induction too.

Given the above qualifications and others, to determine compatible methods judgement calls

are required based on expert knowledge. DEXiPM is a qualitative multi-criteria assessment tool that requires inputs that are preferably quantitative to be categorised from high to low within a parameter range appropriate to the crop system being considered. The utility of the outcome is dependent on these basic parameter ranges being defined and data being as objective as possible.

For pesticides inputs are defined as TPI units (Total Pesticide Index) where the full recommended dose of each active ingredient (or product) is 1.0 and the total for the crop in the whole season is calculated. Similar indices are calculated for other measurable inputs whilst others are classed as low to high by more subjective assessments.

Ex-ante assessments were carried out on the planned treatments and these are summarised in the figures below. Ex-post assessments are then made based on the actual inputs and the outputs such as yield and quality.

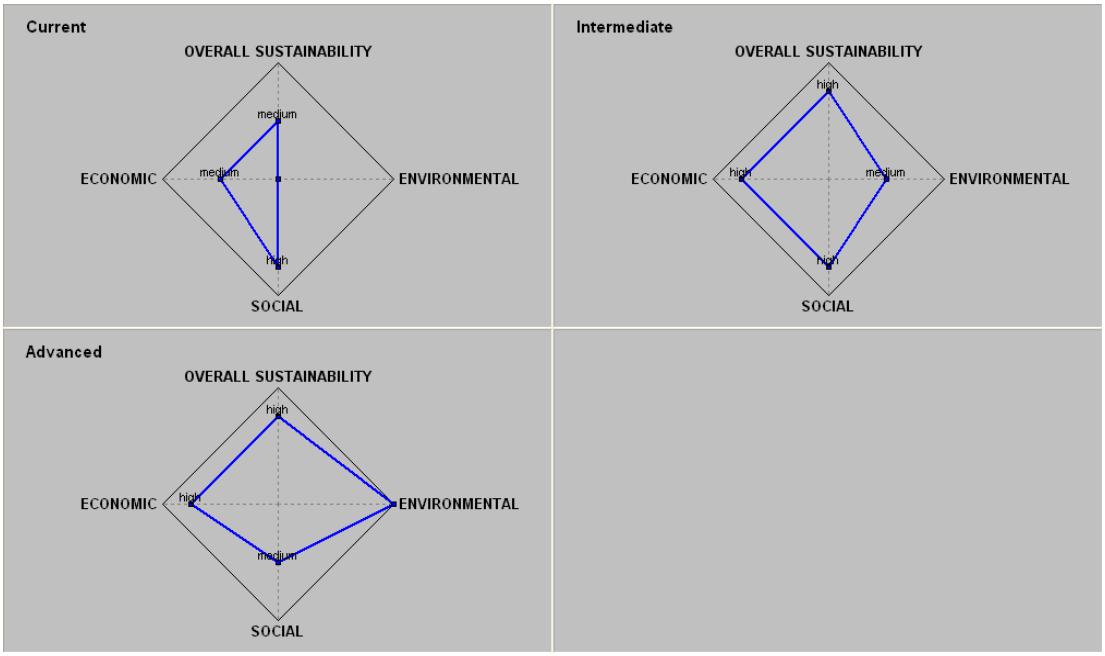

Figure 1. Ex-ante assessment of overall sustainability rating of Current, Intermediate and Advanced wheat-based rotation treatments for sustainability using DEXiPM.

The ex-ante assessment showed overall sustainability of Intermediate and Advanced treatments is improved (Figure 1), the former on environmental and economic dimensions whilst the latter biased towards the environmental at a cost to the social dimension, attributable largely to the reduction in TPI from using resistance elicitors instead of fungicides. At a slightly more detailed level the environmental sustainability shows improvements on the biodiversity and environmental quality dimensions in the Intermediate treatment, both further enhanced in the advanced treatments (Figure 2). Other graphics and tables identify the main individual components contributing changes.

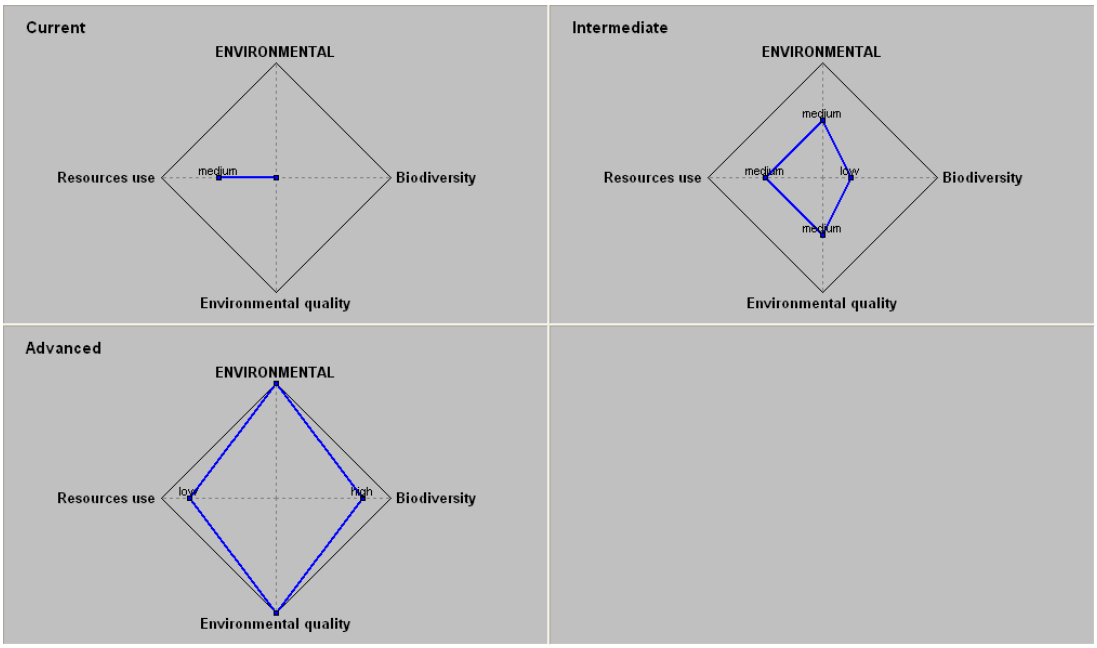

Figure 2. Ex-ante assessment of environmental sustainability rating of Current, Intermediate and Advanced wheat-based rotation treatments for sustainability using DEXiPM.

A preliminary ex-post assessments showed little change to the economic and social dimensions as the planned treatments were carried out largely unchanged. However, disease and yield are highly responsive to the environment and inputs with direct economic consequences. In the first year disease was severe and the elicitor treatments were not adequate in the advanced treatment. Furthermore, weeds were not adequately controlled in the Intermediate and Advanced treatments creating problems for the following year. In summary, the overall sustainability tended towards the current practice, economic performance having been sacrificed for gains in social and environmental criteria.

DISCUSSION

The DEXiPM tool proved very useful for defining sustainability hypotheses then testing them. Ex-ante assessment showed whether economic, environmental or social dimensions should be affected by changes in cropping practice and to what degree. Ex-post assessment was used to validate the hypotheses and identified where judgements proved inaccurate and what the consequences were. Economic, environmental and social dimensions have different drivers and so changes are made using different instruments such as environmental payments for carrying out different practices. Where, for example, yield is excessively damaged it will be clear that the reduction in the economic dimension is not adequately compensated for by increase in the other dimensions. Where this is not adequately reflected then the decision model rules such as parameter weightings and aggregation need to be re-assessed or the parameter ranges revised for the cropping system being assessed. Nevertheless, DEXiPM assessment is a promising approach for understanding and managing new approaches for improving crop system sustainability, interfacing farming, policy, environmental, social, scientific and economic areas.

ACKNOWLEDGEMENTS

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APPLICATION OF PLANT DEFENCE ELICITORS ON HORTICULTURAL CROPS TO CONTROL BACTERIAL PATHOGENS

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Summary: Plant defence elicitors can be used to induce systemic resistance and control pathogens in a number of different crops. Our previous trials provided evidence that some experimental elicitors can control bacterial pathogens in allium and brassica crops. The work is now being expanded with trials in 2013 to test whether commercial products with potential elicitor activity also provide protection. The aim is to obtain robust evidence for products that are commercially available to growers in the UK now, or are likely to be available in the near future. Data from the first of a two-year trial shows that application of elicitors can reduce disease in radish, and can also affect the yield (fresh weight) of broccoli.

INTRODUCTION

Plant defence elicitors trigger natural resistance in the plant and have proven credentials against bacterial and fungal pathogens. Experimental elicitors such as ASM and BABA have been shown to control *Pseudomonas fluorescens*-mediated soft rot in broccoli, under controlled conditions (Pajot & Silue, 2005) and a separate study showed protection in apple seedlings against *Erwinia amylovora* (Brisset *et al.*, 2000). In Asia, Probenazole (Orzymate) is approved for use against bacterial and fungal blight in rice. However, the majority of elicitor trial work has targeted fungal infections, e.g. application of BABA was shown to control fungal infection of lettuce by triggering plant defence genes (Pajot *et al.*, 2001). In oil seed rape trials in Scotland, a combination of cis-jasmone, Bion and BABA was found to be highly effective against the fungal disease light leaf spot (Oxley & Walters, 2012). For bacterial diseases, there are few other feasible options. Although antibiotics are used in some countries, their application is banned in many others, including the UK. Plant defence elicitors generally have little or no toxicity and are not microbiocidal. Instead they work through priming the plant's own defence mechanisms and as such have lower eco-toxicity and a negligible effect on soil health. In addition, the action of elicitors on plant resistance is an ideal way to defend against weak (opportunistic) pathogens. Successful use of plant defence elicitors in a commercial setting is likely to vary between crops and disease systems. It is most likely that such elicitors will aid in crop protection as part of an integrated management system (Walters *et al.* 2013).

Many plant defence elicitors remain experimental, i.e. unlicensed for use in UK and / or further afield. Some fungicide active ingredients, such as azoxystrobin are known to induce resistance pathways (Herms *et al.*, 2002) and, under various trade names (e.g. Amistar), are licensed for use on brassicas and allium in the UK. Other active ingredients with elicitor activity are

marketed as fertilizers or nutritional supplements, e.g. chitosan-containing products and as such are not subject to approval status.

Brassica and allium crops suffer from a number of important fungal and bacterial diseases. Bacterial pathogens are a serious concern because available control options are very limited in choice and their efficacy depends on appropriate application. Trials have been initiated to test whether plant defence elicitors can be used to provide protection against bacterial and fungal pathogens in five different horticultural crop for commercially important diseases. These include head rot of broccoli caused by a combination of soil-borne bacteria (*Pseudomonas fluorescens, Ps. marginalis* and *Pectobacterium carotovorum* (Cui & Harling, 2006) and bacterial blight / leaf scorching in radish caused by *Pseudomonas cannabina* (Bull *et al.*, 2010). Post-harvest storage often exacerbates the problem or result in manifestation of disease symptoms (e.g. in the case of radish bunches).

The aim of the project is to test whether elicitors can limit bacterial and fungal infection and symptomatic disease in commercially important horticultural crops, using field and glasshouse trials. The work uses active ingredients with potential elicitor activity that are commercially available to growers. Preliminary information is provided for year 1 of a two-year trial for radish and broccoli.

MATERIALS AND METHODS

Experimental trials

Experimental field trials for broccoli and cabbage, and glasshouse trials for radish were established at an experimental field station in Scotland. Treatments were tested in replicate plots of three and the bacterial inoculum was applied at 10^6 cfu/ml by foliar spray, until run-off. Broccoli (var. Parthenon) was grown on a 100 m x 25 m site. Radish (vars. Celesta and Expo) were grown from seed for four-five weeks in compost, in glasshouses. Twenty replicate plants were assessed per treatment. Broccoli were infected with a cocktail of *Pseudomonas fluorescens, Ps. marginalis* and *Pectobacterium carotovorum* (collectively known as head-rot bacteria) and radish was infected with *Pseudomonas cannabina pv. alisalensis* (*Pca*).

Applications

Elicitors were applied as the sole treatment for broccoli and either applied independently or in conjugation with two fungicides for radish (one application of azoxystrobin (Amistar) at 7 – 14 days and one application of pyraclostrobin (Signum) at 14 – 21 days). The timing of application was dependent on plant development and all treatments were applied with hand-held sprayer. Two applications of elicitors were applied to broccoli at 14-day intervals, 40 days after the transplants were established, or to radish at 7 days intervals between 7 and 10 days after seedling emergence. Bacteria inoculum was applied to both broccoli and radish mid-way between the first and second elicitor treatment. Elicitor and fungicides were applied at the same rates and concentrations as previous trials (Holden *et al.*, 2012) and included the following: Stobulurin (Amistar, Signum); Bion (ASM); Regalia; Chitosan (Softguard); Seaweed extracts (Algal600, vacciplant); Harpin; SitKO-SA; Probenazole. Controls included the no treatment control (NTC), no bacteria control (NBC) and no treatment no bacteria control (NBNTC); standard fungicide programme (SFP).

Laboratory validation, disease assessment and analysis

PCR amplification was used to detect pseudomonads (Spasenovski *et al*., 2009). Disease was assessed visually: the incidence of symptomatic disease was scored as 'Healthy' or 'Diseased' and the extent assessed on 5-point scale of symptoms. Broccoli heads were harvested at maturity (~ 80 days after transplant establishment). Analysis of variance was carried out using MS Excel or Genstat computer programmes.

RESULTS

Radish leaves spray-inoculated with *Pca* developed blight-like symptoms on the leaves that in some instances became necrotic (Figure 1). Elicitors were tested on radish either independently, or incorporated into a fungicide programme. Preliminary results show a decrease in the extent of symptomatic disease with the application of SitKO-SA (Figure 2). The decrease was significant for radish var. Celesta, where there was a high extent of disease, but not for var. Expo, where the extent of disease was lower. Interestingly, the elicitors tended to have a greater beneficial effect on disease in the absence of other fungicides.

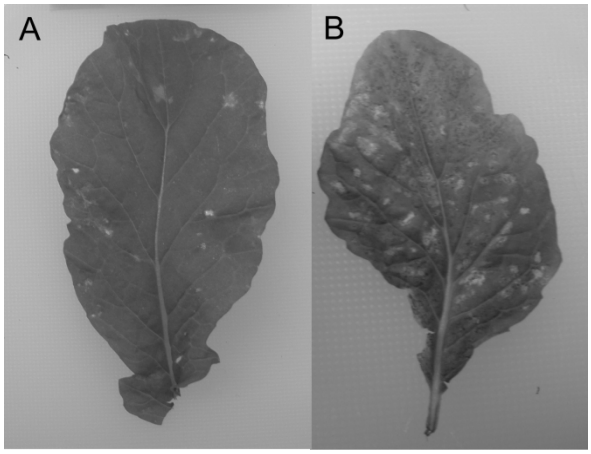

Figure 1. *Pca* symptoms on radish leaves. Radish plants were spray inoculated with *Pca* and disease assessed at the point of harvest. Symptoms of low (A) and high (B) severity.

Elicitors were also used on broccoli to assess their effect on plant development and on symptomatic disease from opportunistic head-rot bacteria. A significant increase in the yield (measured as 'head weight') was found with some elicitors (Figure 3). Those with the greatest head weight also showed symptoms of 'hollow stem' disorder most frequently. Application of head-rot bacteria did not result in symptomatic disease for the majority of plants, which meant that it was not possible to carry out statistical analysis of the effect of the elicitors. However, the least amount of disease was seen with application of Amistar, which was also found in previous trials (Holden *et al.* 2012).

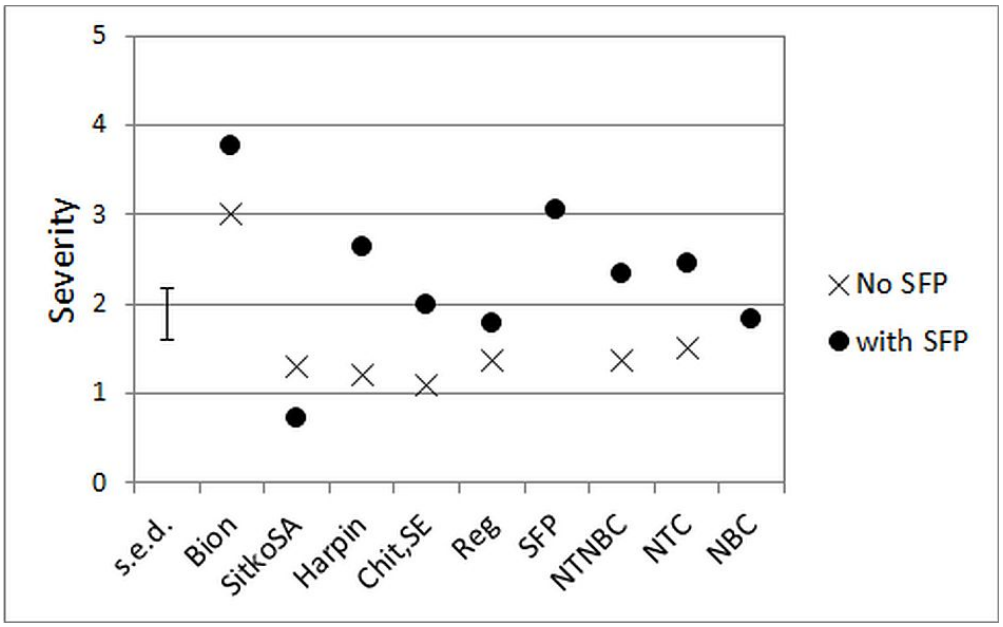

Figure 2. The effect of elicitors on the number of radish plants showing symptoms of *Pca* and the extent of disease. Disease severity was measured on a 0 (no disease) to 5 (maximum disease) scale. The average severity is shown for elicitor treatments incorporated into the fungicide programme (circles) or used independently (crosses). The error bar represents the standard error of the difference. Values are also provided for the controls (SFP; NTNBC; NTC; NBC).

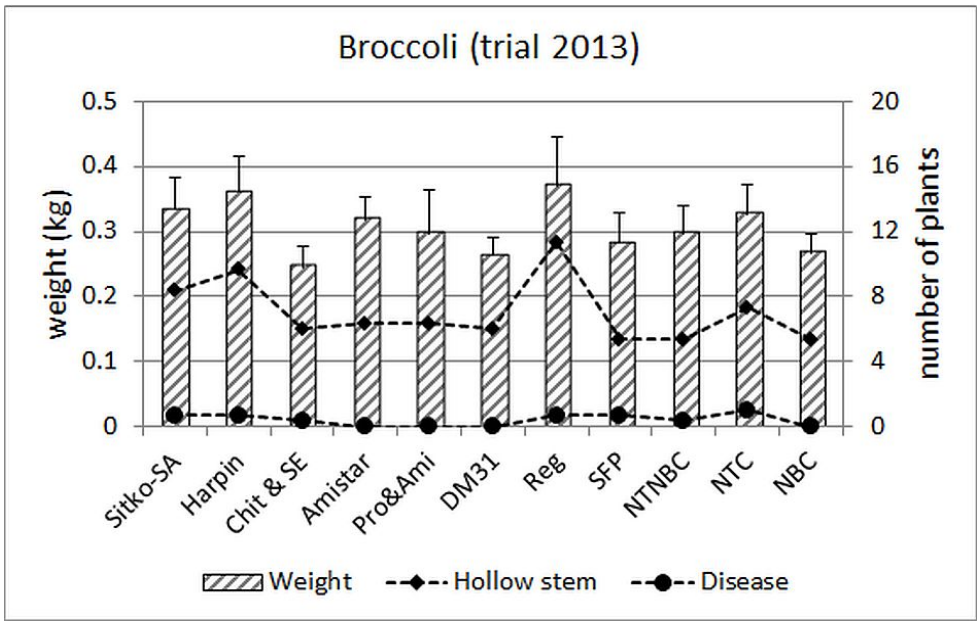

Figure 3. Effect of elicitor treatment on broccoli. The chart shows the average yield of fresh weight (hatched bars) with standard deviation; the number of plants showing hollow stem disorder (diamonds) and symptomatic disease (circles).

DISCUSSION

The effect of elicitors was tested on broccoli and radish plants infected with phytopathogenic bacteria. Preliminary data from the first of a two-year trial shows that one of the elicitors has a beneficial effect on disease symptom reduction in radish. Interactions are evident between variety type (Celesta vs. Expo) and the presence / absence of fungicides (Amistar, Signum). Application of elicitors was also shown to affect yield of broccoli, although those treatments that showed the highest yield also suffered from the highest incidence of hollow stem, a disorder associated with rapid growth. Symptomatic disease was rare on broccoli, despite the addition of head-rot bacteria that were grown under disease-inducing conditions. Since the bacteria were able to cause symptomatic disease under laboratory conditions, it is most likely that the environmental conditions were not conducive for disease, in this case. It is also possible that other microbes associated with the plants were able to compete with the head-rot bacteria.

Experimental trials have shown some success using salicylic acid mimics, such as ASM in the control bacterial phytpathogens in orchard trees, lettuce, broccoli and tomato (Pajot & Silue 2005; Graham & Myers, 2011; Yigit, 2011; Balajoo *et al.,* 2012). Sitko-SA contains a combination of SA and phosphite, which on its own has also been shown to induce systemic resistance (Lobato *et al.,* 2011). Therefore, it is perhaps unsurprising that SitKO-SA also shows a beneficial effect in the control of *Pca* in radish. Regalia is an extract of giant knotweed (*Reynoutria sachalinensis*) and although its mode of action is unclear, it is thought to induce multiple defence pathways in the host plant. It is recognised to have pharmaceutical properties and has been shown to induce phytoalexins which may aid in the control of fungal pathogens (La Torre *et al.,* 2004; Peng *et al.,* 2013). It is interesting that it had a significant growth effect on broccoli, although this was coupled with a trade-off in the incidence of hollow stem disorder, an undesirable property for producers that can also lead to stem rot.

On-going work aims to extend the trials to better assess the potential effect of elicitors in horticultural crops. There is a growing need to understand the molecular basis for the action of elicitors, so that they can be used as part of an integrated programme of crop management, in a more targeted manner.

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INSECTICIDE USE ON SCOTTISH OILSEED RAPE CROPS: HISTORICAL USE PATTERNS AND PEST CONTROL OPTIONS IN THE ABSENCE OF NEONICOTINOID SEED TREATMENTS

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Summary: Oilseed rape is a profitable break crop and an important component of Scottish arable agriculture. This crop has a wide range of insect pests, resulting in high insecticide input which combines seed treatments and foliar sprays. The organochlorine seed dressings used in the 1990s were replaced with neonicotinoid treatments from 2002 onwards; whereas over the last two decades sprays have been consistently dominated by pyrethroid insecticides. The restriction of neonicotinoid seed treatments will leave only foliar sprays available for crop protection which will limit the options for controlling pests of newly emerged crops.

INTRODUCTION

Oilseed rape has been cultivated in Scotland since the early 1980s (Anon, 1984) and during the last decade approximately 35,000 hectares have been grown each year (Anon, 2012a). In 2012 oilseed rape was the third most commonly grown arable crop in Scotland, accounting for 7% of the total combinable crop area.

Oilseed rape is grown as a break crop in arable rotations, helping to suppress the build up of weeds, disease and insect pests associated with cereals and potatoes. Its cultivation also offers farmers the opportunity to use plant protection products with different modes of action to combat existing pest populations.

Oilseed rape is predominately used to produce food grade oil and recently a market has developed for locally produced cold pressed rapeseed oil. A smaller area of crop is grown for biodiesel production. The pulp left after oil extraction is used for protein meal for animal feed. The market value of the 2012 Scottish crop was £39.4 million (Anon, 2013) and in recent years high market prices have led to oilseed rape becoming a more profitable break crop than peas or beans, which together accounted for less than 1% of the 2012 combinable crop area.

However, oilseed rape has a wide range of insect pests and crops are at risk from damage from seedling establishment through to seed development. The pest pressure oilseed crops are exposed to is reflected by their high insecticide input. In 2012, 94% of winter oilseed rape was treated with an insecticidal seed treatment and 70% of the crop received a foliar insecticide. In comparison, insecticidal seed treatments and sprays were applied to only 6 and 37% of the winter wheat crop respectively (Watson *et al*., 2013). This paper looks at trends in oilseed rape cultivation and insecticide use over the last 20 years and discusses the options available in the future, particularly in relation to the potential absence of neonicotinoid seed treatments.

METHODS

The pesticide use data presented in this paper are taken from the dataset collected by the Pesticide Survey Unit at Science and Advice for Scottish Agriculture (SASA), a division of the Scottish Government's Agriculture, Food and Rural Communities Directorate.

Biennial surveys of pesticide use on arable crops are conducted as part of the UK Government's statutory post-approval monitoring programme. The surveys are conducted by collecting data from a random sample of Scottish farms stratified by size and geographic region. Estimates of total pesticide use are produced from these sample data by ratio estimation; the data are multiplied by raising factors calculated by comparing the sampled area of each crop in each stratum with the total crop areas recorded in that year's agricultural census. Details of data collection and estimation can be found in the individual survey reports published on the SASA website (http://www.sasa.gov.uk/pesticides/pesticide-usage).

RESULTS AND DISCUSSION

Scottish Oilseed Rape Cultivation 1992-2012

Oilseed rape is an important component of the arable crop rotation. The overall area, and the proportion of spring and winter varieties grown, has varied over time in relation to climatic conditions at planting, market prices and changes in agricultural subsidies. However, over the last decade cultivation has been fairly steady at ca 35,000 ha, with more than 80% being winter sown varieties, with a mean yield of ca 3.5 t/ha (Figure 1). The yield increase from 2000 onwards was primarily due to greater cultivation of winter varieties which are higher yielding than spring sown crops (Figure 1). The poor yield in 2012 has been attributed to the difficult climatic circumstances in that year. Overcast conditions extend flowering and shorten the maturation period resulting in smaller seeds with lower oil content. Heavy rain can also result in pod shattering and premature seed shed.

Key Insect Pests and Historical Insecticide Use (1992-2012)

Oilseed rape has a wide range of insect pests and crops are at risk from damage throughout their life cycle. At seedling establishment, winter crops are vulnerable to damage from flea beetles, mainly the cabbage stem flea beetle (CSFB) *Psylliodes chrysocephala* whose larvae bore into the stems allowing water to enter the plant which can cause winter kill if it freezes. Other autumn pests include adult *Phyllotreta* spp. flea beetles which feed on rape foliage, rape winter stem weevil (*Ceutorhynchus picitarsis*) larvae which feed in crop stems and aphids such as *Myzus persicae* which transmits *Turnips yellow virus* (TuYV). Oilseed rape is also vulnerable to pollen beetle (*Meligethes aeneus*) damage at flowering and cabbage seed weevil (*Ceutorhynchus assimilis*) and brassica pod midge (*Dasineura brassicae*) damage during seed pod development.

In order to combat this wide range of pests, oilseed rape has historically been treated with a combination of seed treatments, to protect against insects colonising the crop at emergence, and foliar sprays to prevent damage from species occurring later in the season (Figure 2).

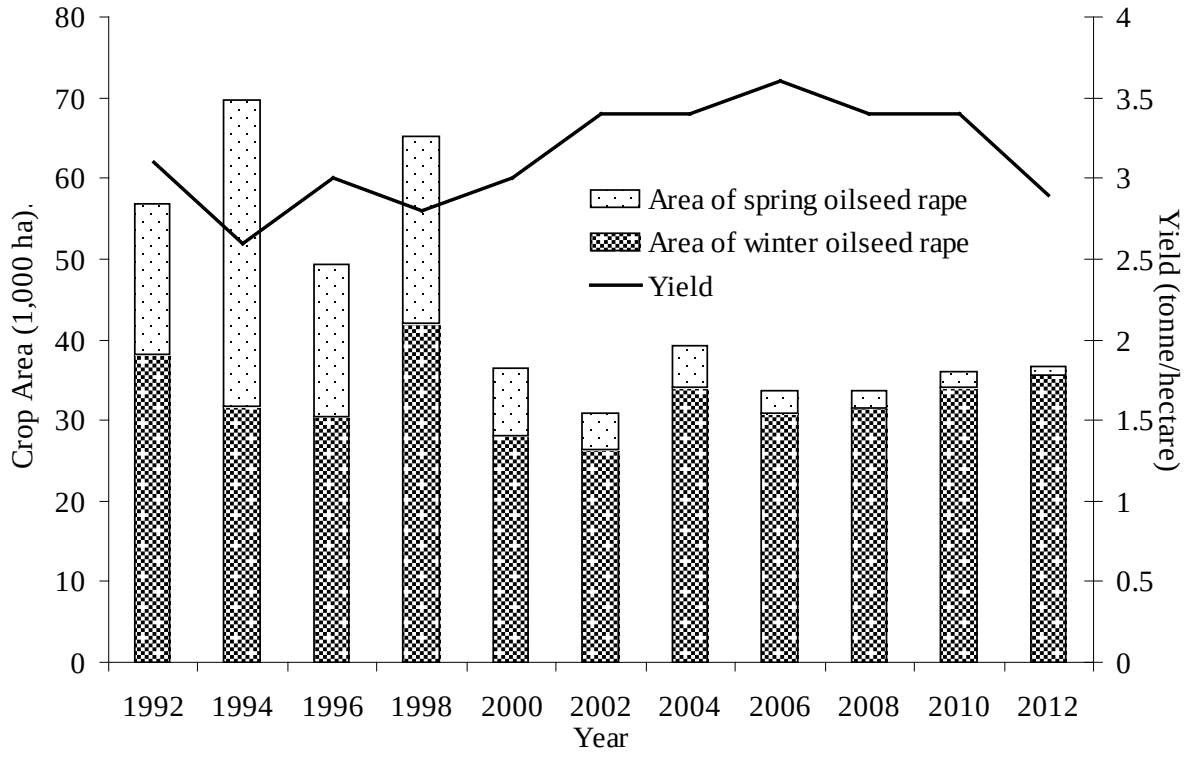

Figure 1. Oilseed rape cultivation area and yield 1992-2012 (data source: Anon, 2012b).

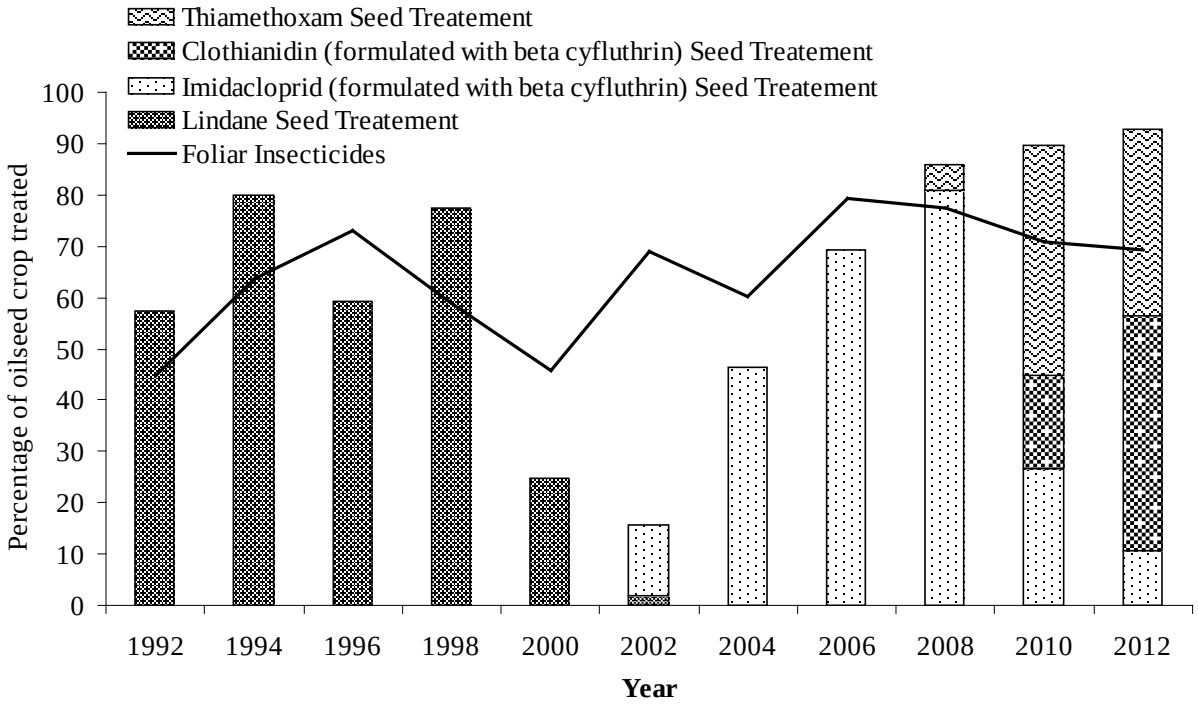

Figure 2. Percentage of oilseed rape crop treated with insecticidal seed treatments and foliar sprays 1992-2012.

Oilseed rape crops have received consistently high insecticide input over the last two decades (Figure 2) and, unlike cereals, insecticidal seed treatments are a basic component of the crop protection programme. In the 1990s, the seed treatment used was lindane, an organochlorine compound which was applied to between 60 and 80% of crops. Lindane approval was withdrawn in 1999 and its use declined to 20% in 2000 in anticipation of its 2001 final use date. As lindane was being withdrawn, imidacloprid, a new broad spectrum systemic neonicotinoid insecticide, was approved for use on oilseed rape crops. Imidacloprid was approved in 2000 and first encountered, on 14% of the crop, in 2002. The area of crop treated with imidacloprid increased over time, with an estimated 81% treated in 2008. Two other neonicotinoid compounds, thiamethoxam and clothianidin, were subsequently approved for use on oilseed rape crops in 2007 and 2008 respectively and have since overtaken imidacloprid use. In 2012, imidacloprid, thiamethoxam and clothianidin were applied to 11, 36 and 46% of the crop respectively, representing total neonicotinoid coverage of 93%. Both imidacloprid and clothianidin are co-formulated with the pyrethroid beta-cyfluthrin.

In relation to foliar applications, over the last 20 years the percentage of the crop treated has varied between 44 and 79% (Figure 2). Foliar sprays are reactive so display temporal variation based on pest pressure. Figure 3 presents the total area of sprays, including multiple applications to the same area. Foliar applications are dominated by pyrethroid compounds, which have accounted for more than 90% of the total spray area throughout the presented period. The pyrethroid compounds used have varied over time. In the 1990s alpha-cypermethrin and cypermethrin were the principal compounds applied (accounting for 60 to 90% of spray area); whilst in the 2000s the majority of pyrethroid use was lambda cyhalothrin and tau-fluvalinate (accounting for 60 to 70% of spray area).

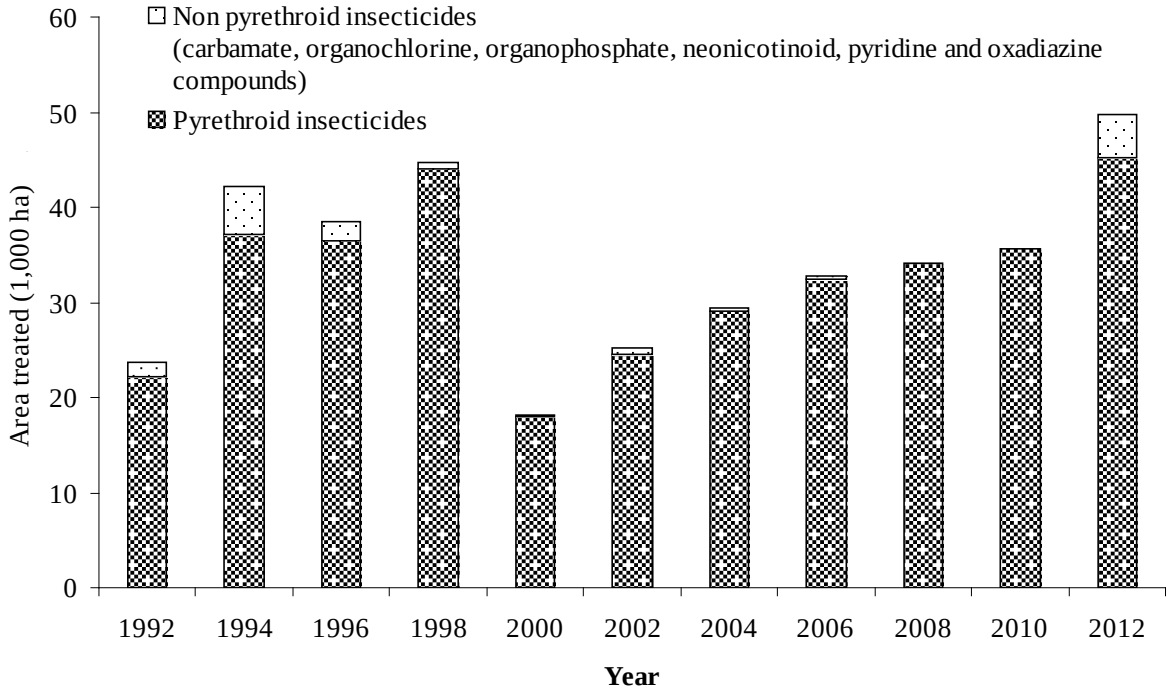

Figure 3. Total spray area of foliar insecticides, including multiple treatments to the same area, 1992-2012.

This dependence on pyrethroids reflects that most insecticides approved for use on oilseed rape belong to this class. Currently, pyrethroids account for 54 and 76% of approved actives and products respectively (Table 1). In addition, multiple pyrethroid applications may be made during the growing season, whereas the neonicotinoids (acetamiprid and thiacloprid) and indoxacarb are limited to a single application. Most pyrethroids also have approval for use during flowering, unlike indoxacarb and pymetrozine.

The non-pyrethroid sprays have also varied over time; in the early 1990s they were almost exclusively lindane (organochlorine, last approved use in 2001) and pirimicarb (carbamate). Organophosphate (dimethoate and chlorpyrifos) sprays were applied in the early 2000s until their approval was withdrawn for use on oilseed crops. In the mid to late 2000s very few non-pyrethroid sprays were recorded, those that were being pirimicarb and pymetrozine (which was approved in 2003). However, in 2012 foliar use of non-pyrethroids increased from the 0 to 5% displayed in the previous eight surveys to 9% of the total spray area. The compounds recorded in 2012 were pirimicarb, pymetrozine and for the first time the neonicotinoids acetamiprid and thiacloprid and the oxadiazine compound indoxacarb.

Table 1. Foliar insecticides with current approval for use on oilseed rape.

Chemical Group	Active Ingredient	No. Products	Example product(s)
Carbamate	Pirimicarb	16	Aphox
Neonicotinoid	Acetamiprid	1	Insyst
	Thiacloprid	3	Biscaya
Oxadiazine	Indoxacarb	3	Steward
Pyrethroid	Alpha cypermethrin	9	Alert, Fastac
	Beta cyfluthrin	1	Gandalf
	Cypermethrin	10	Permasect C, Toppel 100
	Deltamethrin	14	Bandu, Decis
	Lambda cyhalothrin	31	Hallmark, Komodo
	Tau-fluvalinate	4	Mavrik
	Zeta cypermethrin	6	Fury 10 EW, Minuet EW
Pyrethrum	Pyrethrins	2	Pyrethrum 5 EC
Pyridine	Pymetrozine	2	Plenum

Future Options for Pest Control

The European Union has adopted a proposal (Regulation (EU) No 540/2011) prohibiting the use of clothianidin, imidacloprid and thiamethoxam on oilseed rape from December 2013. This restriction will be reviewed, although not necessarily rescinded, after two years. As there are no alternative insecticidal seed treatments approved for use on oilseed rape, only foliar use of the compounds listed in Table 1 will be available for crops sown in 2014. The absence of seed treatments has implications for control of pests colonising newly emerged crops. Whilst these can be treated with sprays, there are inherent difficulties in doing so. Effective management of early season pests will require a quick response, allowing control of CSFB before they lay eggs and aphids before they transmit viruses. This will require increased vigilance by farmers and as sprays cannot be applied until crops have sufficient leaf area, they may be of limited use when pest colonisation occurs at cotyledon or first leaf stage. In addition, spray applications are reliant on weather conditions, and if delayed may be too late to prevent damage.

Another concern is the resistance status of the two main autumn pests. Whilst there is currently no reported resistance to neonicotinoids in the UK, resistance to both pyrethroids and pirimicarb are reported to be prevalent in *Myzus persicae* (Anon, 2012c) leaving very few options for their control. In addition, CSFB resistance to pyrethroids has been reported in Germany (Heimbach & Muller, 2012) leading to concern that it may also develop in the UK. Where pyrethroids are ineffective, this may lead to increased use of the other foliar options such as pymetrozine. However, the loss of the neonicotinoids will increase resistance pressure on all remaining active ingredients.

Overall, the absence of neonicotinoid seed treatments may make control of autumn pests challenging. The economic impact is difficult to estimate, although some reports have suggested it might be significant. A greater number of sprays will be required, although this will be offset to some extent by a reduction in the cost of applying a seed treatment. However, higher seed rates may be necessary to mitigate damage by autumn pests and yields may be affected. The average yield loss on untreated crops from CSFB is around 1% and from TuYV is 15% (Nicholls, 2013).

In conclusion, insecticide input is integral to oilseed production and historically growers have used a combination of seed treatments and foliar sprays. In the absence of seed treatments, farmers will have to rely on sprays, and this may have a detrimental effect on profitability. The extent of the effect on rape cultivation and economic return will not be clear until the 2014/15 crops are harvested. However, when considered in the wider context of other potential restrictions to oilseed rape pesticides, such as metaldehyde and key rape herbicides, due to implementation of the Water Framework Directive (Twining & Clarke, 2009), oilseed rape growers face considerable uncertainty about the availability of future pest control options.

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PESTICIDES IN THE RIVER UGIE - RESULTS FROM TWO YEARS OF MONITORING TO INFORM THE DEVELOPMENT OF A CATCHMENT MANAGEMENT METHODOLOGY

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Summary: Scottish Water started detailed monitoring of raw water quality in the River Ugie catchment in North East Scotland in June 2011 due to concerns over elevated pesticide concentrations above the drinking water standard. Two years of monitoring have demonstrated strong temporal variations in pesticide concentrations and loads across the catchment. Elevated concentrations largely coincide with periods of increased rainfall and river discharge, reflecting the hydrologically-driven nature of pesticide transport. Spatio-temporally diverse monitoring has allowed the delineation of pesticide loads at a sub-catchment level and this has been related to cropping patterns and pesticide use. Scottish Water has been able to use these data to focus catchment management activities in the Ugie.

INTRODUCTION

The River Ugie catchment in the North East of Scotland is used by Scottish Water as a drinking water source for the town of Peterhead and its surrounding area, supplying a population of approximately 40,000 people. On occasions, pesticide concentrations in the river have exceeded drinking water standards (0.1µg L^{-1} for individual pesticides and 0.5µg $L^{-1}$for total pesticides) as set by the EU Drinking Water Directive (98/83/EC). The removal of such contaminants from raw water has increased Scottish Water's operational and capital expenditure (OPEX and CAPEX). Recognising this issue, Scottish Water has adopted a catchment management strategy termed Sustainable Land Management (SLM) that uses an incentive scheme to encourage land owners in the catchment to adopt practices and mitigation measures aimed at reducing the likelihood of pesticides entering the river. As a part of this project, catchment monitoring has been initiated to provide a baseline of water quality to better target interventions. This paper follows on from Gillman *et al*. (2012) by outlining the results of two years of monitoring. It examines what Scottish Water and catchment stakeholders can learn about the nature of the problem and how this understanding informs the development of the catchment management approach in the Ugie.

CATCHMENT MONITORING

A brief summary of the catchment sampling strategy is provided in this paper, with a more detailed outline presented in Gillman *et al*. (2012).

Water quality sampling

Although raw water quality samples are collected at the water treatment works for regulatory purposes, the SLM project recognised that a higher spatial array of sampling points would give a better indication of which areas of the catchment are contributing the highest frequency and load of different pesticides. An on-going sampling programme was therefore started in mid-2011. A total of 10 sampling points were selected covering both the North and South Ugie, and the sub-catchments of the major tributaries (Figure 1).

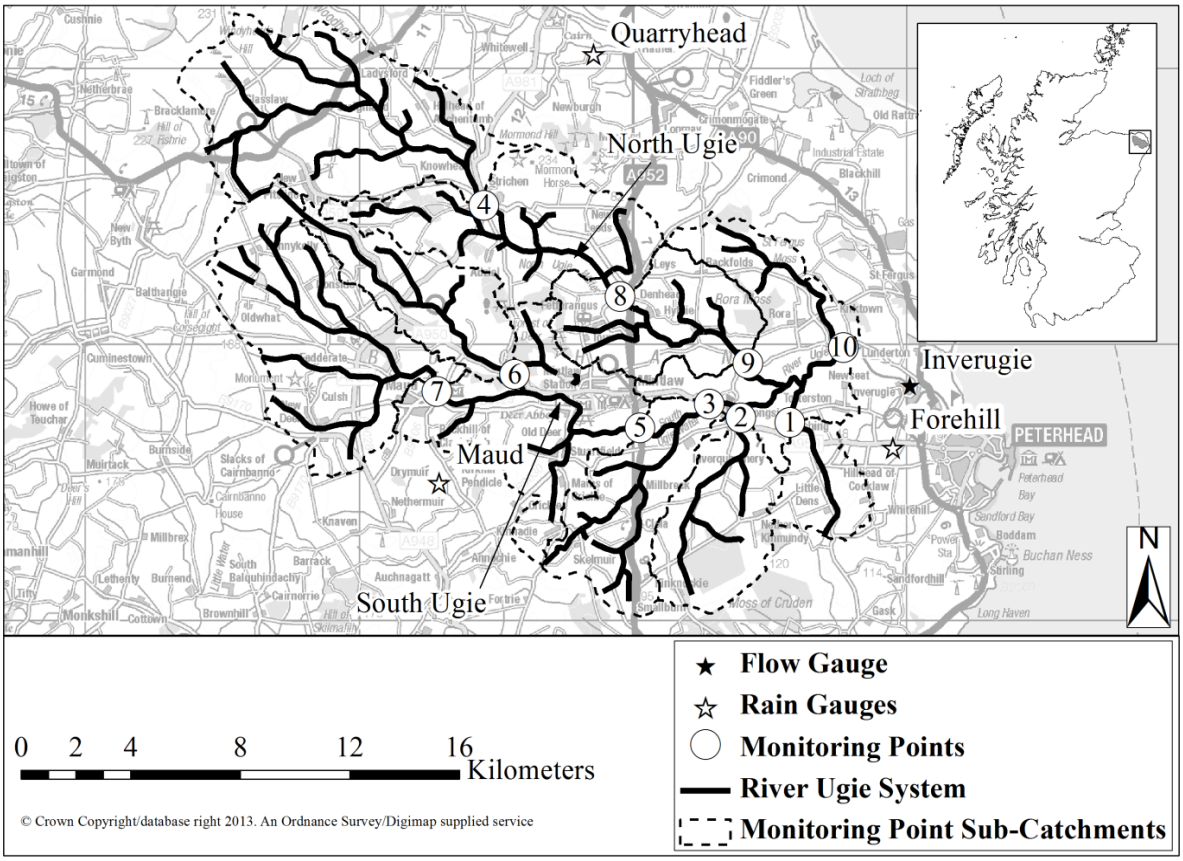

Figure 1. The River Ugie catchment showing the location of Scottish Water's Sustainable Land Management monitoring points of water quality.

The initial sampling frequency was bi-weekly, switching to a weekly sampling timetable in September 2011. Samples collected by Scottish Water sampling teams were returned to the laboratories and analysed within 24 hours of collection. The suite of determinands chosen for analysis is listed in Gillman *et al*. (2012).

Hydrological and meteorological data

Discharge data at a 15-minute time-step were obtained from the Scottish Environment Protection Agency (SEPA) operated velocity-area station at Inverugie, downstream of sample point 10 in Figure 1. The Low Flows Enterprise (LFE) model (Wallingford Hydro Solutions, 2013) was used to derive daily average flow values at each of the ungauged monitoring points. The LFE model procedure used to produce mean daily flow values for ungauged sites is detailed in Goody *et al*. (2010).

Rainfall data were obtained from three separate gauges at Quarryhead, Forehill, and Maud, and areal weighted (rainfall values interpolated by area covered) for each monitoring point (Figure 1). Data on a 15-minute time-step were available for Quarryhead and Forehill, and a total daily rainfall for Maud. Instantaneous load and total annual loads were derived for each pesticide using equations 1 and 2 respectively.

$$L_i = C_i Q_i \quad (1)$$

$$L_t = \frac{\bar{L_i}\, t}{1000} \quad (2)$$

Where L_i refers to instantaneous load (µg s^{-1}), C_i to measured concentration (µg $litre^{-1}$), Q_i to instantaneous flow measurement (litre s^{-1}) (as derived from the LFE model for each monitoring point). Equation 2 uses the mean instantaneous load ($\bar{L_i}$) as calculated by equation 1 to derive the total annual load (L_t) in grams by multiplying by time (t) in seconds.

RESULTS

Temporal trends in pesticide detection

During the first two years of sampling (June 2011 – June 2013), six pesticides were detected above the permitted concentration value (PCV) i.e. 0.1 µg L^{-1} at the catchment outlet (sample point 10). The temporal trends at the outlet for these pesticides were examined against the areal weighted rainfall and discharge at the outlet (Figure 2).

Seasonal trends were observed for a number of the determinands recorded above the PCV. Chlorotoluron, which had the highest concentration during sampling, was largely present from the late autumn to late winter in both years; although concentrations were higher in 2011-12 than 2012-13. Conversely metaldehyde concentrations were higher in the autumn of 2012 than the same period in 2011. Elevated metazachlor concentrations were observed in the autumn periods of both 2011 and 2012 with two concentrations above PCV in 2011 and one in 2012. Two springtime CMPP peaks were observed in May 2012 and May 2013. However, there tended to be less distinct seasonal trends in MCPA with peaks observed in both winter and summer.

Generally, peaks in pesticide concentration coincided with periods of recorded rainfall and subsequent elevated discharge. For example, the highest recorded concentration of chlorotoluron (1.31 µg L^{-1}) on 08/12/2011 was preceded by 15.8mm of rainfall (at the Forehill gauge) in the 48 hours prior to sample collection, and an increase in discharge from 3.3 m^3 s^{-1} on 6/12/2011 to a peak of 25.1 m^3 s^{-1} just after sample collection. A notable exception is the CMPP peak on 21/05/2013 which was not preceded by significant rainfall in the day prior to sampling (although rainfall followed in the days after). The total annual load calculated at the catchment outlet for the six pesticides in 2011-2012 (4499 g) was almost double that (2617 g) in 2012-13. Individually 2,4-D, chlorotoluron, MCPA, and metazachlor had higher loadings in the 2011-2012 period while CMPP and metaldehyde had higher loadings in the 2012-2013 period. The largest total annual load was for chlorotoluron with 3007 g in the 2011-2012 period and 1196 g in the 2012-2013 period.

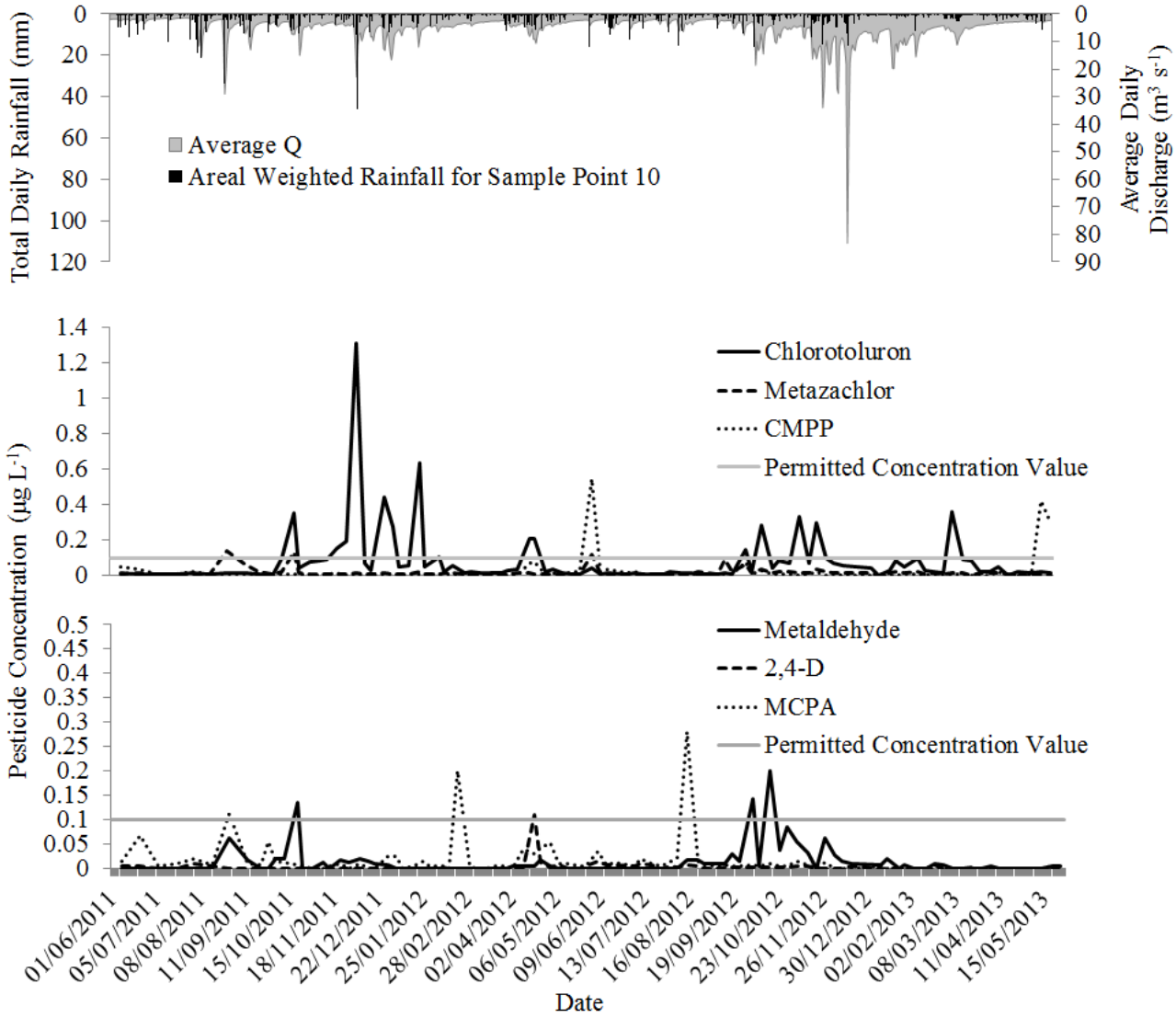

Figure 2. Time series of six pesticides which recorded at least one value above 0.1 µg L^{-1}at sample point 10 on the Ugie catchment, plotted against areal weighted rainfall and discharge at the catchment outlet, 06/2011 – 06/2013.

Spatial trends in pesticide detection

At least one pesticide was detected above the PCV limit at each of the ten monitoring locations during sampling. High spatial heterogeneity was exhibited between pesticides detected (>0.1 µg L^{-1}) at each sample point (Figure 3). For example, 2,4-D was only found in the South Ugie (sample points 2, 3, 5, 6 and 7) and metaldehyde at sample points 2, 3, 9, 8 and 10, whereas chlorotoluron was found at each sample point with the exception of sample point 4.

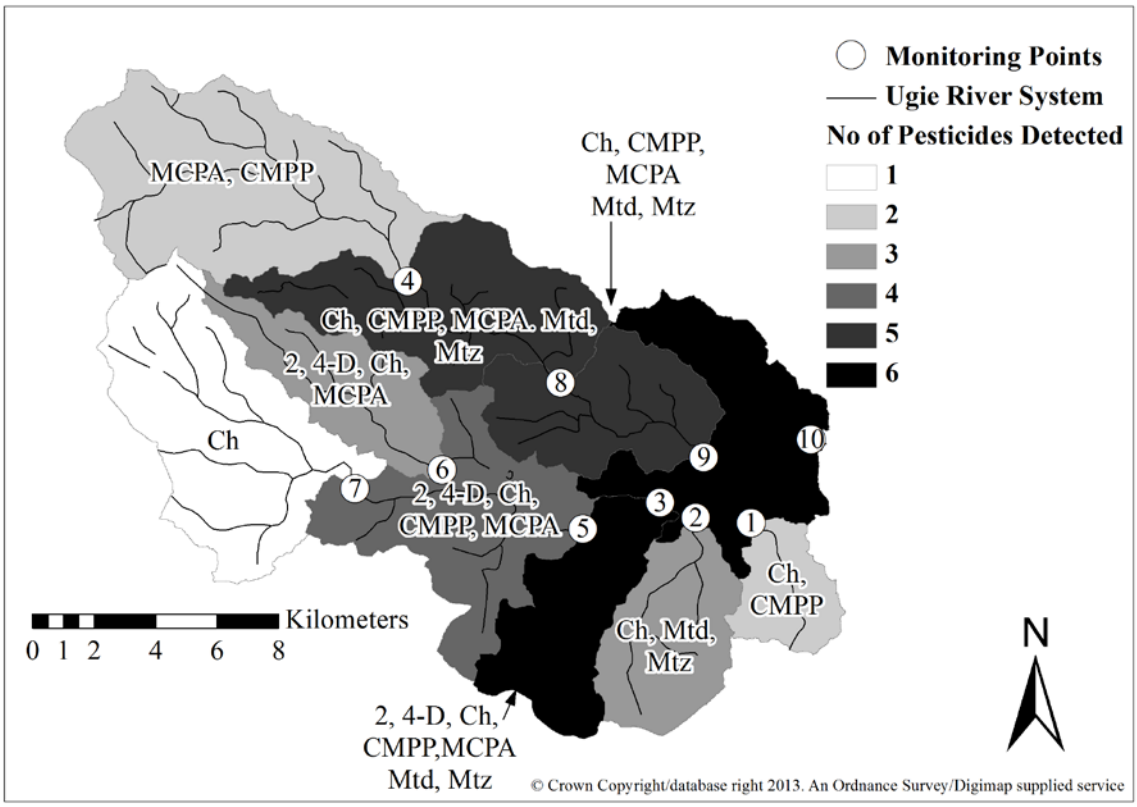

Figure 3. A spatial representation of pesticides detected >0.1 µg L^{-1}at each monitoring location (06/2011 – 06/2013). Ch = Chlorotoluron, Mtd = Metaldehyde and Mtz = Metazachlor

Total annual load values can be used to identify which parts of the catchment contribute higher levels of pesticides than others. For example, during the 2011-2012 sampling period, the sub-catchment draining to point 2 contributed 3.82 g km^{-2} yr^{-1} of metaldehyde compared to 0.95 g km^{-2} yr^{-1} for the catchment as a whole (derived from sample point 10). Similarly, the 2011-2012 loadings of chlorotoluron are higher in the sub catchments draining to points 1, 2 and 3 (19.08, 6.93 and 9.83 g km^{-2} yr^{-1} respectively) than points 8 and 9 (2.85 and 9.81 g km^{-2} yr^{-1} respectively), suggesting that although concentrations were high across the whole catchment, the largest loads were derived from the South Ugie and its tributaries (sample points 2, 3, 5, 6 and 7).

DISCUSSION, CONCLUSIONS AND FUTURE WORK

The temporal trends observed from the monitoring largely align to pesticide application dates in the catchment. For example chlorotoluron is largely applied to cereal crops such as barley, a crop grown widely in the catchment. Applications are made pre- and post-emergence in the late autumn and early spring. High concentrations and loads can also be linked to catchment conditions as is the case with the metaldehyde peaks in the autumn of 2012 which were recorded at the same time as a particularly wet and mild period, conditions known to be linked with high slug populations (Choi *et al.*, 2004) and pollutant mobilisation. The results showed that the majority of peaks in pesticide concentration coincided with periods of recorded

rainfall. Authors such as Tediosi *et al.* (2012) have reported that rainfall events following pesticide application are important in determining pesticide transport and subsequent loss.

The spatial variability in both concentrations and loads also largely reflects patterns of land use. Disproportionate loadings of metaldehyde in sub-catchments 2 and 3 for example reflect the dominance of arable land cover types and more specifically the cultivation of oilseed rape. It is therefore important to note that the high risk areas of the catchment may change with future crop rotations.

The collection and analysis of the monitoring data reported here can guide Scottish Water in the development of a catchment management programme. The observed links between activity and impact highlights the potential effectiveness of catchment management in the Ugie. Quantifying the impacts of pesticides on raw water could also prove particularly useful for disseminating drinking water protection concerns to other catchment stakeholders. The spatial delineation of pesticide loadings from different sub-catchments could also help identify which parts of the catchment should be prioritised for interventions. It also provides a baseline for gauging any improvements in raw water quality following selection and implementation of management measures.

Future work lies in developing tools that aim to be proactive rather than reactive in identifying pesticide raw water contamination risks at higher spatial and temporal resolutions.

ACKNOWLEDGEMENTS

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CHLORPYRIFOS: SAY NO TO DRIFT STEWARDSHIP CAMPAIGN

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Summary: Chlorpyrifos is a key insecticide active ingredient in the control of many economically important insect pests, some of which with no adequate alternative control strategies currently available. When applied through conventional flat fan nozzles, chlorpyrifos no longer passes a recently revised regulatory risk assessment for exposure of aquatic invertebrates from spray drift, and the current policy of the United Kingdom Chemicals Regulation Directorate only considers applications through conventional flat fan nozzles when risk assessments are conducted. The 'Chlorpyrifos: Say No to Drift' Stewardship Campaign has alerted chlorpyrifos users to the requirement to make all applications through '3 star' low-drift nozzles, with appropriate buffer zones next to watercourses, and emphasised the importance of doing so in the hope of securing future approvals for chlorpyrifos products. Research trials by Dow AgroSciences Ltd. have confirmed comparable efficacy for applications of chlorpyrifos made through conventional nozzles and '3 star' low-drift nozzles.

INTRODUCTION

Insecticide products containing the active ingredient chlorpyrifos (e.g. Dursban® WG insecticide, 75% w/w chlorpyrifos-ethyl, water dispersible granule, Dow AgroSciences Ltd.) are very effective in the control of a range of insect pests; evidenced by their proven track record over the last 40 years. Key insect pests controlled by chlorpyrifos, some with no reliable alternative means of control, include: wheat bulb fly (*Delia coarctata*) in wheat; leatherjackets (*Tipula paludosa*) in cereals, grassland and sugar beet; frit fly (*Oscinella frit*) in grassland and maize; and codling moth (*Cydia pomonella*) and tortrix moth (*Adoxophyes* spp.) in apples and pears. However, the chances of spray drift droplets containing chlorpyrifos reaching watercourses must be minimised due to the recognised risk to aquatic invertebrates, many of which are important food sources for other aquatic animals.

When applied through conventional flat fan nozzles, chlorpyrifos no longer passes a recently revised regulatory risk assessment for exposure of aquatic invertebrates from spray drift. The current policy of the United Kingdom Chemicals Regulation Directorate (CRD) only considers applications through conventional flat fan nozzles when risk assessments are conducted. Therefore, it has not been possible to re-register chlorpyrifos insecticides in the United Kingdom.

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The ‘Chlorpyrifos: Say No to Drift’ Stewardship Campaign was instigated in October 2011 by a consortium of approval holders (Dow AgroSciences Ltd., Headland Agrochemicals Ltd., Makhteshim Agan UK Ltd.) with the aims of providing clear guidance to users of chlorpyrifos insecticides on the need to keep spray drift away from watercourses, and to provide evidence to CRD which would enable low-drift nozzles to be used in risk assessments, as is already the position with many European regulatory authorities. The recommendations of the stewardship campaign are that applications of chlorpyrifos must always be made through Local Environment Risk Assessment for Pesticides (LERAP) ‘3 star’ low-drift nozzles, leaving an unsprayed buffer zone next to watercourses of 20 metres in arable crops and 50 metres when applications of chlorpyrifos are made through orchard sprayers.

Additionally, field trials have been conducted by Dow AgroSciences Ltd. to investigate whether the performance of chlorpyrifos insecticides, when applied through LERAP ‘3 star’ low-drift nozzles, is comparable with that achieved using conventional flat fan nozzles. The field trials reported in this paper studied effects on control of wheat bulb fly (*D. coarctata*) in wheat and codling moth (*C. pomonella*) in apple orchards.

MATERIALS AND METHODS

Stewardship Campaign

The ‘Chlorpyrifos: Say No to Drift’ Stewardship Campaign involved an intensive effort by many members of the approval holders’ consortium, based on thorough communication of the importance of the campaign to the continuing availability of chlorpyrifos. The aim was to develop a lasting partnership with growers, agronomists, agrochemical distributors, spraying contractors, relevant trade associations, produce buyers and appropriate publications. This communication effort was conducted in many ways including a specific campaign website, stands at trade events, grower meetings and training days, email bulletins and presentations at relevant conferences. National Register of Sprayer Operators (NRoSO) and British Agrochemicals Standards Inspection Scheme (BASIS) courses were also prepared and delivered to interested parties.

Campaign support materials were also produced including technical literature, pens and other merchandise; free samples of water-sensitive paper to show spray deposition; and 200 free sets of Albuz TVI low-drift nozzles for orchard sprayers.

Survey

To gauge current understanding and adoption of low-drift nozzles by chlorpyrifos users in the UK, independent surveys were conducted by the Department for Environment, Food and Rural Affairs (DEFRA) Pesticides Usage Survey Group (PUSG). A postal survey in late 2012 was responded to by 282 chlorpyrifos users across many crop types. Additionally, 200 fruit growers took part in face-to-face interviews during the early months of 2013, to investigate their use of low-drift nozzles for applying chlorpyrifos in orchards before the launch of the ‘Chlorpyrifos: Say No to Drift’ stewardship campaign (2011), their awareness of the campaign, and their intentions to use low-drift nozzles in future (2013).

Efficacy Trials

Four efficacy trials were established during 2012 to compare the performance of conventional nozzles with alternative low-drift nozzles. Two trials in the UK assessed control of wheat bulb

fly (*D. coarctata*) in winter wheat, and two trials in Italy investigated performance against codling moth (*C. pomonella*) in apple orchards.

The two UK trials were in winter wheat crops following sugar beet, a situation with high potential for infestation with wheat bulb fly, with treatments of chlorpyrifos applied on 30th January 2012. Applications of Dursban® WG insecticide (75% w/w chlorpyrifos-ethyl, water dispersible granule, Dow AgroSciences Ltd.) at 0.6 kg/ha were made using precision plot sprayers applying a volume of 200 litres/ha. Treatments were applied at 1.9 bar pressure using conventional Teejet XR110-025 flat fan nozzles or low-drift, air induction Billericay Farm Services (BFS) Bubble Jet 025 nozzles with a LERAP '3 star' rating. Reliable infestation with wheat bulb fly only occurred in one of the trials and that data is presented in this paper.

Two codling moth trials were established in apple orchards in Verona province, north-east Italy, an area where codling moth is an endemic and serious pest, frequently causing economic damage to fruit if appropriate pest management is not undertaken. A randomised complete block trial design was used, with four replicates, at both sites. The chlorpyrifos used in these trials was Dursban® 480EC insecticide (480 g a.i./litre chlorpyrifos-ethyl, emulsifiable concentrate, Dow AgroSciences Ltd.) applied at 52.8 g a.i. per 100 L water using conventional Albuz ATR or low-drift Albuz TVI (air induction) nozzles. A spray volume of 1,500 litres/ha was delivered at 150 kPa pressure with all applications made to second generation codling moth larvae on 10th July 2012.

RESULTS

Stewardship Campaign and Survey

The 'Chlorpyrifos: Say No to Drift' Stewardship Campaign received extensive press coverage with over 120 articles published to date. The campaign was represented at key industry events and many interactive workshops were conducted to educate and inform chlorpyrifos users and sprayer operators. Also, over 1,700 users, advisors and sprayer operators have signed a declaration in support of the campaign, committing to use only low-drift nozzles for chlorpyrifos applications. Results of the 'Chlorpyrifos: Say No to Drift' Stewardship Campaign can be seen in the responses to the independently run surveys.

Of the 282 chlorpyrifos users responding to the postal survey in 2012, an average of 90% were aware of the 'Chlorpyrifos: Say No to Drift' Stewardship Campaign, with some variability over crop types. Campaign awareness, by crop type, is shown in Figure 1.

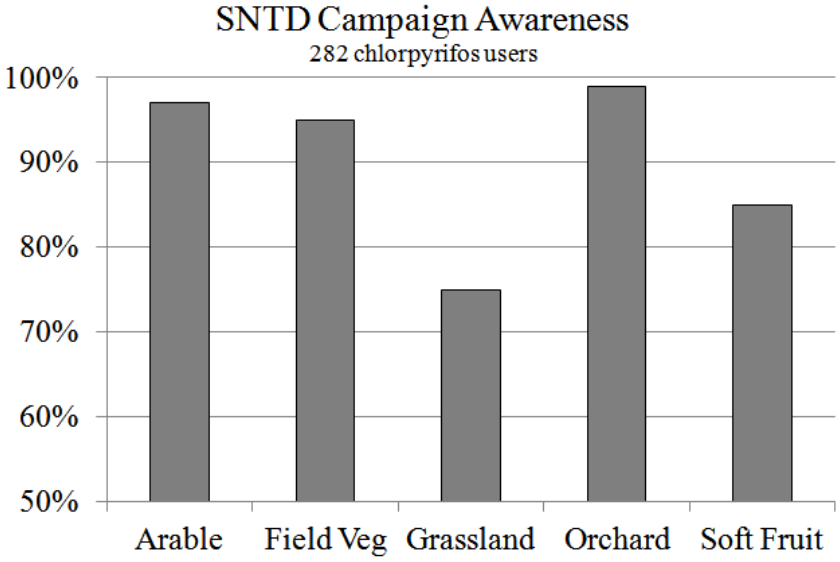

Figure 1. 'Chlorpyrifos: Say No to Drift' Stewardship Campaign awareness (2012).

The adoption, current or planned, of low-drift nozzle technology by the postal survey respondents showed current usage to be quite good in general, with intentions to increase usage of these nozzles for future applications of chlorpyrifos. The results are shown in Figure 2.

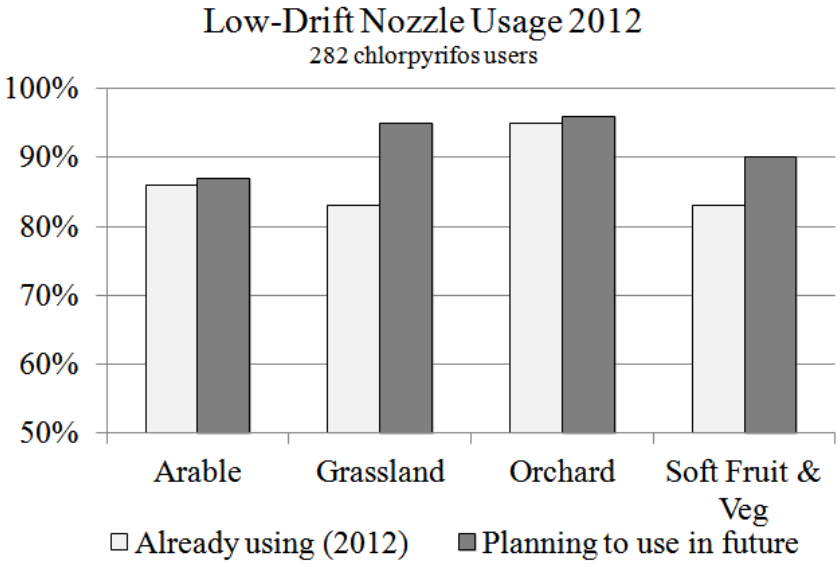

Figure 2. Current and planned usage of low-drift nozzles by crop type (2012).

The results of the face-to-face interviews with 200 users of chlorpyrifos in UK orchards showed excellent awareness (99% of respondents) of the 'Chlorpyrifos: Say No to Drift' Stewardship Campaign by 2013, and a sharp increase in the usage of low-drift nozzle technology by this sector (from 6% in 2011 to 95% in 2012, with 96% planning to use in 2013).

Efficacy Trials

Assessments were conducted, and expressed as percent deadheart symptoms, in the untreated and treated areas in the wheat bulb fly trial 44 days after treatment. Percent deadheart symptoms were compared from applications of chlorpyrifos made with conventional flat fan nozzles (110-25FF) and LERAP '3 star' rated low-drift nozzles (3* LDN). Both chlorpyrifos treatments were significantly better than the untreated (P=0.05), but not significantly different (P=0.05) from each other. These results are presented in Figure 3.

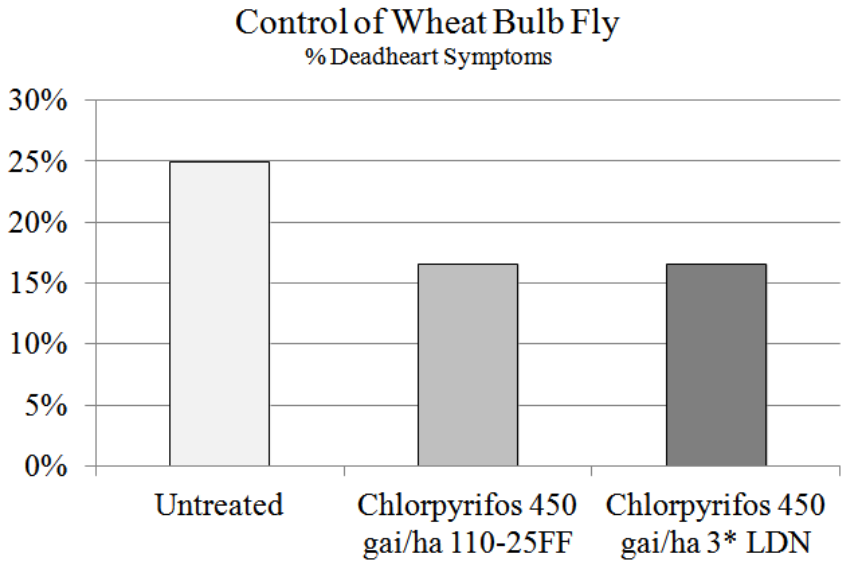

Figure 3. Percent deadheart symptoms 44 days after treatment.

In the apple orchard trials on codling moth, assessments were conducted for percent damage to fruit and percent larval control at both sites in Verona. Results were compared from applications of chlorpyrifos made with conventional flat fan nozzles (Conv.) and LERAP '3 star' rated low-drift nozzles (3* LDN). Both chlorpyrifos treatments were significantly better than the untreated (P=0.05), but not significantly different (P=0.05) from each other. These results are presented in Figure 4.

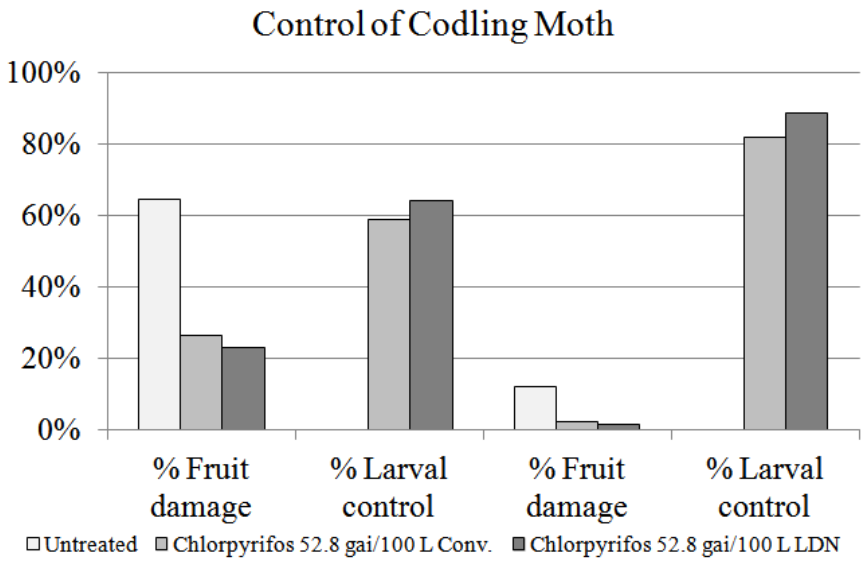

Figure 4. Influence of nozzle type on percent fruit damage and larval control.

These data confirm comparable levels of control of target pests can be achieved when appropriate low-drift nozzles are used in place of conventional nozzles to apply chlorpyrifos.

CONCLUSIONS

The 'Chlorpyrifos: Say No to Drift' Stewardship Campaign has achieved measurable successes since its inception, including greater awareness of the need to use LERAP '3 star' low-drift nozzles, allied with appropriate buffer zones next to watercourses, for all applications of chlorpyrifos, with increased acknowledgement and commitment by chlorpyrifos users to adopt low-drift nozzles, compared with the position before the campaign began.

Research trials by Dow AgroSciences Ltd confirm control of insect pests is comparable whether conventional or '3 star' low-drift nozzles are used to make appropriately timed applications of chlorpyrifos.

ACKNOWLEDGEMENTS

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FACTORS AFFECTING THE HEALTH OF SCOTTISH HONEY BEE COLONIES

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Summary: Pollination of agricultural crops by insects, including honey bees, generates an estimated £400 million to the UK economy. Managed stocks of honey bees in Scotland have experienced significant overwintering losses in recent years. A survey of honey bee colonies was therefore conducted by the Scottish Government in 2012 and 2013 to determine the incidence and severity of diseases and to examine factors affecting the overall health of colonies. Infection by the bacteria causing European Foulbrood was found in 14.5% of apiaries in 2012 and 5.4% in 2013. Findings were confined to localities where the disease was known. In contrast, American Foulbrood was only found by random sampling outside these areas. The severity of Varroa mite infestations and weather were determined to be important contributors to colony losses during the winter.

INTRODUCTION

Insects play an essential role in pollinating some agricultural crops and this is estimated to generate £400 million for the United Kingdom (UK) economy (Breeze *et al*, 2011). These pollinators also provide unquantified environmental benefits via the pollination of wild plants (POST, 2010). The European honey bee (*Apis mellifera*) has been managed by beekeepers throughout recorded history to produce honey and wax and to act as a pollinator of crops; as a result, it is the best understood of all the UK pollinators.

The UK's largest outbreak of European Foulbrood (EFB), a notifiable bacterial disease of honey bees, was identified in the county of Angus, Scotland in June 2009. More than 250 colonies (hives) were found to be infected by the bacteria. Most of these belonged to seven businesses operating exclusively in the east of Scotland, and in response Scottish Government (SG) Bee Inspectors were deployed to manage a programme of eradication and disease control under the framework of the EFB control strategy (Scottish Government, 2013a). However, minimal information on the incidence of the disease was available for the rest of Scotland.

The Scottish Honey Bee Health Survey was instigated in 2012 to gain a better understanding of the incidence of bacterial brood diseases in Scotland and to estimate the importance of other factors affecting Scottish honey bee health. Potential factors examined included 'weather', 'husbandry' (queen health/age, nutrition and colony size), 'diseases (Nosema, Acarine disease, Varroa mites, Foulbroods and viruses) and their control' and 'external factors' (local environment, wildlife and pesticides). The results of the surveys in 2012 and 2013 are discussed.

MATERIALS AND METHODS

A random sample of 10% of Scottish beekeepers registered on the BeeBase database was selected for the survey each year. An SG Bee Inspector used a questionnaire to establish the degree of experience and training of the beekeeper, overwintering colony losses, suspected reasons for those losses and methods of husbandry. The inspector also conducted a full inspection of each apiary for symptoms of diseases, took a sample of 60 adult bees from one colony for further disease testing and collected debris from the colony over a period of 7 days using a floor insert.

The incidence of Foulbrood diseases affecting colonies in the randomised survey was compared with the incidence recorded during 'targeted' inspections of apiaries known or suspected to have Foulbrood in the same year. Targeted inspections of an apiary were conducted according to the following criteria: previous Foulbrood infection at the apiary, infection present at another apiary owned by the same beekeeper, suspicion of disease reported by a beekeeper or infection recorded within 5km of the apiary.

Samples taken from an apiary were sent to the SASA laboratory for analysis. If Foulbrood was suspected, larvae were tested for the presence of the bacteria *Melissococcus plutonius* and *Paenibacillus larvae* (the causative agents of EFB and American Foulbrood (AFB) respectively) using negative staining and high powered light microscopy (x1000). Adult bees were killed and examined for symptoms of Acarine disease (tracheal scarring caused by infestation by the mite *Acarapis woodi*) by dissection under light microscopy (x10). Abdomens were removed from the bees and crushed to extract gut contents, which were examined under high powered microscopy (x400) for the presence of Nosema spores (the infectious stage of the microsporidial pathogens *Nosema apis* and *Nosema ceranae)*. The debris from each hive was examined for the presence of *Varroa destructor* mites (Varroa) using light microscopy (x10).

RESULTS AND DISCUSSION

Foulbrood

EFB was not found in any of the randomly selected apiaries inspected in 2012 and 2013 (Table 1). However, 44 EFB-affected apiaries were found during targeted inspections in these years. These results indicate that EFB is rare in areas outwith localities of known infection.

Table 1. Number of apiaries infected by bacteria causing European Foulbrood (EFB) and American Foulbrood (AFB) in targeted and randomly selected inspections in 2012 and 2013

	2012				2013			
Type of inspection	Total inspected	Not infected	EFB Positive	AFB Positive	Total inspected	Not infected	EFB Positive	AFB Positive
Targeted	235	201	34	4	186	176	10	2
Randomly selected	105	100	0	1	42	37	0	3

Over the two years of the random survey, AFB was found at four apiaries in three different regions of Scotland. Inspection of apiaries at risk from these infected colonies revealed a further six infected by the AFB bacteria. There were no findings of AFB at targeted inspections resulting from previous Foulbrood occurrences. These findings are a concern because AFB is a serious and notifiable disease, and if surveillance had been confined to targeted inspections of known Foulbrood infection during those two years then the outbreaks would not have been identified. This also confirms that infection may be present without being seen by a beekeeper, particularly if infection has been recorded at nearby colonies.

Factors affecting honey bee health

The standard measure of honey bee health is the extent of colony losses incurred during a winter (Van Der Zee *et al*, 2012). In our survey the incidence was 11% for winter 2011-12 and 32% for winter 2012-13. Similar results for the same years were recorded in an independent survey of Scottish bee health conducted by Strathclyde University (Peterson and Gray, 2013).
In line with surveys from other countries (Van Englesdorp *et al*, 2012), beekeepers were asked to suggest possible reasons for their overwintering colony losses. Some reasons suggested (e.g. problems with mating, colony strength and starvation) produce obvious symptoms in the hive, whereas others (e.g. cold and disease) produce less obvious symptoms and, therefore, may be considered a 'best guess' by the beekeeper. The main reasons suggested for winter 2012-13 were weather conditions, reproductive success (mating), colony strength at the start of winter and disease (Figure 1).

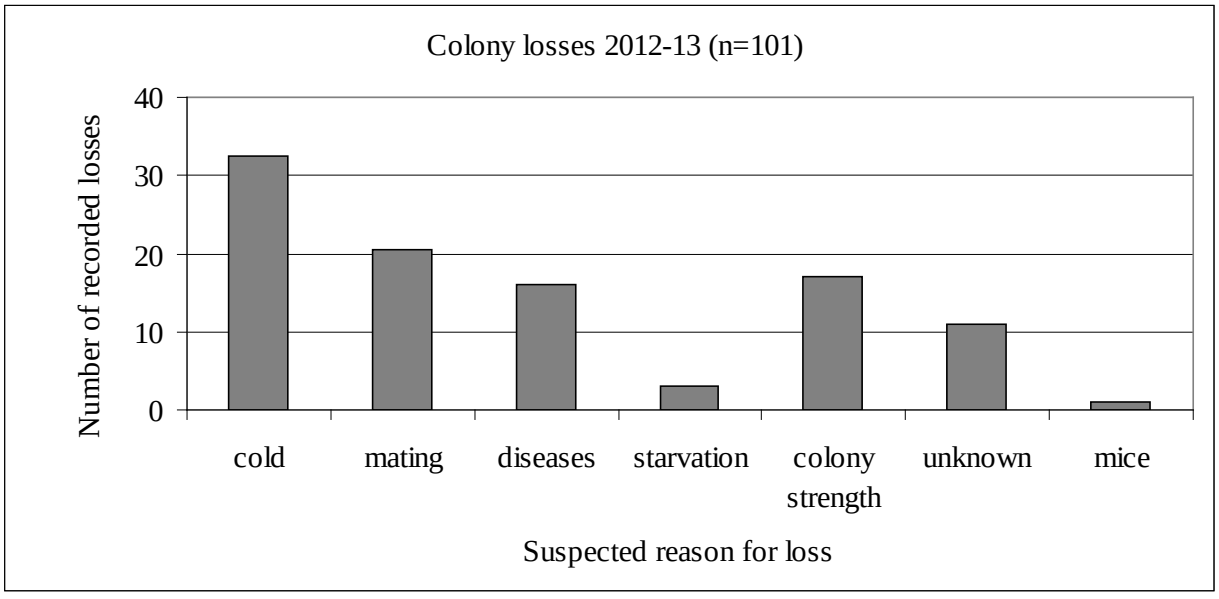

Figure 1. Suggested reasons for overwintering colony losses (n=101) during winter 2012-13

Weather
Overwintering losses are greatly affected by climate; cold weather and extended periods of confinement reduce bee survival and cool, wet summer months can seriously reduce mating success and colony build-up. Seeley and Visscher (1985) reported that the risk of overwintering loss increased when a colony had already been affected by disease or nutritional stress. Data from the Meteorological Office shows that the winter of 2012-13 was colder and

longer than average (between September 2012 and May 2013), and beekeepers suggested that this was directly responsible for 32% of losses recorded by the end of the winter. The meteorological data also shows that the summer of 2012 (June-August) was the wettest in the UK since 1912, and this may have been partly responsible for the 37% losses attributed to poor mating and colony strength at the start of winter.

Husbandry

Beekeepers suggested that 3% of winter losses during 2012-13 were due to starvation. Adjusting husbandry practices to take into account the potential impact of the weather (by checking food stores over winter, merging colonies to ensure adequate colony strength and adopting managed queen rearing programmes) may help to reduce future losses. There was no obvious relationship observed between colony loss and the education/experience of the beekeeper, so additional training may not necessarily mitigate losses.

Diseases

Symptomatic adult bee diseases (Nosema and Acarine disease) were detected in only a small number of colonies during the survey (Table 2). However, this may be an underestimation of the actual incidence because sampling was carried out during the summer when colonies experience a rapid turnover of adult bees and fewer bees show symptoms than at other times of the year. The number of colonies found to be infected by *Nosema* spp. varied between years and also amongst sampling areas, with a significantly higher incidence and severity recorded in the south of Scotland, particularly in Dumfriesshire (Fisher's test P= 0.0184). However, the difference between years was not significant (Fisher's test P=0.7809).

Table 2. Total incidence of adult bee diseases in 2012 and 2013.

	Acarine Disease	Nosema spp.	Disease Free	Total
Scotland	3 (3%)	16 (15%)	91 (82%)	110
Dumfriesshire	0 (0%)	6 (43%)	8 (57%)	14

Table 3. The severity of Varroa infestation in Scottish honey bee colonies over two years.

Severity of infestation (number of mites in 7 day sample)	2012 number of colonies	2012 %	2013 number of colonies	2013 %
Absent	21	30	15	38
Low (1-10)	20	28	14	36
Moderate (11-20)	11	15	3	8
High (21-99)	11	15	5	13
Very High (>100)	8	11	2	5
	n = 71		n = 39	

Varroa mites and the viruses that they spread are known to pose a major threat to honey bee health (Martin, 2012). Moderate to very high numbers of Varroa mites (base line = > 10 mites collected over a 7 day period) were found in debris collected from 36% of colonies, with as

many as 10,000 mites recorded in some colonies (Table 3). Although fewer colonies harboured a moderate to very high number of Varroa mites in summer 2013 compared with 2012, this difference was not statistically significant (Fisher's test P=0.0997).

The incidence of colony losses experienced at an apiary in the previous winter appeared to be directly related to the number of Varroa mites found in debris (Table 4). However, several of the beekeepers who had recorded 100% winter losses during winter 2012-13 had not yet restocked and, therefore, could not provide a sample of hive debris for testing.

Table 4. The incidence of colony losses in the apiary in the previous winter in relation to the incidence of mites in samples of debris.

Percentage of colonies lost during winter 2012-13	**Number of samples (apiaries)**	**Mean number of mites found in debris collected over 7 days in 2013**
0%	22	3.5
1%-49%	12	8.4
50% - 100%	16	35

Recently, resistance to pyrethroid pesticides has been recorded in Varroa mites (Martin, 2004), so an integrated pest management system (IPM) is now recommended to manage mite populations within colonies. Several Varroacide products are available, and most beekeepers use a combination of two or more annually. It was not possible to identify a relationship between the products used and the severity of infestation in the colony because many combinations of products were used and the numbers of beekeepers using specific combinations were relatively small.

External factors

Mice are known to pose a threat to overwintering honey bees as they can invade and destroy a colony by stealing food stores. However, these were only identified as the cause of colony loss on one occasion over the two years. Pesticides were not identified by beekeepers as a cause of colony loss; however, following recent concerns over the effect of several compounds on pollinator health (Goulson, 2013), a sample of comb containing stored pollen/honey/brood was taken from 39 colonies during 2013 for pesticide residue analysis. Results will be reported when available. Land use and the local environment were not directly recorded, although 'pollen sources' were identified by the beekeeper and no obvious correlation between pollen source and colony loss was observed.

CONCLUSIONS

The results indicate that targeted inspections are sufficient to detect apiaries infected by EFB bacteria. However, for the detection and control of AFB in the Scottish bee population, random testing supported by follow-up targeted inspections would appear to be necessary. In apiaries in which Varroa is present, colony losses over winter increase with the severity of the mite infestation. Whilst there are serious concerns about the role that pesticides and land use may play in the decline of pollinators in the UK, a significant percentage of annual honey bee losses can be attributed to the direct effects of cold weather and to the indirect effects of summer weather on colony strength and mating. Many of these factors can be mitigated by introducing

effective disease control and establishing better husbandry practices to alleviate the effects of the increasingly unpredictable Scottish weather.

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CHEMICAL CONTROL OF JAPANESE KNOTWEED AS A TOOL FOR RESTORING NATURAL HABITATS

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Summary: Japanese knotweed is an invasive plant brought to the UK in the mid 19th Century as a highly prized ornamental. Since its introduction it has caused serious problems in many areas. These range from impeding drainage and displacing natural flora and fauna to causing structural damage and an unsightly blight on habitats and communities. Various methods are used for its control including chemical and mechanical. This paper presents data from 11 trials carried out in France and the UK comparing the short and long term efficacy of picloram versus a new formulation of aminopyralid and triclopyr (GF-1883) for Japanese knotweed control.

INTRODUCTION

Japanese knotweed (*Fallopia japonica*) is an invasive plant brought to the UK in the mid 19th Century as a highly prized ornamental. Since then, it has become a serious problem in a range of habitats; particularly roadsides, riverbanks and derelict land where it displaces native flora and causes structural damage (Lourdet, 2010). The plant in the UK is a female clone reproducing by fragments of rhizome, crown or stem. It can spread from a tiny fragment of rhizome weighing less than a gram. This, its vigour, and its lack of natural enemies have contributed to this super-weed's success. The weed causes significant problems including; adverse impact on biodiversity; increased flooding incidence, reduced water quality; interference with societal infrastructure; and compromised public safety (Environment Agency, 2006).

There are three species of invasive knotweed in the UK: Japanese knotweed (*Fallopia japonica*), the most widespread, Giant knotweed (*Fallopia sachalinensis*) and hybrid knotweed (*Fallopia* x *bohemica*) a cross between Japanese knotweed and giant knotweed. Japanese knotweed can grow up to 3 to 4m tall. The stem is green with red or purple specks and forms dense cane-like clumps. Leaves are heart shaped, up to 12cm long (Child and Wade, 2000).
Two pieces of legislation are applicable to this weed:

In the Wildlife and Countryside Act 1981, it is listed under Schedule 9, Section14; making it an offence to plant or otherwise cause the species to grow in the wild.

In the Environmental Protection Act 1990, Japanese Knotweed is classed as "controlled waste" and as such must be disposed of safely at a licensed landfill site according to the Environmental Protection Act (Duty of Care) Regulations 1991. Soil containing rhizome material can be regarded as contaminated and, if taken off a site must be disposed of at a suitable licensed landfill site and buried to a depth of at least 5m.

Various methods have been used to control Japanese knotweed. Non-chemical methods include cutting, pulling and removal of stems, regular mowing, carefully controlled digs, sifting through soil and removing rhizomes for incineration, grazing and, more recently biological control through the introduction of the non-native psylid *Aphalara itadori*.

It is widely recognised that chemical control (with or without non-chemical methods) is often the most effective option for the control of Japanese knotweed, when used as a programmed, long term approach. For habitats not near water the active ingredient picloram is widely used and provides good control. Unlike some herbicides it provides selective control allowing survival of grasses and regeneration of natural vegetation after the Japanese knotweed has died. Picloram is systemic, being rapidly absorbed by foliage and roots, and also has some residual effect. It is the industry standard and is manufactured by Dow AgroSciences. GF-1883, a new product developed by Dow AgroSciences, contains 12grams acid eequivalent/Litre (gae/L) aminopyralid tri-isopropanolammonium and 120 gae/L triclopyr triethylammonium and is a Soluble Liquid Concentrate (SL) formulation. It is currently under review by the Chemical Regulation Directorate. GF-1883 is selective and systemic, being rapidly absorbed by the foliage (stems and leaves). It is hoped that this will provide an alternative option to picloram.

This paper summarises data from various experiments carried out by Dow AgroSciences in France and the UK comparing the efficacy of GF-1883 and picloram on Japanese knotweed.

MATERIALS AND METHODS

Eleven trials were carried out in France and the UK between 2007 and 2009. Trials were designed as randomised complete block with 3 to 4 replications per treatment and contained as many as 10 treatments. Only results for picloram and GF-1883 are presented. Plots were between 12 and 30m^2. Treatments were applied using a compressed air pressurised knapsack sprayer using a 2 to 3m boom (for broadcast applications) or a hand lance with a single nozzle (for spot applications). Broadcast applications were made using water volumes of 200 to 300 L/ha. Spot applications were made using water volumes of 300 to 1000 L/ha, depending on the weed density and height, and applied using a hand lance to achieve maximum foliar coverage just before the point of runoff. Trials were carried out under GEP (Good Experimental Practice) and to EPPO Guidelines (European and Mediterranean Plant Protection Organization). Picloram was applied as a 240 grams acid equivalent per litre (gae/l) SL formulation at a rate of 5.0 to 5.6 Litres per hectare (L/ha) for broadcast applications or 1 Litre per hectolitre (L/HL) for spot treatments. GF-1883 was applied at a rate of 4L/ha for broadcast applications or 2L/HL for spot treatment applications.

The trials all compared control of Japanese knotweed by picloram to GF-1883 but some also had a range of other objectives. Two trials were specifically designed to compare control by an application after the Japanese knotweed had been cut (Trials 1 and 3), one was designed to evaluate a programmed approach over 2 years (Trial 3), and finally one trial evaluated influence of plant height at application on efficacy (Trial 4). In Trial 3 a total of 2 applications were made in sequential years to the same plots. In Trial 4 which evaluated the influence of plant height; a total of 5 separate applications were made as separate treatments i.e., plots only received 1 application each. In some trials picloram or GF-1883 were not applied at certain applications. Where this is the case the entries in the control columns are left blank. Assessments of percentage control were based on a 0 to 100 scale where the non-treated gave 0% control. Assessments were made in season (usually 3 to 5 months after application) and 1 year after application (1YAA) (Table 1.).

RESULTS

Statistical analysis of the combined results is not carried out due to the range of objectives and circumstances of the trials (Table 1). Percentage Data presented are means of 3 or 4 replicates.

Table 1. Japanese knotweed control from picloram and GF-1883, 11 trials, France and UK

Trial Number	Trial Location	Application timing	Application method	Height at Application	Herbicide	Control in season	Control 1 YAA
1	Saone et Loire France	28-Jun-07	Spot	100cm (regrowth after cut)	picloram[1]	77	83
					GF-1883[2]	80	30
2	Saone et Loire France	28-Apr-08	Broadcast	50cm	picloram	95	50
					GF-1883	57	0
3	Saone et Loire France	24-Sep-08	Spot	100cm (regrowth after cut)	picloram	99	97
					GF-1883	99	95
		24-Aug-09	Spot	75cm	picloram		98
					GF-1883		92
4	Saone et Loire France	13-May-09	Broadcast	40cm	picloram		
					GF-1883	50	15
		20-May-09	Broadcast	80cm	picloram		
					GF-1883	94	20
		25-May-09	Broadcast	100cm	picloram		
					GF-1883	70	26
		25-May-09	Spot	100cm	picloram	100	95
					GF-1883	98	50
		03-Jun-09	Broadcast	140cm	picloram		
					GF-1883	20	6
5	Derby UK	29-May-07	Broadcast	100cm	picloram	72	45
					GF-1883	76	50
6	Bedford UK	06-Jun-07	Spot	50cm	picloram	99	76
					GF-1883	99	92
7	Caerphilly UK	17-Jun-08	Broadcast	40cm	picloram	75	20
					GF-1883	56	17
8	Manchester UK	14-May-08	Broadcast	50cm	picloram	70	99
					GF-1883	40	26
9	Manchester UK	04-Jun-08	Spot	100cm	picloram	100	65
					GF-1883	95	100
10	Caerphilly UK	10-Jun-08	Spot	100cm	picloram	99	90
					GF-1883	98	88
11	Essex UK	06-Aug-08	Spot	110cm	picloram	99	90
					GF-1883	99	98

[1] – picloram was applied at 5.0 to 5.6 L/ha for broadcast applications in 200 to 300 L/ha water or 1L/HL for spot treatments in 300 to 1000L/ha water

[2] – GF-1883 was applied as 4L/ha for broadcast applications in 200 to 300 L/ha water or 2L/HL for spot treatments in 300 to 1000L/ha water

DISCUSSION

The most notable result, looking at all trials together, is the difference in efficacy of spot versus broadcast applications. Where spot applications are made the control of Japanese knotweed 1 year after application is generally much higher than where broadcast applications of picloram and GF-1883 were made, (Trials 1, 3, 4, 6, 9, 10 and 11). In season control was generally better with spot application. This can be explained by the increased coverage achieved by spot application and a greater amount of chemical being available for plant uptake and translocated in the plant. Data comparing the ideal height for control from a broadcast application are variable but appeared to be 80 to 100cm. Smaller plants are likely to have less foliage for intercepting the herbicide therefore insufficient chemical is absorbed and hence transported through the plant leading to poorer efficacy. This effect is also replicated in Trials 7 and 8 where efficacy is reduced due to small plant height. Plants over this size are likely to be too great in size to be able to achieve a complete systemic kill. They will also be close to flowering and movement within the plant will be upwards towards the flowers therefore reducing the systemic efficiency of the chemical.

We recommend an approach of making spot applications in 2 consecutive years to provide desired level of knotweed control. Control after 1 year of application was good, but application the subsequent year provided excellent control.

Application following cutting (trials 1 and 3) when regrowth was sufficient appeared to provide good control.

With regard to the efficacy of picloram versus GF-1883 there seems very little difference between the two products. Broadcast applied picloram provided better long term control than GF-1883 (trials 2, 7 and 8). Where spot applications were made picloram and GF-1883 provided the same level of control. As GF-1883 may provide less residual control than picloram potential for regeneration and recovery of desirable plant species could increase Regenerated species recorded in the trials the season following application were predominantly grasses such as *Holcus lanatus* and *Poa annua* but also include broad leaved species such as *Rumex obtusifolius, Urtica dioica* and *Silene vulgaris,*

Japanese knotweed continues to be a threat to the UK on many levels with its control mandated by various legislation. The studies summarised in this paper show that applications of picloram or GF-1883 are extremely useful tools in the control of this noxious weed and can play a valuable part in restoring natural, diverse grassland habitats.

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THE EFFICACY OF DIFFERENT WEED CONTROL METHODS IN SWEDES

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Summary: A field trial was established to compare a number of cultural and herbicide methods to control weeds in a commercial crop of swedes. Band application with glyphosate significantly increased fresh weight yield compared with the untreated. Scarification and band application was good at controlling broad leaved weeds, but were not effective against grass weeds, particularly annual meadow grass. In contrast, metazachlor was very good at controlling grass weeds, but was unable to control some species of broad leaved weeds, including hemp nettle, field pansy and knotgrass. Band application with glyphosate significantly increased fresh weight yield compared with the untreated and was the most cost effective treatment.

INTRODUCTION

Swedes are still an important crop traditionally grown in the north east of Scotland as a fodder crop for livestock throughout the winter months. Despite this local importance they have attracted little research over the last 30 years as they are not a high value crop.

Currently, weed control relies on herbicides developed for other crops and cultural practices, such as inter-row cultivation. As a low value crop, one of the key requirements is to keep input costs low, therefore measures must be cost effective. As swedes are not a competitive crop, failure to control weeds can result in a large reduction in yield (Forbes, 1985a).

Early control of weeds in the crops development is vital if yield looses are to be avoided. Once swedes have developed a canopy approximately 6 weeks after sowing, weed competition is reduced. In field trials, conducted from 1980 to 1982 in Aberdeenshire, hand weeding up to 6 weeks after sowing improved fresh weight yield. However, where weeding of plots continued after this time no improvement in yield was observed (Forbes, 1985b).

Cultivation methods are important in controlling weeds. Ploughing is commonly used prior to growing a swede crop and results in seeds from the previous crop being buried. As many swede crops are sown in drills this means that scarifiers are a popular option for inter-row cultivation. The use of inter row cultivations is particularly effective against young weed seedlings and shallow rooted weeds, such as chickweed (*Stellaria media*), but it does not control weeds growing in the crop rows (Forbes, 1985a).

A number of pre and post emergence herbicides are available for swedes, however, the number

of actives available has been greatly reduced. In 2003 there were four broad spectrum active ingredients used in pre-emergence products, which were metazachlor, propachlor, trifluralin and chlorthal-dimethyl (Davies, 2003). However, in 2013, metazachlor was the only broad spectrum pre-emergence spray still on the market that has an on label approval for swedes. Although a number of post emergence products are available, they are not widely used as they do not target a broad spectrum of weed species.

One option examined in this trial is a band application of glyphosate. The main purpose of band applications is to reduce the amount of herbicide applied, which is good from a financial and environmental point of view. Although this method is not at present approved, it is being sought in other crops, so its inclusion in the trial was deemed suitable.

The aim of this trial was to compare the efficacy of a number of commercially available weed control measures with a band application of glyphosate in a swede crop grown for fodder. The efficacy of different control options were compared on broadleaved and grass weed species and yield benefits were assessed. A cost benefit analysis was performed to see which treatment was financially most suitable for the farm where the trial was performed.

MATERIALS AND METHODS

Trial Design

The field trial was carried out at Wester Badentyre farm, which is located near Turriff in Aberdeenshire. The selected field is currently used in an arable rotation with the trial of swedes being sown after a crop of spring barley.

The field trial was laid out in a randomised block design with six different treatments (Table 1) being replicated three times. The variety used was Gowrie and a precision seed drill was used to sow seed at 15cm intervals on the 26th of May 2012. Each plot measured 7 metres long by 4 drills wide (2.8 metres) with the outer two drills being used as guard rows to reduce the risk of spray drift. At both ends of each plot, a one metre gap was left to the next plot which acted as a guard.

Treatments

Treatments are summarised in Table 1. Hand weeding was performed every two weeks with the last weeding being carried out on the 21st July 2012 (56 days post sowing). Herbicide treatments were applied using a knapsack sprayer. The band application of glyphosate was simulated by adapting a knapsack sprayer, by attaching a long bucket over the nozzle to prevent drift.

Table 1. Details of control measures assessed the field trial. Includes product and rate, date treated and details of application.

Name	Product & Rate	Date treated	Details
Untreated			
Hand weed	Every two weeks	16 days after sowing	Last carried out at 87 days after sowing
Scarify	Tractor Mounted machine	35 days after sowing	
Metazachlor	Butisan S @ 1 l/ha + 450 l/ha water	1 day after sowing	Applied using knapsack
Band application of Glyphosate	Glyphosate 360 @ 1.5 l/ha + 250 l/ha water	36 days after sowing	Applied using knapsack
Metazachlor + Glyphosate	Butisan S @ 1l/ha + 450 l/ha water and Glyphosate 360 @ 1.5 l/ha + 250 l/ha water	Butisan S (1 day after sowing) Glyphosate 360 (36 days after sowing)	Applied using knapsack

Crop assessments

The groundcover of both broadleaved and grass weeds were assessed using a clear acetate sheet with 100 equally sized rectangles printed on it. This was held above the crop and visually aligned with the drills. Ground cover was measured by looking through the grid and counting the number of squares where more than half of one rectangle was dominated by a particular plant species. There were 3 measurements of groundcover for each trial plot. The groundcover was measured over the growing season by performing this assessment every two weeks until the trial was harvested.

Swedes were harvested by hand using a tapner, which removed any soil still on the roots and cut the leaves (tops) off of the swede so that the roots and tops could be weighed separately. This was carried out on the 16th December 2012 and the data converted into t/ha. Swede yield and weed ground cover was analysed using Analysis of Variance with Minitab 16.

Cost benefit analysis

The cost required to carry out each treatment was calculated using a number of different sources including the SAC Farm Management Handbook (SAC Consulting, 2012). These sources provided a guide on the average machinery contractors' charges and the cost of sprays. The band application of glyphosate was based on the cost of using a conventional sprayer with drop bars (Tillett, 2005). The cost of hand weeding was not calculated as this cost would vary depending on the amount of weeds and hand weeding is no longer viable due to the high cost of labour (Forbes, 1985a). For each treatment these values were added together and used to calculate the cost of producing one tonne of fresh weight of swede roots.

RESULTS

The most common species of broadleaved weeds found were fumitory (*Fumaria officinalis)*, knot grass (*Polygonum aviculare)*, chickweed and volunteer oilseed rape (*Brassica napus ssp. Oleifera).* Broadleaved weeds were not seen in the trial until 16 days after sowing (Figure 1) and after this time they increased rapidly. Overall, the untreated plots had significantly ($P<0.05$) higher groundcover of broadleaved weeds than the hand weeded plots. At 113 days after sowing, the untreated plots peaked with a groundcover of 19 % which was significantly higher than the hand weeded treatment. After 113 days after sowing, all of the treatments had a steady decline in the broadleaved weed groundcover due to the crop having a full canopy. Among plots where control measures were applied, differences were not obvious. Although the application of metazachlor seemed to cause a numerical reduction in ground cover, this difference was not significant compared with the other control measures.

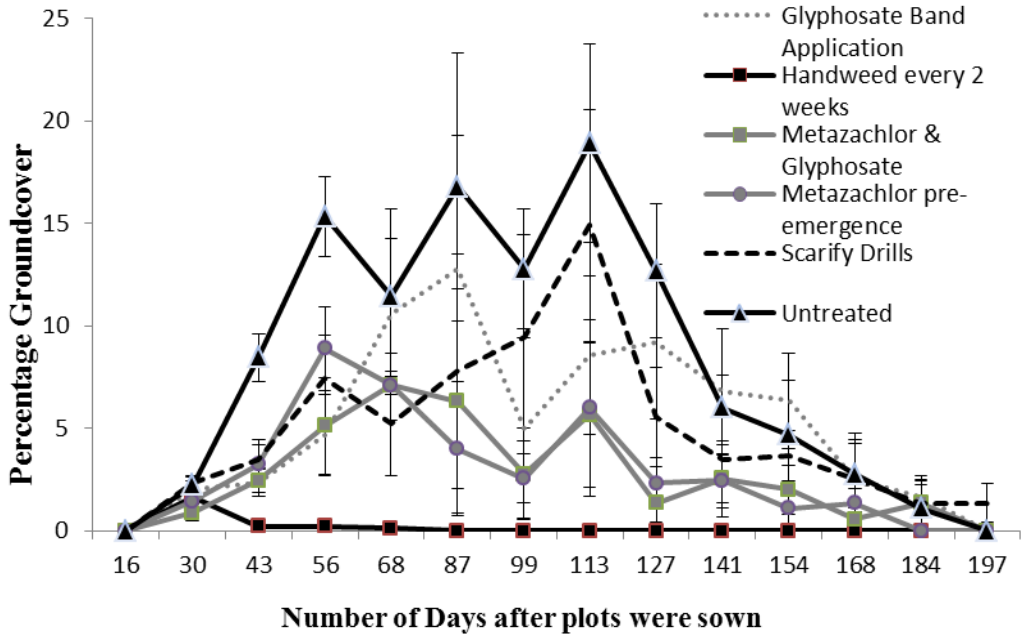

Figure 1. Groundcover of broadleaved weeds throughout the growing season for each treatment. Groundcover was assessed 3 times in each of 3 replicate plots. Error bars represent Standard Error of Mean.

The main species of grass weeds found in the trial were annual meadow grass (*P. annua*) and volunteer barley (*H. vulgare).* Overall the treatment with metazachlor and band application of glyphosate had significantly ($P<0.05$) lower grass weed ground cover than the band application of glyphosate, untreated and scarified treatments (Figure 2).

The mean yield from the hand weeded plots produced the highest fresh weight yield of 89.1 t/ha (Table 2). This treatment was significantly ($P<0.05$) higher than all of the other treatments. The second highest yielding treatment was the band application of glyphosate which produced yields of 81.3 t/ha. There was a significant difference in yield between the band application of glyphosate and the untreated treatment which only yielded 74 t/ha. There were no differences between the other treatments.

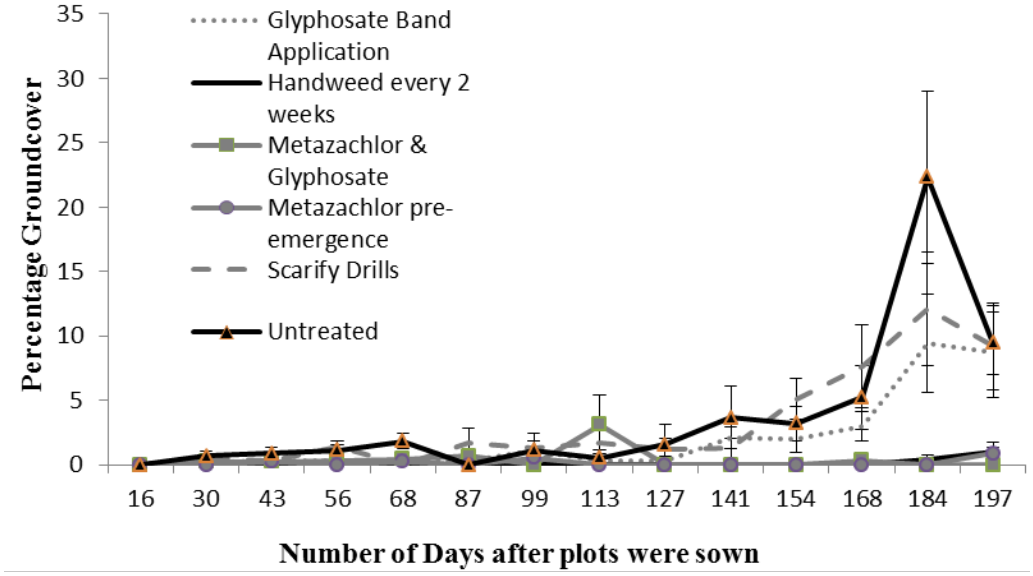

Figure 2. Groundcover of grass weeds throughout the growing season for each treatment. Groundcover was assessed 3 times in each of 3 replicate plots. Error bars represent Standard Error of Mean.

DISCUSSION

In this field trial, both cultural and herbicide methods showed positive weed control compared with the untreated. The degree of efficacy depended on the weed species and the mode of action of the treatment, with no method other than hand weeding controlling all weed species. Although the hand weeded treatment had the best overall control over grass and broadleaved weeds and the highest fresh weight yield, it is not considered viable because of the high cost of labour required (Forbes, 1985a).

Metazachlor was the most effective treatment at controlling annual meadow grass and a range of broad leaved weeds. This is due to its residual mode of action which persists in soil. This prevented annual weeds, such as annual meadow grass germinating in the first six weeks after sowing, until the swede canopy was thick enough to smother the competing weeds. The biggest problem with this treatment was the gaps in its weed control spectrum and so the species knotgrass and fumitory were not effectively controlled. Although it has been noted that knotgrass is moderately resistant to metazachlor (Davies, 2003) no control at all was observed in the present trial. However, for the residual activity to work well soil conditions should be damp and in 2012 it was dry when the product was applied, which may over reduced its efficacy.

The least effective control measures for grass weeds were scarification and the band application of glyphosate. These successfully controlled the weeds growing between the rows, but not in the rows where either no herbicide was applied or scarifying did not occur. The scarified treatment may have been more successful in a drier year, because when this treatment was carried out, the soil conditions were quite moist. This meant that the weeds may not have been killed off properly and the chance of regrowth was likely unless two or three passes were carried out.

Table 2. Fresh weight yield and cost of production in terms of per area (ha) and per tonne of fresh weight in this field trial.

	Untreated	Hand Weeded	Band application of glyphosate	Scarify	Metazachlor	Metazachlor & band application of glyphosate
Fresh weight yield of roots (t/ha)	73.69	89.1	81.30	80.31	77.20	80.62
Cost per tonne	£6.49		£6.07	£6.50	£6.65	£6.55

In this study, the only control measure that significantly increased yield was the band application of glyphosate (Table 2). The cost benefit analysis revealed that band application with glyphosate was the cheapest, as the active is relatively cheap compared with a selective herbicide, such as metazachlor. At present, band application of glyphosate is not approved on swedes and it is suggested that future work should be performed to consider this. Drop leg nozzles are used for band application by some growers, but there are also a range of tractor mounted band spraying equipment which has been specially developed for this purpose, which could be considered in future.

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THE SPREAD AND MOVEMENT OF THE NEW ZEALAND FLATWORM (*ARTHURDENDYUS TRIANGULATUS*) IN SCOTLAND

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Summary: It is now over 20 years since the New Zealand flatworm was identified as an alien organism which could potentially have a detrimental impact on Scotland's soils and wildlife. Since then, over 2000 records have been received by the James Hutton Institute (formally SCRI). These reports strongly suggest it was introduced into Scotland via botanic gardens, then from commercial outlets to domestic households. More recently, it has spread to farmland. The mechanism by which it is spread between domestic gardens is still probably via containerised plants but other potential routes may occasionally occur e.g. on the fur of dogs and cats. In rural areas, flatworms have been found attached to silage and hay bales, on the ground sheets of tents and in kick samples from streams. Once established in a field, the New Zealand flatworm has the capacity to migrate up to one metre each day.

INTRODUCTION

The mechanism by which potentially harmful alien species are introduced and spread must be understood if future introductions are to be stopped and the detrimental impacts of those organisms already here are to be minimised or reduced (Manchester & Bullock, 2000). The New Zealand flatworm has possibly been introduced into the British Isles on at least two separate occasions (Dynes *et al*., 2001). The fact that in Scotland it was first found at Edinburgh Botanic Gardens suggested it could have been introduced when plants were collected in New Zealand. However, Willis & Edwards (1977), Bloch (1992) and Blackshaw & Stewart (1992) suggested it may have come in on ornamental plants. The senior author has found it in a rhododendron nursery on the Banks Peninsula, New Zealand, which exported plants to Scotland. To date, *Arthurdendyus triangulatus* has only been recorded from agricultural fields in western Scotland.

A retrospective survey, undertaken using a questionnaire, showed that in Scotland, until 1970, the New Zealand flatworm had only been found in botanic gardens and garden centres. From 1971 onwards, it was recorded in domestic gardens and only a decade later, it was found in agricultural land (Boag *et al*., 1994). Samples and records received by the senior author for the last 22 years have been mainly from domestic gardens and are lodged with NBN (National Biodiversity Network; http://www.nbn.org.uk/; Figure 1).

The rate at which the New Zealand flatworm can move was investigated by Mather & Christensen (1995) who found a maximum rate of 16.9 m h^{-1} under artificial conditions in the Faroe Islands, while Gibson & Cosens (1998) reported a more realistic maximum rate of 1.8 m

d^{-1} in an allotment in Edinburgh. However these results were not obtained under field conditions and therefore extrapolation of these findings to what happens in farmland in the west of Scotland could be misleading.

The purpose of this paper is to use data collated over 20 years from records sent to the James Hutton Institute to investigate how the New Zealand flatworm had been, and is being, spread and to assess the rate of migration, once it has become established, under field conditions.

MATERIALS AND METHODS

The data used in this paper were mainly obtained from the general public (usually gardeners) contacting the senior author over the last 22 years after they were made aware of the New Zealand flatworm via posters, TV, radio and press interest. A retrospective survey was undertaken in 1992-1993 by sending questionnaires to those who had previously identified and left records with a number of organisations e.g. National Museums of Scotland, BRISC (Biological Recording in Scotland), Edinburgh University etc.

A field experiment on the movement of the New Zealand flatworm was undertaken in 1999 in an uninfested part of a field (which already had a part infested with flatworm) at a farm at Sandbank, near Dunoon on the west coast of Scotland (Ordnance Survey Grid Reference NS 145 819). The experimental design was in the form of a cross. Traps (black polythene bags 600 mm x 300 mm filled with 5 kg of sand) were laid out at distances of 1, 3, 6, 9, 12, 15, 18, 21 and 24 m from the centre in a north, south, east and west directions. After being set out for a month and no flatworm being found associated with these traps, 154 flatworms were released under the central trap on 16th October and their movement to other traps monitored until 7th January. A repeat experiment was undertaken using 231 flatworms starting on May 10th and finishing on 13th June of the following year.

RESULTS

Data collected from the general public

The data from the records sent to the James Hutton Institute (formerly the Scottish Crop Research Institute) were of a similar nature over the 20 year period. Confirmation of the identity of the specimens was a major concern and initially many specimens were sent by post but more recently photos attached to emails are the norm. Misidentifications of specimens sent to the institute included over 100 specimens of horse-leech (probably *Haemopis sanguisuga*), numerous earthworms and even a slow worm (*Anguis fragilis*) which had been run over by a car. Accompanying notes submitted with records indicated that the respondents had few earthworms in the soil and enquired as to how to control the flatworms and increase earthworm populations. Records also highlighted unexpected modes of spread e.g. flatworm being found on the fur of cats and dogs, in kick samples from rivers and on the underside of tent ground sheets. In rural areas where agricultural fields are known to be infested with New Zealand flatworms, silage bales and hay bales were found to have the flatworm adhering to them, suggesting it could be spread when these bales are relocated to other fields/farms. The most recent NBN map showing the distribution of the New Zealand flatworm indicates that it has

continued to spread in Scotland and now can be found in all the major Hebridean Islands as well as in Orkney and Shetland.

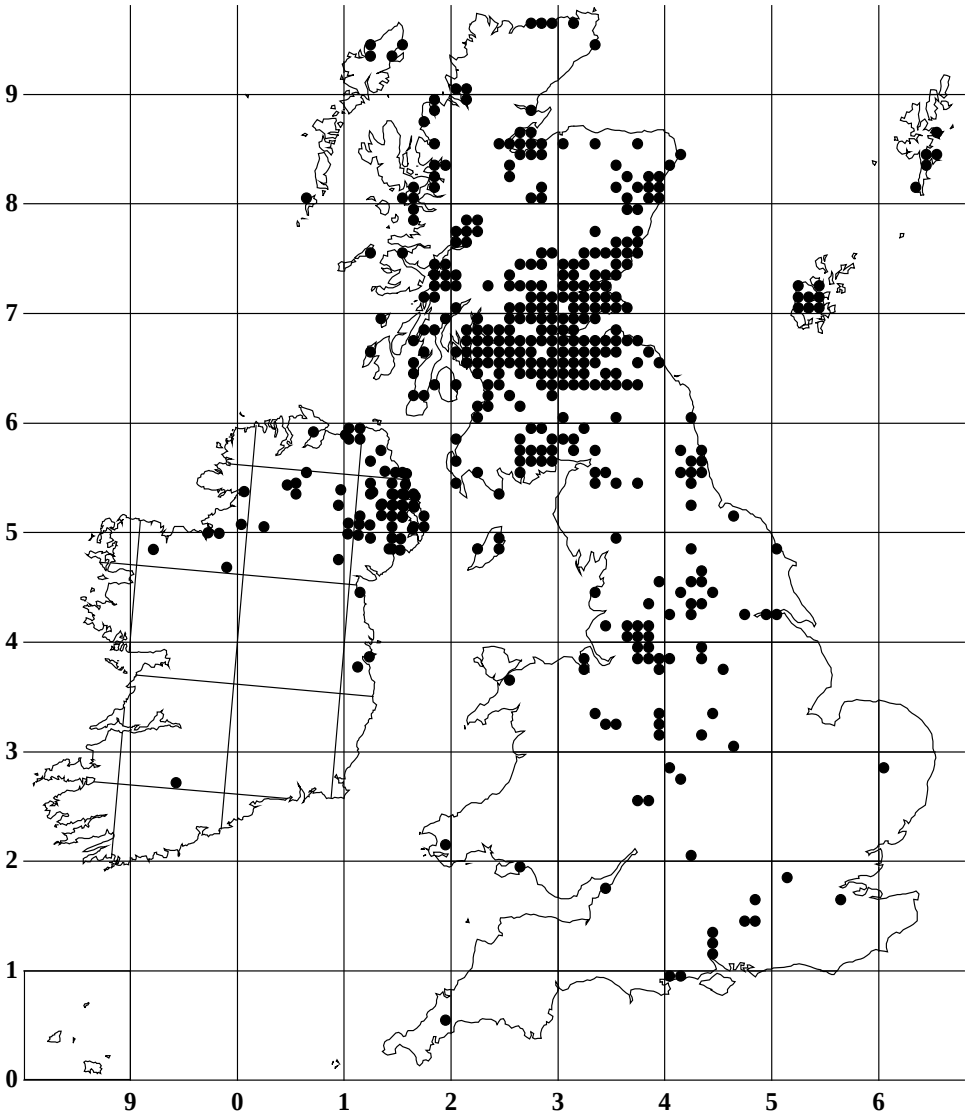

Figure 1. Distribution of New Zealand flatworm, (*Arthurdendyus triangulatus*) in the British Isles at 10km grid resolution.

Flatworm migration experiment

The results of the flatworm migration experiment indicated that, under field conditions, the New Zealand flatworms released on 16th October 1999 migrated 24 m in 26 days (0.92 m d^{-1}) while comparable data for those released on 10th May 2000 showed they migrated 24 m in 21 days (1.14 m d^{-1}) (Table 1). Within both experiments the fastest recorded movement of a single flatworm was 15 m in 7 days (2.14 m d^{-1}).

Table 1. Rate of movement of the New Zealand flatworm, *Arthurdendyus triangulatus*, in a field in western Scotland, 1999 and 2000.

Oct–Jan	16 Oct	22 Oct	28 Oct	04 Nov	11 Nov	17 Nov	26 Nov	06 Dec	07 Jan
Max distance travelled (m)	0	9	12	15	24	24	24	24	24
Mean distance travelled (m)	0	0.9	1.6	2.9	5.7	4.1	5.3	6.5	4.3

May-June	09 May	16 May	22 May	30 May	07 Jun	13 Jun	14 Jun
Max distance travelled (m)	0	15	18	18	24	24	24
Mean distance travelled (m)	0	2.7	4.7	5.9	6.7	8.6	11.1

DISCUSSION

The results from scrutinising 2000 records sent to the institute confirm that the New Zealand flatworm is still mainly a problem confined to domestic gardens. In Scotland, in 1991, 56 of the 217 botanic gardens, nurseries and garden centres (26%) were infested with New Zealand flatworm (Boag *et al*., 1994). Since then, anecdotal evidence suggests that, as the reputations of these establishments were at stake, the proportion of infested botanic gardens, garden centres and nurseries decreased and the main conduit of spread of the New Zealand flatworm is now via containerised plants being exchanged between neighbours, relatives and friends. However, the number of fields/farms infested in Scotland is probably underreported as farmers are often unaware of the presence of the New Zealand flatworm. In Northern Ireland, a survey undertaken in 1991 found 4% of fields infested but a repeat survey in 1998/1999 found 70 % of the same 60 farms surveyed were infested (Murchie *et al*., 2003). No comparable follow up, second survey has been undertaken in Scotland.

The results of the flatworm migration experiment indicated that the New Zealand flatworm is relatively mobile and has the potential to colonise fields relatively quickly. The rate of movement recorded after the May release was faster than after the October release. This may have been due to higher temperatures over the spring/summer, which concurs with Yeates *et al*. (1998) who suggested that the rate of movement was greater with elevated temperatures. Under

artificial laboratory conditions, the New Zealand flatworm has been recorded as travelling up to 12 m h^{-1} (Gibson & Cosens, 1998) and 17m h^{-1} (Mather & Christensen, 1995). However these data are of theoretical interest only. The rates obtained by Gibson & Cosens (1998) in an Edinburgh allotment varied between 3.9 m d^{-1} and 0.4 m d^{-1}, which is similar to those recorded during the field experiments, with 0.92 m d^{-1} and 1.14 m d^{-1}. There is also evidence to suggest that the spread of the New Zealand flatworm within a field may not be uniform. A survey of the distribution of the flatworm in an infested field in the west of Scotland has shown its spread to be patchy (Boag *et a*l., 1999). The reason for this is not clear but may be due to the lack of refugia or the moisture content of the soil (Boag *et al*., 2005).

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SUSTAINABLE CROP PROTECTION: THE STORM BEFORE THE CALM?

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Summary: Current systems for crop production and protection are under threat due to the convergence of biological, commercial and environmental forces. The adaptation and evolution of pests, diseases and weeds under selection by monocultures and agrochemicals is an increasing problem. Globalisation of trade in plants and plant products poses threats to the biosecurity of both crops and natural plant communities. Environmental change, especially climate change and extreme weather events, imposes further stresses on agricultural systems. The exceptional weather in the UK in 2012 impacted on the yield and quality of arable crops due to direct effects on plant development and record levels of some diseases, such as Septoria leaf blotch and Fusarium ear blight. Climate models predict that such extreme episodes will become more common. There is a need to increase the resilience of crops and production systems to biotic and abiotic stresses. Advances in genetics and genomics, gene discovery, new technologies for crop improvement, and more precise tools for the detection and diagnosis of pest and diseases and emerging variants, should lead to more durable methods for crop protection. Improved understanding of the ecology and evolution of biotic agents in agricultural systems, including beneficial species, should also aid development of more sustainable integrated approaches to disease and pest management.

INTRODUCTION

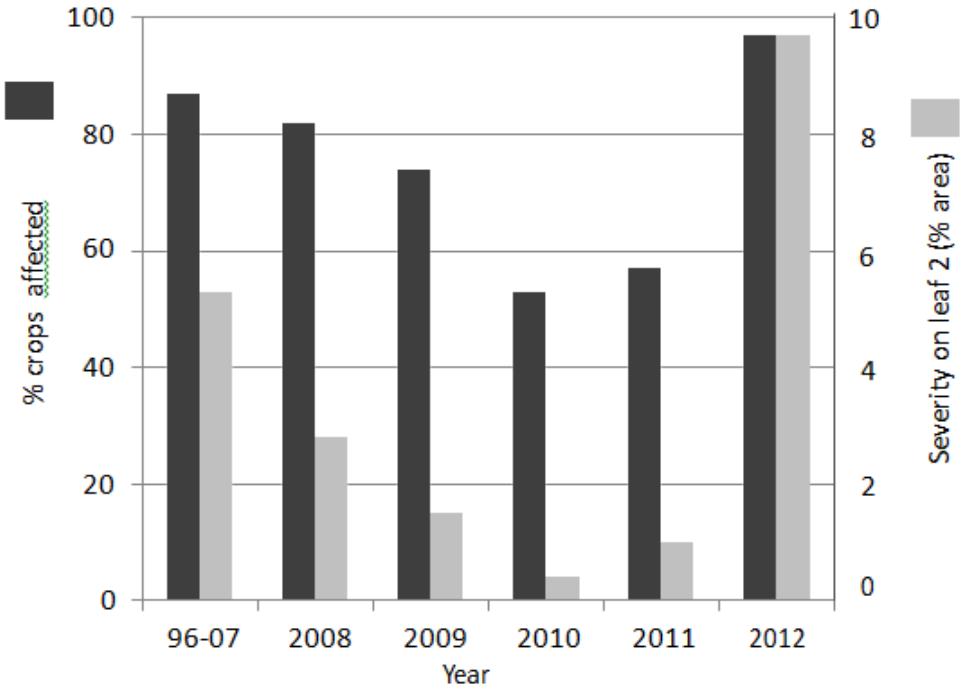
The substantial increases in the productivity of major crops over the past 50 years have been attained through the application of science and technology; selective breeding of improved cultivars, better agronomy, and optimised inputs of fertilizers and agrochemicals. Effective crop protection has also played a key role. The targeted use of fungicides in cereals, for example, has made an important contribution to yield and quality since their introduction around 40 years ago. Growers have had access to a diversity of highly active and affordable chemistry that controlled most of the key diseases and in the majority of seasons gave a financial return. But this scenario may now be changing. The adverse regulatory environment in Europe is likely to reduce the diversity of actives available, while the product pipeline is diminishing. At the same time the ongoing process of microbial evolution has compromised the effectiveness of several classes of fungicides, with the selection and spread of resistant biotypes (Brent & Hollomon, 2000). A similar situation is developing with control of major weeds and insect pests. Alongside these biological challenges is the spectre of climate change, and the prospect of more extreme and unpredictable weather events. While there is considerable uncertainty about the scale of such meteorological changes, there is evidence that

extreme weather is increasing in frequency (Coumou & Ramsdorf, 2012), and climate models predict that this trend will continue into the future (Orlwosky & Seneviratne, 2011).

A PORTENT OF THINGS TO COME?

The exceptional conditions in the UK in 2012 posed a major challenge for growers. After a spring drought in the southern part of the UK, record levels of rainfall were recorded across the whole country over the summer months. This impacted on crop development, disease pressure, and control programmes. The incidence of Septoria leaf blotch and Fusarium stem base infections and ear blight (Fig. 1) on winter wheat was the highest ever recorded in the national disease survey since it began in 1975 (CropMonitor). The number of fungicide sprays applied was also a record (an average of 3.7 applications), despite the difficulties of effectively timing treatments. The national average wheat yield was reduced from around 8 tonnes to 6.7 tonnes per hectare with concomitant effects on quality. Plot trials at Rothamsted with a susceptible wheat variety (cv Consort) and a two spray programme (T1 and T2 spray timing; products applied at full label recommended rate) illustrated the difficulty of controlling Septoria leaf blotch (Fig. 2). Disease levels on leaf 2 and the flag leaf were high with most treatments, with the exception of formulations containing the new Succinate Dehydrogenase Inhibitor (SDHI) fungicides, with corresponding effects on yield. Results highlighted our potential reliance on SDHI chemistry to manage Septoria in high disease seasons, a cause for concern in the context of fungicide resistance management (see below). The wet conditions continued into the autumn and winter, with knock-on effects on following crops, many of which were drilled late, with patchy emergence and poor establishment.

A



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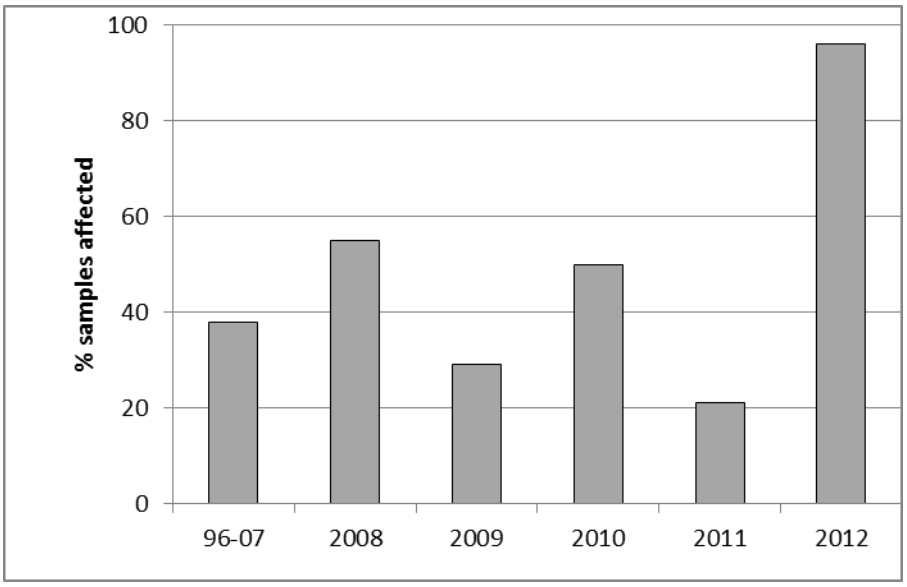

Figure 1. Incidence and severity of Septoria leaf blotch (A) and Fusarium ear blight (B) in commercial winter wheat crops in England. Data from CropMonitor, the Defra funded winter wheat disease survey (source Fera).

The overall area of winter wheat planted was down 19% with many growers switching to spring cropping (AHDB/HGCA survey 2013). The negative impacts of 2012 were not only confined to cereal crops. The volume of production of potatoes was down 28 per cent, the lowest over the past 30 years, and sugar beet was 14 per cent lower than 2011 (Defra, 2012 Agriculture in the United Kingdom, 2012). The only exception was winter barley where yields were relatively unaffected in England, but reduced in Scotland.

Overall, the 2012 season illustrated the vulnerability of UK arable agriculture to extreme weather, and highlighted the need for more resilient crops and production systems in the face of likely future threats. Fortunately, the more favourable conditions in summer 2013 allowed the industry to recover some of the potential losses predicted from the difficulties encountered the previous season.

THE THREAT OF ACCELERATED EVOLUTION

Modern, intensive agriculture exerts high selection pressure on associated biotic agents, favouring biotypes that can survive and reproduce in the crop environment. Examples include directional selection for virulence on common crop genotypes, and the emergence and spread of pesticide resistant insects, fungi and weeds. In UK agriculture, the incidence of pesticide resistance has continued to increase over the past decade, both in terms of the number of pest, fungal and weed species affected, and the extent of resistance in populations. In black grass, *Alopecurus myosuroides*, the major annual grass weed in Western Europe, combined resistance to more than one class of herbicides with different modes of action is an emerging threat. Target site resistance to the two most commonly used classes, the acetolactase (ALS) and acetyl coenzyme A carboxylase (ACCase) inhibitors is a particular concern (Marshall *et al*. 2013). Multiple herbicide resistance (MHR) in this weed has also been linked to an enhanced

ability to metabolise inhibitors from different chemical classes, analogous to multiple-drug resistance in microorganisms or tumour cells. Recently, it has been shown that a specific glutathione transferase enzyme plays a key regulatory role in MHR black grass, a finding that suggests a possible chemical intervention to restore herbicide efficacy by inhibition of this enzyme target (Cummins *et al*., 2013). But the extent of resistance of various sorts in black grass populations is now such that the weed is unlikely to be adequately managed without recourse to agronomic measures (Lutman *et al*., 2013). The continuing development of resistance to insecticides in key agricultural and horticultural pests is also a threat; recent examples include the emergence of neonicotinoid resistance in the peach-potato aphid *Myzus persicae* in Spain and France, and knock-down resistance to pyrethroids in the grain aphid, *Sitobion avenae*, (Fig. 3) that is now present at many sites in the UK (Foster *et al*. 2012).

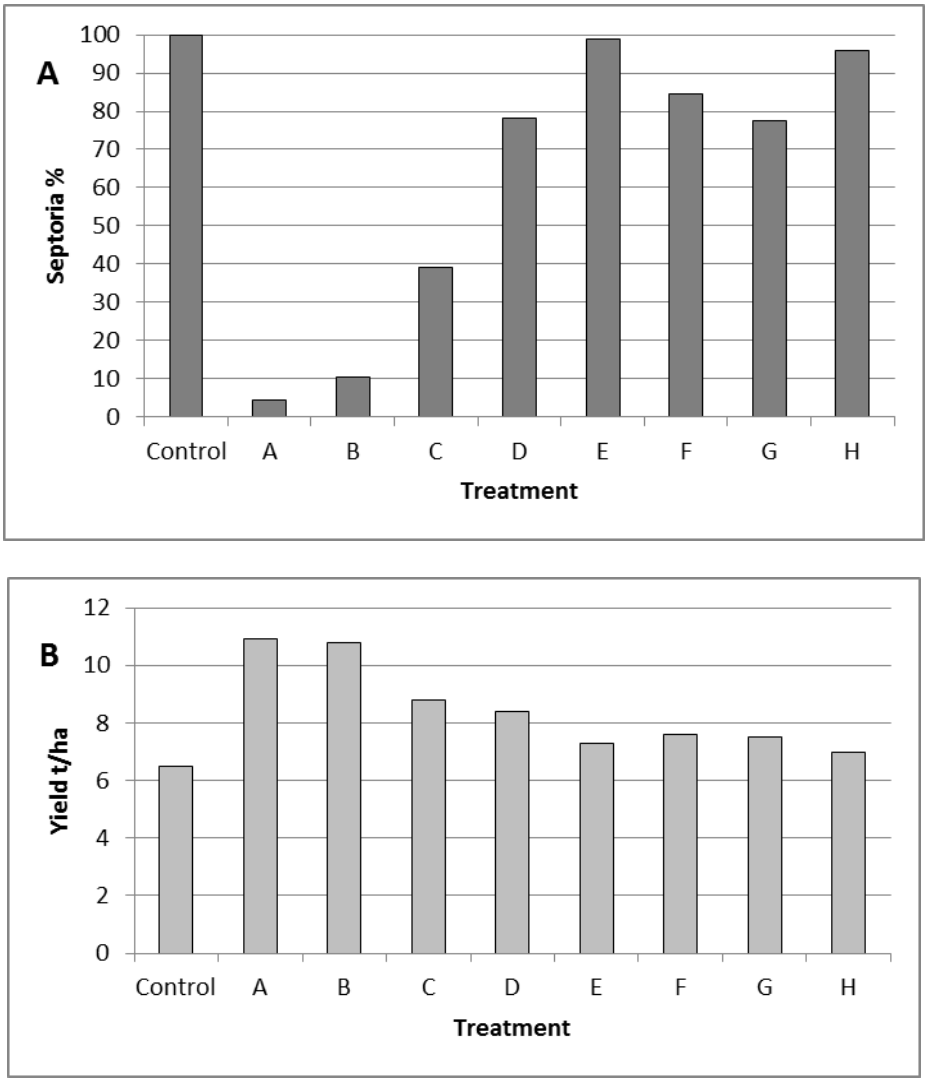

Figure 2. Severity of Septoria leaf blotch in July 2012 on leaf 2 (A) in field plots of wheat cv Consort treated with different fungicides, and average plot yields (B). Treatments A bixafen + prothioconazole, B fluxapyroxad + epoxiconazole, C prothioconazole, D epoxiconazole, E prochloraz, F tebuconazole, G chlorothalonil, H pyraclostrobin. Only treatments A and B, containing SDHI fungicides, gave high levels of control, and fully protected yield.

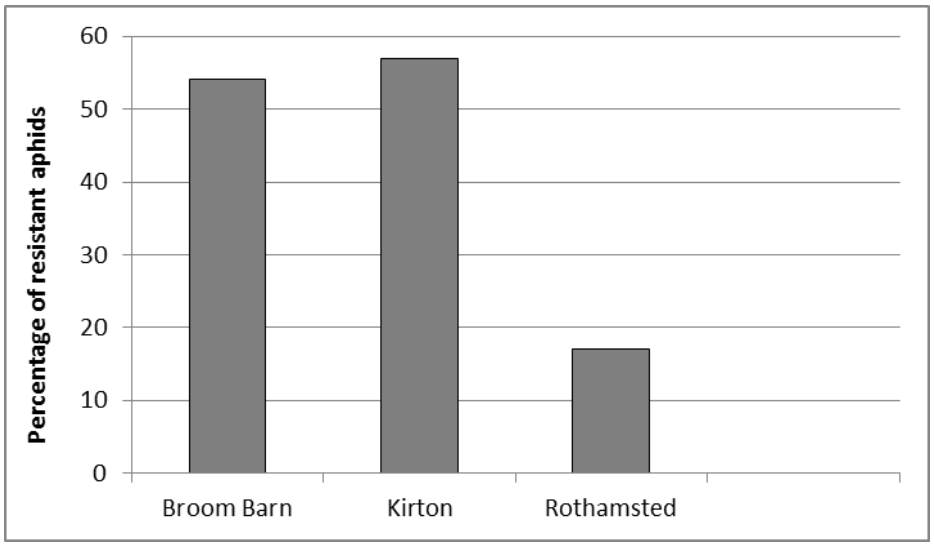

Figure 3. Incidence of grain aphids (*Sitobion avenae*) carrying the kdr mutation for resistance to pyrethroid insecticides in aphid traps at three sites in the UK in 2012. Data courtesy of Richard Harrington, Stephen Foster and Martin Williamson.

The recent history of control of Septoria leaf blotch, caused by *Mycosphaerella graminicola*, further illustrates the increasing difficulties posed by the evolution of resistance to agrochemicals. Thirty years ago, first generation site-specific fungicides such as the methyl benzimidazoles (MBCs) gave good control of Septoria. A single mutation in the β-tubulin fungicide target conferring high levels of resistance to MBCs emerged and quickly became common in the pathogen population. This mutation (E198A) has no discernible effect on pathogen fitness and remains common in current UK populations of *M. graminicola* in the absence of any strong selection by MBCs. Fortunately the azole fungicides then became available for use in cereal fungicide programmes and initially gave effective control of Septoria.

The options for chemical control of the disease increased during the 1990s with the introduction of the highly active QoI (strobilurin) fungicides that also boosted yields by prolonging green leaf area. By the end of the millennium, fungicide programmes based on site-specific inhibitors such as the azoles and QoIs, together with the multisite fungicide chlorothalonil, provided flexible and reliable management of Septoria, even on susceptible wheat varieties. This all changed from 2002 when resistance to the QoIs was first detected in the UK, again conferred by a single amino acid substitution (G143A) in the cytochrome β fungicide target site. Within two seasons the G143A mutation had reached high levels in the pathogen population (Fraaije et.al, 2005), despite attempts to reduce selection, such as restricting the number of fungicide sprays, and mixing QoIs with fungicides with a different mode of action.

By 2005, chemical control of Septoria again relied predominantly on chlorothalonil and the azoles, and especially the two most active triazoles, epoxiconazole and prothioconazole. By this time the efficacy of several of the older azoles on Septoria had declined (Clark, 2006), such that higher doses were needed to control the disease. The annual fungicide performance trials sponsored by HGCA revealed a gradual decline in the activity of azole fungicides on

Septoria, while analysis of *M. graminicola* populations in the UK and Europe showed a stepwise accumulation of changes in the fungicide target protein, a 14α demethylase enzyme (CYP51) involved in sterol synthesis. Further investigation showed that some of these changes were linked to reduced sensitivity to azoles, with differential impacts on different members of the azole class (Fraaije *et al*., 2007; Leroux *et al*., 2007). However, until recently, the most active azoles still controlled the disease, if used at appropriate doses. Since 2010 there have been there have been further changes in the *M. graminicola* population, with novel combinations of target site mutations (Leroux & Walker, 2011; Cools & Fraaije, 2013), and a new more resistant phenotype associated with over-expression of the CYP51 protein (Cools & Fraaije, 2012). This continuing evolution of the pathogen under selection by fungicides is now impacting the efficacy of the best azoles, a scenario amply demonstrated during the severe 2012 Septoria epidemic (Fig 2). Fortunately, co-formulations with the new SDHI fungicides performed well, and as yet there is no indication of resistance to this chemical class emerging in *M. graminicola* in the field. However, we are now in a situation where should high level resistance to SDHI develop, we would have to rely on older, less effective, multisite fungicides as the only chemical intervention able to provide some control of this important disease.

The capacity of pathogens such as *M. graminicola* to adapt to fungicides used for their control has refocused attention on breeding wheat cultivars with higher levels of genetic resistance to disease, although in the case of Septoria it has, to date, proved difficult to introduce durable resistance into elite cultivars without some yield penalty and most current varieties are rated as moderately susceptible. It is hoped that new approaches based on genomics, marker assisted selection and association genetics will help to uncouple these traits (Kollers *et al*. 2013).

THE IMPACT OF ENVIRONMENTAL CHANGE

Beyond the regional problems posed by specific pests and diseases are the global challenges due to population growth, limited renewable resources, and environmental change, especially climate change (Wheeler & Von Braun, 2013). One key conclusion of the UK government Foresight project on Food and Farming futures (www.bis.gov.uk/assets/foresight/docs/food-and-farming/11-547-future-of-food-and-farming-summary.pdf) is the need for a more integrated approach to food production and the sustainability of production systems. This includes not only use of new technology to produce food more efficiently, but also the need to keep pace with evolving threats such as the emergence of new and more virulent pests and diseases. Recent experience with invasive species and novel biotypes of plant pathogens suggests that there is an increasing incidence of emerging infectious diseases that threaten not only agricultural production and food security, but also the health of natural ecosystems (Fisher *et al.*, 2012).

In the UK we have experienced an unprecedented number of new biotic threats to native trees and forests, associated with introductions via the increasingly global plant trade, but also possible effects of climate change. An analysis of pest and disease distribution maps and records, corrected for likely observational bias, has shown that many important crop pests pathogens and disease vectors have indeed undergone positive latitudinal shifts in recent decades, a trend that concurs with models of likely changes in species ranges under a warming climate (Bebber *et al*., 2013). This, together with the propensity of spore-forming pathogens to occasionally undergo long distance aerial dispersal (Brown & Hovmoller, 2002), in some cases spreading more aggressive pathotypes into new regions (Hovmoller *et al.*, 2008), suggests that

the biosecurity risks to crops and food security will continue to increase, and pose a challenge for crop protection for the foreseeable future.

CAN NEW SCIENCE DELIVER FRESH SOLUTIONS?

These increased threats to both crops and native plant species are occurring at the same time as a revolution in the biological sciences. The advent of low cost, high throughput sequencing and associated molecular technologies, along with improving bioinformatic tools for genome analysis, means that we now have access to unprecedented amounts of information on the genetic blueprints of many plant pathogens and several types of pests. These include a range of organisms with contrasting life histories and different ways of inflicting damage to crops. New insights into the mechanisms by which these agents survive, multiply, and attack their hosts will undoubtedly be gained. There is also an exponentially increasing pipeline of crop plant genomes, including globally important staples such as rice and wheat (Brenchley *et al*.,

2012). Mining these genomic resources, coupled with improved tools for validating gene function will lead to a deeper understanding of the genetic and molecular basis of plant development, resource use efficiency, and resistance to biotic and abiotic stress. Dissecting the molecular architecture of the plant immune system is already suggesting biotechnological strategies to improve the durability of disease resistance (Dangl *et al*., 2013). Comparison within and between the gene pools of different crop species will more rapidly reveal the extent of variation in key traits that can be exploited by plant breeders, and thereby extend and accelerate the breeding process. Together these advances will aid the production of more resilient crops and lead to novel approaches and solutions in the field of plant protection (Lucas, 2012).

An additional perspective originates from recognition that many of the problems encountered with the durability of current methods for pest and disease control derive from insufficient understanding of the evolutionary processes operating in agro-ecosystems, and the need for a more integrated holistic approach (Thrall *et al*., 2011). Ideally this should incorporate measures to reduce directional selection, and ecological principles to take advantage of natural mechanisms of pest predation and disease suppression (Andrews *et al*., 2012). Predictive modelling of the potential outcomes of different agricultural practices, from within fields to wider spatial scales, will also be of value, for instance in optimising the management of pesticide resistance (e.g. Van den Bosch *et al*., 2013).

HOW QUICKLY CAN SCIENCE DELIVER SOLUTIONS TO THE CHALLENGES FACED BY FARMERS AND THE AGRICULTURAL INDUSTRY?

The most immediate practical applications of genomics have already come in the area of pathogen detection and disease diagnosis (Studholme *et al*., 2011). Next generation sequencing technologies can accelerate the identification of novel disease agents, or novel variants of known pests and pathogens, for instance strains with genetic changes conferring virulence or resistance to pesticides. This information can in turn be translated into molecular diagnostic tests able to track such changes in field populations, thereby informing pest and disease management decisions. There is also the expectation that genomic information, and especially comparative analysis of pest and pathogen genomes, will identify novel targets for

chemical intervention (Cools and Hammond-Kosack, 2013). Molecular modelling with high-throughput screening of chemical libraries should aid design of the next generation of agrochemicals, or re-design of existing chemistry to circumvent resistance. These new technologies promise to aid the discovery process and re-invigorate the product pipeline.
An example of the speed with which information can now be gained on a new disease threat comes from the recent invasion of the UK by the ash-dieback pathogen, *Chalara fraxinea*. Within one year of its first occurrence scientists have not only a complete genome sequence of the pathogen, but also of a resistant ash tree that survived the epidemic in Denmark, for comparison with the sequence of disease susceptible trees (see http://oadb.tsl.ac.uk/ and http://www.ashgenome.org/). This should provide clues to the basis of host resistance as well as mechanisms of pathogenicity in the fungus. But the challenge of translating these insights into practical control measures in the field to contain the epidemic remains formidable.

INCREASING THE RESILIENCE OF PRODUCTION SYSTEMS

The drive for sustainable intensification of agriculture to ensure food security requires a series of integrated measures, scientific, socio-economic and political, to increase production, ensure more equitable distribution, and to reduce waste (Garnett *et al*., 2013). On the production side, steps to narrow the gap between potential yield and actual yields on the farm are essential. This is particularly the case with crops such as wheat where yields have stagnated in recent years (Hawkesford *et al.*, 2013). Effective management of pests, diseases and weeds protects yield potential and reduces waste. Advances in these areas need to be achieved without increasing inputs or environmental impact, whilst ensuring the resilience of crops to biotic and abiotic stress. A concerted effort to introduce or improve such traits via genetics is required, while alternative approaches such as the use of beneficial microorganisms for disease suppression, pest control or to supplement nutrients available to crops, ideally delivered via the seed, can make a contribution (Andrews *et al.*, 2011).

Progress also needs to be made on two other fronts. The current regulatory environment in Europe is a significant obstacle to providing the diversity of tools and approaches required to achieve sustainable crop protection. This applies not only to the portfolio of available chemicals, but also novel solutions based on biotechnology. Almost 20 years after the first deployment of GM crops with resistance to insects or viruses, there are still major constraints to use of such technology in the field in Europe (Halford, 2012). If the advances in understanding of the molecular genetics of plant immunity are to deliver practical dividends (Dangl *et al*., 2013), this anomaly needs to be resolved. The second issue concerns more effective communication and integration of the crop protection community, as well as support for translational science to ensure efficient transfer of new approaches to the farmer. It is encouraging that the recently launched UK Agricultural Technology Strategy (https://www.gov.uk/government/publications/uk-agricultural-technologies-strategy)
recognizes this need and recommends the establishment of Centres for Agricultural Innovation, including innovation in crop protection. This will be an important step towards developing and implementing a national crop protection strategy, founded on the principles of integrated management.

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MANAGEMENT OF DISEASES IN BARLEY IN NORTHERN BRITAIN

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Summary: In order to maximize returns from winter and spring barley crops in northern areas of the UK it is necessary to implement robust disease management strategies involving cultural measures alongside effective chemistry. Azoles such as prothioconazole continue to give excellent control of the main barley diseases and remain the backbone of barley disease management. Alongside the azoles, new generation SDHI chemistry and the strobilurin group also give robust control. Ramularia, has developed single-step field resistance to strobilurins conferred by mutation of target site at G143A, and net blotch, which has developed partial resistance to strobilurins in some areas due to the F129L mutation. The fact that all three of these major groups remain effective against some or all the major barley diseases gives excellent opportunities for effective control and resistance management strategies. This paper presents recent trials data from Scotland, over three very different seasons, to demonstrate the effectiveness of the key active substances prothioconazole, bixafen and fluoxastrobin and discusses best practice for management of barley disease in northern areas of the United Kingdom.

INTRODUCTION

The foliar diseases *Rhynchosporium commune*, *Ramularia collo-cygni* and *Pyrenophora teres* are the major foliar diseases infecting barley crop in Scotland and northern England. These diseases along with *Fusaria spp*. and *Microdochium spp*. have a significant negative impact on yield and quality. Rhynchosporium remains the most potentially damaging disease in the north according to Scottish Government funded monitoring records at SRUC (www.sruc.ac.uk/crops). Varietal resistance has improved, however the disease still occurs widely when conditions are favourable and can lead to significant losses (Walters *et al*., 2012). Ramularia is a disease that has been poorly understood until recently. The disease is becoming increasingly important in northern England and Scotland leading to average losses of 0.4 t/ha (Walters *et al*., 2008) and is also increasing in importance in the rest of the United Kingdom. Control of this disease has been made difficult through lack of information on varietal resistance alongside widespread resistance to the strobilurin group of fungicides. *Pyrenophora teres* is a disease that occurs more sporadically, but where susceptible varieties are grown, epidemics can lead to large yield losses where not controlled adequately (Shaw & Royle, 2008).

In order to maximize returns from winter and spring barley crops in northern areas of the UK it is necessary to implement robust disease management strategies involving cultural measures alongside effective chemistry. Azoles such as prothioconazole continue to give excellent

control of the main barley diseases and remain the backbone of barley disease management. Alongside the azoles, new generation SDHI chemistry and the strobilurin group also give excellent control with the exception of Ramularia which displays widespread field resistance to strobilurins, having developed a single-step resistance mutation (Fountaine *et al*., 2011) and net blotch which displays partial resistance in some areas to strobilurins (Sierotzki *et al*., 2007). The fact that all three of these major groups remain effective against some or all the major barley diseases gives excellent opportunities for effective control and resistance management strategies. The data presented is taken from trials carried out in 2011, 2012 and 2013, seasons which differed in extremes of weather. 2012 was exceptionally wet with waterlogging, reduced temperatures and sunlight in the summer, and 2013 had the coldest spring and warmest July for many years. Disease pressure also differed widely between seasons. This variability increases uncertainty for growers as to best management options.

MATERIALS AND METHODS

Field trials

A total of six winter barley and six spring barley field trials were sown over three seasons (harvest year 2011, 2012 and 2013). The treatments were fully randomised within blocks with three replicates per treatment. Varieties were chosen which were susceptible to disease. Saffron was used for winter barley trials and Optic or Concerto for spring barley trials. Plots measured 2 x 12 metres. All inputs and agronomic practices, apart from fungicides, were in line with local practice. Information on treatments, varieties and sites is presented in subsequent tables.

Table 1. Fungicide treatments applied to trials.

Trade name	Active ingredients	gai/l	Max. indiv. dose (l ha^{-1})
Proline275	prothioconazole	275	0.72
Bravo	chlorothalonil	500	2.00
Siltra Xpro	bixafen + prothioconazole	60 + 200	1.00
Fandango	fluoxastrobin + prothioconazole	100 + 100	1.25
Bontima	isopyrazam + cyprodinil	62.5 + 187	2.0
Seguris	izopyrazam + epoxiconazole	125 + 90	1.0
Adexar	fluxpyroxad + epoxiconazole	62.5 + 62.5	2.0

Not all treatments were evaluated in all trials and this is indicated by blanks in tables 5, 6, 7 and 8. Disease and green leaf area were assessed regularly through the season by leaf layer on 10 plants per plot as % surface area infected. Yield, visual disease and green leaf area were analysed using analysis of variance using Genstat for windows 8th edition or ARM. All other agronomic inputs were as per local standard practice.

Winter barley treatments

Table 2. Fungicide treatments applied to trials (as product shown in Table1).

Treat-ment	Active ingredients applied at GS30/31	Rate l ha^{-1}	Active ingredients applied at GS49-55	Rate l ha^{-1}
1	Untreated	-	Untreated	-
2*	prothioconazole	0.36/0.44	prothioconazole + chlorothalonil	0.27/0.29
3	bixafen + prothioconazole	0.6	bixafen + prothioconazole	0.4
4	bixafen + prothioconazole	0.6	bixafen + prothioconazole	0.6
6	bixafen + prothioconazole	0.6	bixafen + prothioconazole + chlorothalonil	0.4 + 1.0
7	fluoxastrobin + prothioconazole	1.0	bixafen + prothioconazole	0.6
8	bixafen + prothioconazole	0.6	fluoxastrobin + prothioconazole	0.75
9	fluoxastrobin + prothioconazole	1.0	fluoxastrobin + prothioconazole	0.75
10	bixafen + prothioconazole	0.6	fluoxastrobin + prothioconazole + chlorothalonil	0.75 + 1.0

*prothioconazole rate in 2011 / and in 2012

Spring barley treatments

Table 3. Fungicide treatments applied to trials (as product shown in Table1).

Treat -ment	Active ingredients applied at GS30/31	Rate l ha^{-1}	Active ingredients applied at GS49-55	Rate l ha^{-1}
1	untreated	-	untreated	-
2	bixafen + prothioconazole	0.4	bixafen + prothioconazole	0.4
3	bixafen + prothioconazole	0.4	bixafen + prothioconazole	0.6
4	bixafen + prothioconazole	0.4	bixafen + prothioconazole	0.8
5	bixafen + prothioconazole	0.6	bixafen + prothioconazole	0.6
6	bixafen + prothioconazole	0.6	bixafen + prothioconazole + chlorothalonil	0.6 + 1.0
7	fluoxastrobin + prothioconazole	0.75	bixafen + prothioconazole	0.6
8	fluoxastrobin + prothioconazole	0.75	fluoxastrobin + prothioconazole	1.0
9	izopyrazam + cyprodinil	0.8	izopyrazam + cyprodinil	1.6
10	fluxpyroxad + epoxiconazole	0.8	fluxpyroxad + epoxiconazole	1.0

RESULTS

Winter barley

Table 4. Impact of fungicides on yield in winter barley trials (t ha^{-1}).

Treatment	2011 Lanark	2011 Dundee	2012 Perth	2012 Lanark	2013 Lanark	2013 Perth
1	7.96	6.82	5.36	5.36	5.0	7.8
2	8.50	7.29	6.05	6.18	-	-
3	8.84	7.40	5.97	6.29	5.1	8.1
4	8.79	7.32	6.01	6.40	5.5	7.6
6	9.05	7.44	6.38	7.14	5.4	7.9
7	8.80	7.49	6.07	7.17	-	-
8	8.49	7.36	6.27	5.73	-	-
9	8.56	7.21	6.15	6.86	5.1	7.4
10	9.06	7.45	6.20	6.44	5.2	7.7
LSD	0.658	0.753	0.490	0.867	0.760	1.050
P	0.082	0.960	0.063	0.003	0.563	0.949

Yield responses (Table 4) to the fungicides tested ranged from -0.4 to 1.8 t ha $^{-1}$. The mean yield response in 2011 was 0.68 t ha $^{-1}$. In 2012 (high disease pressure) the response was 0.96 t ha^{-1} and in 2012 just 0.1 t ha $^{-1}$. A negative yield response was only noted in the 2013 Perth trial where untreated yield was exceptionally high due to very low disease levels and record levels of sunshine in July.

Table 5. Impact of fungicides on Rhynchosporium in winter barley trials.

Treatment	2011 L2, GS72 14 June Lanark	2011 L3, GS83 14 June Dundee	2012 L3, GS55-59, 29 May Perth	2012 L3, GS 72, 19 June Lanark	2013 L3, GS77, 17 July Lanark	2013 L3, GS85, 2 July Perth
1	9.0	10.5	12.0	16.7	10.0	11.0
2	2.5	3.0	1.3	3.7	-	-
3	1.0	1.5	0.7	2.2	1.7	2.2
4	1.8	2.0	1.5	2.3	2.3	1.8
5	1.2	1.8	2.5	3.0	1.0	2.8
6	0.5	1.5	0.3	2.7	0.0	2.0
7	1.0	1.8	1.8	2.7	-	-
8	1.6	2.8	0.0	2.3	-	-
9	1.5	1.5	4.0	3.3	1.7	2.5
10	0.4	1.2	0.7	2.3	2.7	4.0
LSD	1.79	1.96	3.27	4.62	5.95	4.28
Sig.	<0.001	<0.001	<0.001	<0.001	0.003	<0.001

Fungicide treatments gave very highly significant reductions in Rhynchosporium in all six trials (Table 5) compared to untreated controls. There was a trend in some years to some

bixafen + prothioconazole treatments demonstrating better control than Fandango – significant in the Perth trial 2012.

Table 6. Impact of fungicides on yield in spring barley trials (t ha^{-1}).

Treatment	2011 Dundee	2011 E Lothian	2012 Lanark	2012 M Lothian	2013 M Lothian	2013 Lanark
1	6.70	5.74	3.29	3.91	6.7	6.3
2	7.12	6.73	4.67	4.38	6.2	6.5
3	7.45	7.12	4.91	4.38	6.7	6.9
4	7.45	6.56	5.12	4.44	6.7	7.0
5	7.74	6.83	4.42	4.57	6.6	6.9
6	7.47	7.08	4.89	4.36	6.3	7.1
7	7.08	7.45	5.14	4.39	6.4	6.5
8	7.23	6.73	4.82	4.42	6.4	6.8
9	6.75	6.34	4.62	4.46	6.7	-
10	-	-	4.42	4.29	6.2	7.2
LSD	0.879	0.602	0.649	0.464	0.64	0.190
Sig.	0.462	<0.001	<0.001	0.347	0.777	0.043

There were significant increases in spring barley yield (Table 6) in three out of the six trials and a non-significant trend to increased yield in the remaining three. Yield benefits ranged between -0.4 to 1.85 and as with the winter barley trial yields the response in 2013 (low disease pressure, high untreated yield) was low.

Table 7. Impact of fungicides on disease or green leaf area in spring barley trials (% surface area affected).

Treatment	2011 % GLA L2 GS64 5 July Dundee	2011 % GLA L3 GS53 15 June E Lothian	2012 % Rhyn L2 GS82 2 August Lanark	2012 % Ram L2, GS72, 12 July M Lothian	2013 % GLA GS51 26 June M Lothian	2013 % Rhyn GS83 9 Aug Lanark
1	66.0	85.5	41.2	10.0	99.7	11.7
2	95.3	94.2	6.5	1.5	100	3.0
3	90.7	92.5	10.5	1.3	100	1.0
4	87.3	91.0	8.2	1.0	100	2.3
5	89.7	93.5	7.2	1.2	100	3.3
6	87.0	93.8	5.5	0.8	100	6.3
7	90.3	92.8	7.0	2.3	100	2.0
8	86.3	92.5	12.0	2.7	99.3	3.7
9	85.0	89.5	15.8	1.0	-	-
10	-	-	15.2	3.0	100	6.7
LSD	15.75	5.65	7.00	1.70	-	10.12
Sig.	0.093	<0.001	<0.001	<0.001	NS	0.286

There were significant reductions in Rhynchosporium and Ramularia as well as significant improvements in green leaf area. There was a trend to lower disease levels in bixafen + prothioconazole treatments.

DISCUSSION

The trial series demonstrated the variability of yield potential and disease pressure in barley crops between seasons which increases the uncertainty and risk for growers in trying to grown and manage a profitable crop. The disease pressure in 2012 was very high but the season was exceptionally wet and yield potential much reduced commercially. In 2013 the reverse was the case, with low disease pressure and above average sunshine so untreated yields were high. Prothioconazole, bixafen, fluoxastrobin and chlorothaonil, applied in programmes gave highly significant reductions in the key diseases and proportionate yield responses. SDHI treatment gave improved disease control.

The ability to use effective fungicides from different activity groups provides additional security in reducing the risk of fungicide resistance. The addition of chlorothalonil to programmes particularly at T2 targetting Ramularia added to disease control and yield in most trials except those which were strongly eradicant. This provides an additional mode of action and further robust stewardship. The low yields attained in high disease pressure situations highlights the importance of fungicides in sustainable and profitable barley production in the north of the UK. The use of fungicides in balanced mixtures to properly steward and retain effective fungicides for the long-term is therefore vital.

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HOW DO FUNGICIDES INCREASE YIELD OF SPRING BARLEY WHEN DISEASE IS LOW OR ABSENT?

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Summary: Field experiments were conducted at two sites over several seasons to investigate how fungicides increase the yield of spring barley when visible disease is low or absent. The fungicide active ingredients prothioconazole (Proline®), pyraclostrobin (Comet 200®) and chlorothalonil (Bravo 500®), believed to differ in their potential physiological effects, were compared. All fungicides increased mean grain weight through the control of visible disease when disease was present post-anthesis, but only prothioconazole and pyraclostrobin significantly increased grain numbers. The increase occurred in the absence of visible disease pre-anthesis and could not be explained by the control of asymptomatic pathogen infection, control of leaf surface saprophytes, increase in leaf growth, or delayed leaf senescence. The evidence points to other direct effects of the fungicides on plant metabolism. A single application of prothioconazole plus pyraclostrobin at T2 can elicit the response and may be justified economically even if disease fails to develop post-anthesis.

INTRODUCTION

The yield of barley in the UK is considered to be sink-limited: limited by the number and storage capacity of grains rather than the supply of assimilate to fill them (Bingham *et al.*, 2007). The aim of disease management in barley, therefore, is first to protect the development of sink capacity and then protect photosynthetic activity during grain filling (Bingham *et al*., 2010; HGCA, 2013). However, it has been demonstrated that yield responses to fungicide in barley are variable and do not relate well to the amount of visible disease present, which suggests that fungicides may influence the development of sink capacity in ways other than through the control of visible disease (Bingham *et al.,* 2010; Bingham *et al*., 2012). Treatment of low, or sometimes apparently nil, disease can result in substantial increases in grains/m^2, and hence in sink capacity and yield (Bingham *et al.,* 2012). Visual assessment of disease is subjective, but the extent of the discrepancy between disease severity and yield is too large to be attributed to assessment error. This has important implications for the rational use of fungicides, because it means that the requirement for fungicide treatment, expressed in terms of likely improvement in yield or quality, cannot be predicted just from an assessment of the amount of visible disease present in the crop, or the risk of a disease epidemic developing.

Grain numbers are determined by developmental events pre-anthesis. There are several possible mechanisms that might account for yield responses to fungicide involving an increase in grain numbers where there is little or no visible disease. These include: 1) a high sensitivity of grain numbers to small changes in pre-anthesis light interception resulting from disease control or effects of fungicide on canopy expansion, 2) control of symptomless pathogen infection, 3) control of leaf surface saprophytes and 4) direct effects of fungicides on host plant metabolism. The aim of experiments reported here was to determine how fungicides increase yield in spring barley and the implications for management of disease in low disease-risk situations.

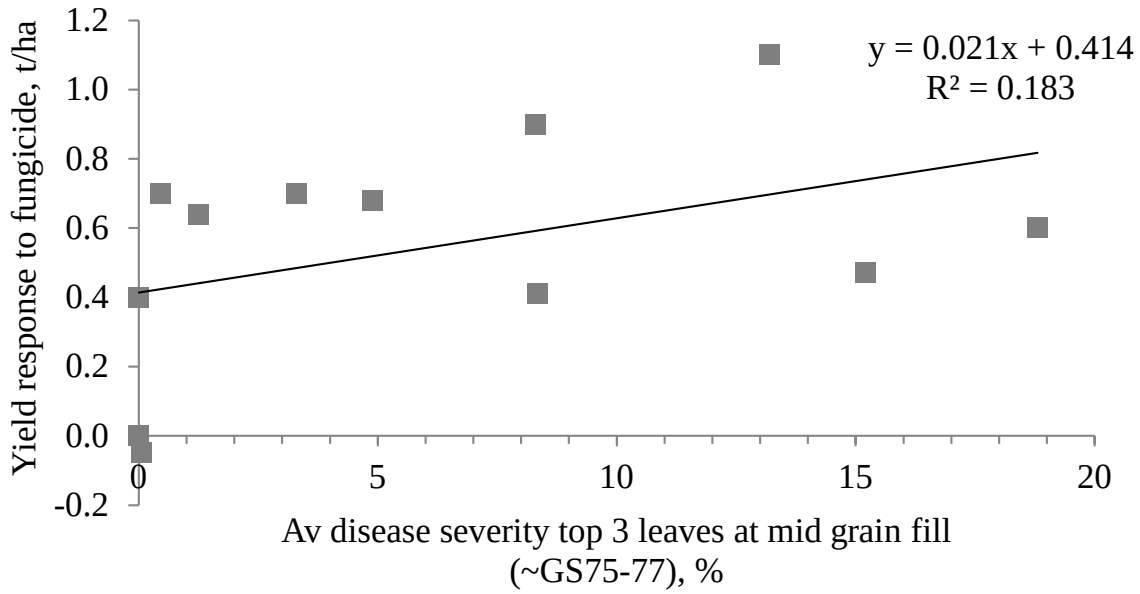

Figure 1. The relationship between the yield response (t/ha @85% dry matter) of spring barley to an application of prothioconazole plus pyraclostrobin at T1 and T2 and the average disease severity of untreated plots at mid grain fill. The intercept on the y axis is significant at $P<0.01$, indicating a significant yield response in the absence of visible disease. All except two data points are values for the variety Westminster; two are the mean response of three disease resistant varieties.

MATERIALS AND METHODS

Full details of the experimental methods can be found in Bingham *et al*. (2013). Only a brief summary is given here. Two series of experiments were conducted. The first compared the effects of different fungicide chemistries on photosynthetically active radiation (PAR) interception, asymptomatic pathogen infection, leaf surface saprophyte abundance and yield formation of spring barley (*Hordeum vulgare* L.) cv Westminster in the absence of pre-anthesis visible disease. The fungicide active ingredients (a.i.) were prothioconazole, pyraclostrobin and chlorothalonil. These are representative examples of the triazole, strobilurin and chlorophenyl groups of fungicides respectively. Some triazoles have been reported to have anti-gibberellin activity (Rademacher, 2000), whilst the strobilurins have been reported to influence cytokinin

and ethylene metabolism (Grossman *et al.*, 1999). Chlorothalonil, on the other hand, has not been linked to effects on plant metabolism. In the second series of experiments the effects of different timings of prothioconazole plus pyraclostrobin on grain numbers was determined.

Experiments to compare fungicide a.i. were conducted between 2009 and 2011 at two sites ADAS Rosemaund (Herefordshire) and SRUC Edinburgh. In 2012 there was an additional site at SRUC, Aberdeen. The experimental design was a randomised block with 4 replicates. Fungicide treatments were chlorothalonil (Bravo 500® @ 1.0 l/ha), prothioconazole (Proline® @ 0.4 l/ l/ha), pyraclostrobin (Comet 200® @ 0.63 l/ha), prothioconazole plus pyraclostrobin (at the same rates as when applied singularly). Untreated plots served as controls. All fungicides were applied at both T1 (GS30–31) and T2 (GS45–49). Visible disease was assessed by leaf layer on 10 randomly selected plants per plot every two weeks from GS31 onwards and absolute leaf area measured by leaf area meter (Li-Cor Biosciences, USA). Photosynthetically active radiation (PAR) interception was measured using a Sunscan Canopy Analysis System (Delta T Devices Ltd, UK) and estimates of PAR interception by healthy tissue made according to Bingham (2013). At GS39 and 59 samples of the top 4 leaves of 20 randomly selected plants per plot were taken and bulked for quantitative PCR analysis of *Rhynchosporium commune* and *Ramularia collo-cygni*. At SRUC Edinburgh 2012, flag leaves were sampled at GS39 and 59 for determination of *Cladosporium* sp. (the major genus of barley leaf surface saprophytes) by quantitative PCR.

Effects of prothioconazole plus pyraclostrobin (rates and products as above) applied at T1, T2 or T1 and T2 were investigated on three varieties Westminster, Quench and Garner at two sites in 2011 (Rosemaund, Edinburgh) and three sites in 2012 (Rosemaund, Edinburgh and Aberdeen).

RESULTS

The most comprehensive set of physiological data are available for 2012, thus the following analysis focusses mostly on experiments for that year. In a cross-site analysis all fungicide groups increased yield significantly compared to untreated controls (Table 1). The increase was greatest (12%) with prothioconazole plus pyraclostrobin and least (7%) with chlorothalonil. By contrast chlorothalonil and prothioconazole plus pyraclostrobin increased mean grain weight (MGW) to a similar extent (4.5%); effects close to statistical significance at the 5% level. Thus the greater effect of prothioconazole plus pyraclostrobin on yield compared to chlorothalonil was the result of the impact on grain number/m^2. Prothioconazole plus pyraclostrobin increased grain number/m^2 by nearly 1000 relative to untreated controls (a 7.5% increase), whilst there was no significant effect of chlorothalonil. When applied separately, prothioconazole and pyraclostrobin were equally effective at increasing grain numbers. None of the treatments influenced the number of ears/m^2. A separate cross-site analysis was conducted on data from experiments at ADAS and SRUC in 2009–2011 with comparable results.

Table 1. Effects of fungicide products with contrasting chemistries on the yield and yield components of spring barley cv. Westminster. Values are means from a cross-site ANOVA of experiments at SRUC Aberdeen, SRUC Edinburgh and ADAS Rosemaund in 2012.

Fungicide	Yield t/ha @85% DM	Ears/m^2	Grains/m^2	MGW, mg @85% DM
Chlorothalonil	5.61	910	12979	43.50
Pyraclostrobin (Pyr)	5.71	947	13443	42.94
Prothioconazole (Pro)	5.71	906	13355	43.16
Pro + Pyr	5.89	968	13678	43.54
Untreated	5.25	937	12718	41.62
P	<0.001	0.274	0.050	0.064
LSD (5%)	0.202	63.4	678.6	1.444

In 2012 there was no significant visible disease prior to ear emergence. After anthesis, visible symptoms of rhynchosporium and ramularia developed. Control of visible disease resulted in a significant increase in post-anthesis PAR interception by healthy (green) tissue. Chlorothalonil, prothioconazole, and prothioconazole plus pyraclostrobin increased PAR interception to a comparable extent, although pyraclostrobin on its own was less effective. There was no significant effect of any fungicide treatment on pre-anthesis PAR interception by healthy tissue.

There was no significant effect of fungicide treatment on the amount of rhynchosporium or ramularia DNA in leaf extracts at GS39 (Table 2). At GS59 there was a significant reduction compared to untreated controls in the absence of visible symptoms of disease, but chlorothalonil was as effective as prothioconazole plus pyraclostrobin at reducing the infection. Chlorothalonil and prothioconazole also reduced the abundance of leaf surface fungi and *Cladosporium sp* DNA in leaf washings to a comparable extent.

In the second series of experiments increased grain numbers were observed in the absence of pre-anthesis disease following applications of prothioconazole plus pyraclostrobin at T1 or T2, although the response to a T2 application was more consistent than that to a T1 across sites and years. There was no interaction between fungicide treatment and variety in these experiments, indicating that all the varieties tested responded to fungicide in the same way.

DISCUSSION

The results show that fungicides can increase grain numbers of spring barley in the absence of visible pre-anthesis disease, but that the effect depends on fungicide chemistry. The triazole and strobilurin active ingredients prothioconazole and pyraclostrobin elicited an increase, but chlorothalonil did not.

Table 2. Quantities of ramularia and rhynchosporium DNA (pg 100 ng^{-1} total DNA) in extracts of bulked leaf samples from the top 4 leaf layers determined by qPCR. Results are from a cross-site analysis of experiments in 2012 where site was analysed as a random effect. Values within a column followed by a different letter are significantly different at P=0.05.

	Ramularia			Rhyncho		
	GS 39	GS 59		GS 39	GS 59	
Chlorothalonil	1.88	1.32	a	0.08	0.21	a
Pyraclostrobin (Pyr)	2.49	3.54	a	0.06	0.56	a
Prothioconazole (Pro)	1.43	2.24	a	0.05	0.19	a
Pro + Pyr	0.84	1.96	a	0.30	0.17	a
Untreated	1.29	9.91	b	0.53	2.55	b
P	0.385	<0.001		0.142	0.017	

The effect on grain numbers cannot be explained in terms of the control of asymptomatic pathogen infection or leaf surface saprophytes, because chlorothalonil was as effective in reducing these fungal populations as prothioconazole and pyraclostrobin yet had no effect on grain numbers. Nor can the response be ascribed to the effects of fungicides on leaf growth and pre-anthesis PAR interception as neither were increased by any of the fungicide treatments. The evidence points to other direct effects of the fungicides on plant metabolism. Interestingly, the response was achieved most consistently with a T2 application late in ear development suggesting effects on floret survival and grain numbers per ear. The mechanisms underlying the effects on grain numbers reported here differ markedly from the physiological effects of fungicides reported for wheat. In wheat, yield increases in the absence of disease are usually attributed to the delayed leaf senescence and extended post-anthesis canopy duration resulting from fungicide application (Grossman *et al.*, 1999; Rademacher 2000). In the current study, an additional increase in yield was observed via an increase in mean grain weight, but this was associated with the control of post-anthesis disease by each of the fungicide groups investigated.

These findings support the following strategy for fungicide management in low disease risk situations. The crop should be monitored for disease and treated for disease if it appears. However, if no visible disease has developed by booting, a single application of fungicide at the T2 timing (GS45–49) will protect against late disease. Application of prothioconazole plus pyraclostrobin is likely to give economic yield benefits, even if no late visible disease develops, by increasing grain number formation. Whether other triazoles and strobilurin active ingredients are able to elicit the grain number response has still to be tested.

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THE INFLUENCE OF SDHI FUNGICIDES ON YIELD POTENTIAL, DISEASE CONTROL AND PHOTOSYNTHETIC ACTIVITY IN SPRING BARLEY AND WINTER WHEAT

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Summary: The introduction of fungicides from the SDHI group of chemistry has brought improved efficacy to cereal fungicide programmes, and are applied against a wide spectrum of diseases. They have also brought increases in yield in wheat and barley. Not all of the yield benefits can be explained by disease control and this paper reports measurable improvements in the photosynthetic activity of leaves from wheat and spring barley field trials. Fluxapyroxad containing fungicides improved the longevity and chlorophyll content of leaves and the data presented suggested that responses were larger in barley than in wheat. There was preliminary evidence that SDHI chemistry might improve photosynthetic efficiency, specifically through the efficiency of carboxylation.

INTRODUCTION

The new generation of succinate dehydrogenase inhibiting fungicides (SDHIs), which were launched in commercial practice over the past four seasons, has broad spectrum and long lasting efficacy against a range foliar pathogens in wheat and barley. They were launched commercially in the UK over low disease pressure seasons and, even in the absence of disease, yield enhancements in wheat of over 0.3 t/ha were observed compared to conventional chemistry (Waterhouse & Semar, 2012). Improved green leaf retention is one effect from the chemistry, with much interest in how this could improve yield production and resource use efficiency. Improved understanding would allow best practices to be developed to maximise the physiological benefits of SDHI chemistry. The work reported here was designed to test the potential yield enhancing properties of SDHI fungicide in winter wheat (in 2011) and spring barley (in 2012). An aim was to establish the principles of assessing SDHI fungicide effects on leaf physiology through measurement of: green leaf area, disease, leaf longevity and photosynthetic activity in order to determine the effects of SDHIs on components of photosynthesis, both physical and biochemical.

MATERIALS AND METHODS

Winter wheat trial, 2011

A winter wheat field trial, variety Consort, was established in the autumn of 2010 at a site in Fife. Agronomic details and all inputs apart from fungicides accorded with local practice. Treatments are shown in Table 1 and 2. The trial was laid out in three replicate blocks, two of which were randomised. Treatments were applied according to the details in tables 1 and 2. Disease assessments were made throughout the season and evaluation of fungicide effects on leaf chorophyll, disease and nitrogen % were made on the flag leaf. Leaf chlorophyll was measured *in situ* on 10 leaves per replicate plot (i.e. 30 leaves in total) using a Minolta Spad Meter at growth stages early-medium milk (GS73/75) and early dough (GS83). The Spad Meter reading was taken from the mid section of leaves at GS73/75, and a healthy mid or basal section of leaves at GS83. Assessments of functional leaf area i.e. green area %, chlorotic area % and necrotic(dead) area % were made in the laboratory on detached leaves at GS83 and GS85/87 (soft-hard dough) using 10 leaves per plot. Total leaf nitrogen % was measured in leaves sampled from replicates 1 and 2 at GS73/75, GS83 and GS85/87. Flag leaf photosynthetic activity (net assimilation) and gas exchange was assessed in treatments 1, 2 and 4 in order to test if there was an increase in leaf photosynthetic activity and/or light conversion efficiency (εc) as a consequence of increased and extended net assimilation capacity. A portable gas exchange/photosynthesis unit (LI-COR 6400) was used to examine the response of net assimilation to changes in light (radiation) and ambient CO_2 levels. This was measured at a light saturation point of 1000 µmol $m^{-2}s^{-1}$ (A_{sat}) at a range of internal CO_2 levels (C_i).

Table 1. Fungicide treatments applied to trials

Trade name	Active ingredients		gai/l	Label dose (l ha^{-1})
Ignite	epoxiconazole		83	1.5
Imtrex	fluxapyroxad		62.5	2.0
Adexar	fluxapyroxad epoxiconazole	+	62.5 + 62.5	2.0

Table 2. Fungicide treatments applied to wheat trial 2011

Treat-ment	Active ingredients	Dose l ha^{-1}	Growth stage applied	Date applied
1	untreated	-	-	-
2	epoxiconazole	1.125	32	2 May
3	fluxapyroxad	1.5	32	2 May
4	epoxiconazole + fluxapyroxad	1.5	32	2 May
5	epoxiconazole	1.125	39	27 May
6	fluxapyroxad	1.5	39	27 May
7	epoxiconazole + fluxapyroxad	1.5	39	27 May
8	epoxiconazole	1.125	39-45	3 June
9	fluxapyroxad	1.5	39-45	3 June
10	epoxiconazole + fluxapyroxad	1.5	39-45	3 June

Spring barley, 2012

A spring barley field trial, variety Concerto, was established in the spring of 2012 at a site in Midlothian. Agronomic details and all inputs apart from fungicides accorded with local practice. The trial was laid out in a randomised block design with four replicates of each treatment. Treatments were applied according to the details in Tables 1 and 3. Green leaf area and disease levels in relation to photosynthetic activity and leaf longevity were assessed.

All measurements were made on leaf 2. Leaf chlorophyll was measured *in situ* on 10 leaves per replicate plot (i.e. 40 leaves in total) as described for wheat at growth stages early-medium milk (GS73/75) and early dough (GS83). The Spad Meter reading was taken from the mid section of leaves at GS73, and a healthy mid or basal section of leaves at GS83. Assessments of green area % were made in the laboratory on detached leaves at GS83 and GS85/87 (soft-hard dough) using 10 leaves per plot. Total leaf nitrogen per cent was measured in leaves sampled from replicates 1 and 2 at GS73/75, GS83 and GS85/87. The portable gas exchange/photosynthesis was used to examine the response of net assimilation to changes in light (radiation) and ambient CO_2 levels at two growth stages, firstly, at maximum canopy size (GS73/75) and secondly during canopy decline (GS83). This enabled the effects of SDHIs on photosynthesis across leaf developmental stages to be assessed.

Yield, leaf chorophyll, disease and nitrogen % from leaf two were assessed in all treatments: Leaf two photosynthetic activity (net assimilation) and gas exchange were assessed in treatments 1, 2 and 3, as described for wheat.

Table 3. Fungicide treatments applied to barley trial 2012 and yield at 85% dry matter, treatments applied 24 May and 12 June 2012.

Treat-ment	Active ingredients	Dose l ha^{-1}	Growth stage applied
1	untreated	-	-
2	epoxiconazole	0.75	25/30 and 45
3	epoxiconazole + fluxapyroxad	1.0	25/30 and 45
4	epoxiconazole + fluxapyroxad	1.0	25/30
5	epoxiconazole + fluxapyroxad	1.0	45

RESULTS

Differences in nitrogen % in the flag leaf (wheat) and leaf two (barley) were not significant and are therefore not reported, although in wheat there was a trend to increased nitrogen % in the flag leaf in fluxapyroxad-containing treatments compared to epoxiconazole during the phase of general nitrogen decline (i.e. after GS73). Table 4 presents the leaf chlorophyll and leaf greenness data for wheat. Both fungicide and treatment timing had highly significant effects, with fluxapyroxad-containing treatments significantly better than epoxiconazole alone, especially at later application timings. Septoria control was significantly better with fluxapyroxad containing treatments at the early application timing, with a similar trend at later timings. Yield was significantly better with fluxapyroxad-containing treatments at the flag leaf and delayed flag leaf timings. Figure 1 presents effects on flag leaf net assimilation at GS77. Net assimilation attained maximum rate at approximately 800 µmol mol-1. The maximal rates

of A_{sat} were 15.3 for the untreated, 16.6 for epoxiconazole and 17.2 for fluxapyroxad. The initial linear slopes of the A/C_i curves were slightly steeper in fungicide treated leaves compared with untreated leaves. The slopes were 0.033, 0.040 and 0.038 in untreated, epoxiconazole treated and fluxapyroxad treated leaves, respectively.

Table 4. Flag leaf chlorophyll (SPAD units), leaf greenness (SPAD units x green leaf area) and disease and green leaf area (GLA) as % leaf area and yield (tha^{-1}) adjusted to 85% dry matter.

Treat-ment	Leaf chlorophyll GS73/75	Leaf chlorophyll GS83	Leaf greenness GS83	Septoria % GS 71	GLA % GS83	Yield tha^{-1}
1	32.5	20.8	4.44	16..6	20.9	6.51
2	30.6	21.3	4.56	13.8	21.3	7.45
3	30.3	23.1	8.74	10.9	38.0	7.24
4	32.7	19.0	10.0	7.22	51.8	7.33
5	30.4	19.0	6.34	6.19	33.1	7.91
6	32.6	20.8	9.60	3.87	45.8	8.10
7	33.3	19.2	11.8	7.32	61.7	8.42
8	32.7	18.5	7.85	6.20	42.5	7.49
9	35.6	20.0	10.8	5.41	54.3	8.33
10	34.9	19.9	14.7	3.78	73.7	8.75
P	<0.001	<0.050	<0.001	<0.001	<0.001	0.047
LSD	1.114	1.875	4.852	6.301	11.26	0.658

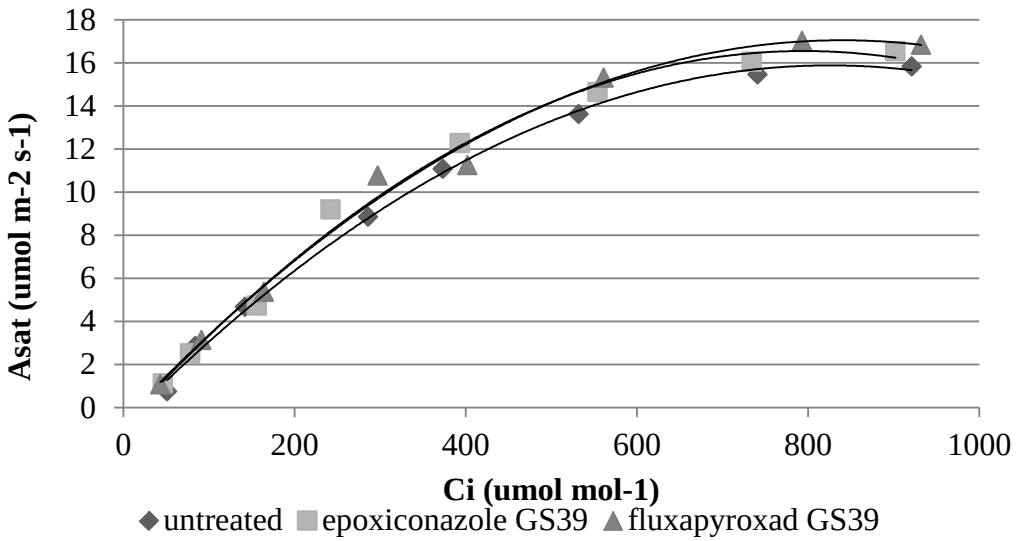

Figure 1. Summary of net assimilation at light saturation (A_{sat}) response to internal CO_2 concentration (C_i). Measurements were made at late milk (GS77)

Table 6. Flag leaf chlorophyll (SPAD units) and leaf greenness (SPAD units x green leaf area) with disease (% leaf area) 26 July 2012, GS79 and yield (tha^{-1}) adjusted to 85% dry matter.

Treat-ment	Leaf chloroph-yll GS73	Leaf chloroph-yll GS83	Leaf green-ness GS73	Leaf green-ness GS83	Ramul-aria % GS79	Rhyncho -sporium % GS79	Yield tha^{-1}
1	27.4	17.6	9.50	5.06	15.0	9.00	4.64
2	29.7	22.9	28.5	11.4	6.50	4.25	5.02
3	31.5	23.3	38.5	14.4	6.25	5.00	4.98
4	30.4	23.5	34.5	11.7	9.75	5.75	5.15
5	30.2	24.4	35.0	13.6	6.50	3.75	5.07
P	<0.001	0.050	<0.001	<0.001	<0.001	0.231	0.072
LSD	1.702	2.831	1.755	2.037	2.951	5.253	0.364

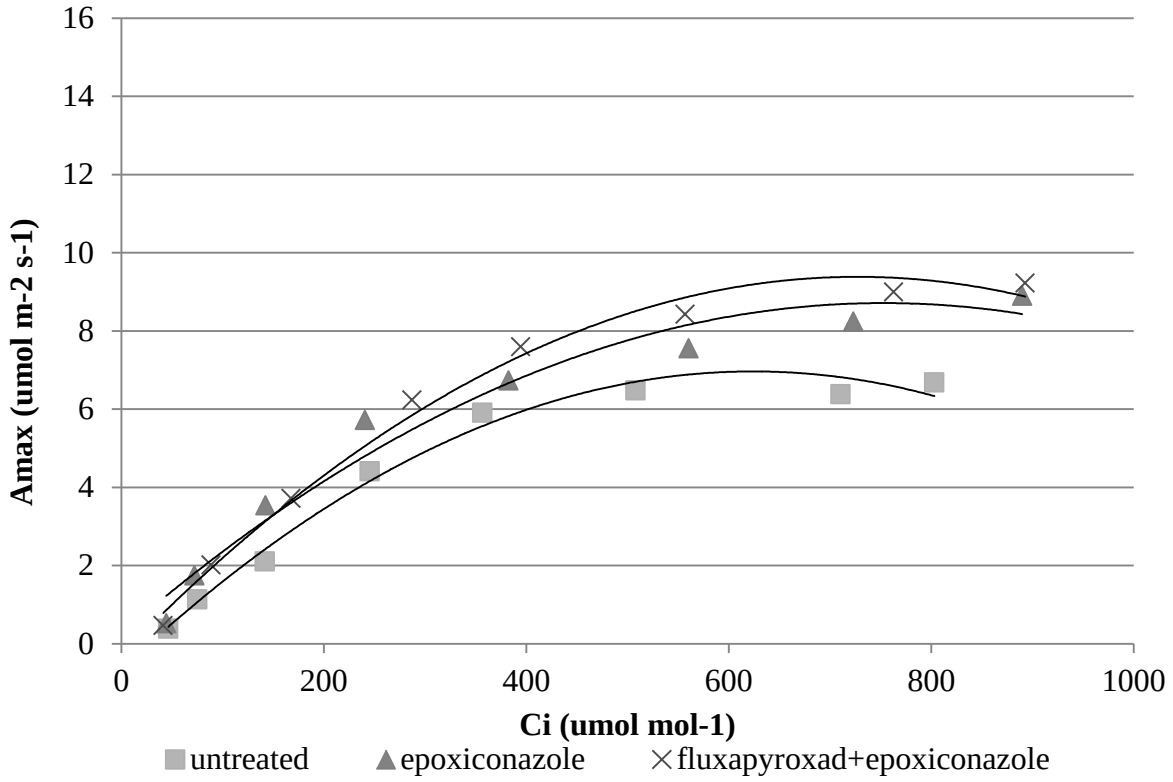

Figure 2. Summary of net assimilation at light saturation (A_{max}) response to internal CO_2. Measurements were made at early dough (GS83).

Table 6 presents treatment effects on leaf two chlorophyll and functional leaf area. Fluxapyroxad + epxiconazole applied twice resulted in significantly higher levels of leaf chlorophyll at GS 73 than epoxiconazole. At GS83 the effects of treatment compared to untreated controls were more pronounced but differences between fungicides were not significant. Differences in leaf greenness were also highly significant and fluxapyroxad +

epoxiconazole applied twice or late was significantly greener than other fungicide treatments. Net assimilation, shown in Figure 2, attained a maximal rate of approximately 7.1 µmol m^{-2} s^{-1} in untreated plants. For fluxapyroxad + epoxiconazole treated plants the maximum rate of A_{sat} was close to 9.4 µmol m^{-2} $s^{-}1$. The initial slope of the A/C_i curve, over the range 0 to 400 µmol mol^{-1} C_i, was slightly steeper for fungicide treated plants, compared to untreated plants. It was evident that at this growth stage the responses of A_{sat} to C_i between the two fungicide treatments were differentiating.

DISCUSSION

The results show early evidence that treatment of wheat with fluxapyroxad containing fungicides prolonged green leaf longevity and that this preserved green leaf was more efficient in terms of light conversion efficiency than a comparable non SDHI treatment (epoxiconazole) because of changes to the underlying biochemical process which could contribute to yield potential. The initial slope of the A/C_i curve indicates the efficiency of carboxylation i.e. the amount of active enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco). The maximal rate of A_{sat}, indicates the point at which assimilation becomes limited by the supply of the substrate ribulose-1, 5-bisphosphate (RuBP). The curve analysis suggested that there was less Rubisco limitation in leaves treated with fluxapyroxad, compared to untreated leaves. The initial slope from the A/C_i curve indicated that fluxapyroxad + epoxiconazole had a steeper response than epoxiconazole. Yield responses of over 2 tha^{-1} were obtained for some treatments, with fluxapyroxad significantly better than epoxiconazole at the delayed flag leaf timing. On barley both epoxiconazole and fluxapyroxad + epoxiconazole treatment increased A_{max} compared to the untreated control at grain filling. The effect on A_{max} on barley was larger than that seen in wheat. At later assessments fluxapyroxad + epoxiconazole treatment increased A_{max}, leaf greenness and chlorophyll content compared to epoxiconazole. Differences in curves may appear small but increases in carboxylation efficiency of even a few percent would be yield enhancing. Yield responses in this trial were small but the crop was very stressed by the wet summer of 2012 and a repetition of this work in less saturated soil would be useful. Both the wheat and barley results suggest interesting new aspects to the understanding of physiological responses in cereals to SDHI chemistry.

ACKNOWLEDGEMENTS

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PHOSPHITE MEDIATED INHIBITION OF THE ASCOMYCETE PATHOGENS *MICRODOCHIUM NIVALE* AND *MICRODOCHIUM MAJUS* IN THE GRAMINEAE

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Summary: *Microdochium nivale* is a major pathogen of many species of the gramineae. Control measures rely on inputs of chemical fungicides, making alternative means of disease reduction desirable. Phosphite (PO_3^{3-}) has proven efficacy in reducing susceptibility to oomycete pathogens. Field trials have exhibited significantly lower percentages ($p<0.01$) of *M. nivale* incidence on PO_3^{3-} treated plots of turfgrass. The mode of this suppression is currently being determined. *In vitro* studies with *M. nivale* and *M. majus* showed that PO_3^{3-} concentrations of 100 $\mu g/ml^{-1}$ and above fully inhibited mycelial growth, with hyphal morphology displaying distinct irregularities in amended PDA. Analysis of PO_3^{3-} treated grass tissues indicated rapid *in planta* accumulation, symplastic mobility and no *in planta* conversion to PO_4^{3-}. Current research will determine if PO_3^{3-} can enhance synthesis of defence related compounds and lead to induction of systemic resistance.

INTRODUCTION

Microdochium nivale (teleomorph *Monographella nivalis* (Schafnitt)) is an ascomycete pathogen and causal agent for many disease complexes in numerous graminaceous species (Smiley *et al*., 1992; Tronsmo *et al*., 2001; Pronczuk *et al*., 2003). *M. nivale* was first thought to be a single species, but research studying the conidial morphology and use of Random Amplified Polymorphic DNA (RAPD) assays divided it into two varieties (var. *nivale* and var. *majus*); (Lees *et al*., 1995; Nicholson *et al*., 1996), and it is now acknowledged to be two distinct species, *M. nivale* and *M. majus* (Glynn *et al*., 2005; Hofgaard *et al*., 2006). *M. majus* differs from *M. nivale* in conidial morphology, with predominantly 3 or more septa, whilst *M. nivale* has 1 to 3 septa. Conidia are produced in large numbers and are readily dispersed by wind and rain splash and, along with soil borne mycelium, are the main source of inoculum (Tronsmo *et al*., 2001).

There have been reports of different host specificity of the two species (Simpson *et al*., 2000). Analyses of turfgrass isolates using RAPD and Restriction Fragment Length Polymorphism (RFLP), found only *M. nivale* (Mahuku *et al*., 1998), while Hofgaard *et al*. (2006) concluded that isolates of *M. nivale* were more pathogenic on *Lolium perenne* than *M. majus.* The majority of research however, shows that both species are major pathogens of winter cereals, responsible for a series of disease complexes causing pre- and post-emergence death of wheat, barley and oat seedlings, leading to reduced establishment and reductions in grain yield. They are causal agents of wheat head blight, foot rot and ear infection of mature plants and *Microdochium* Leaf Blotch of oats (Pettitt *et al.*, 1993; Humphreys *et al*., 1995; Clement & Parry, 1998). In turfgrasses, *M. nivale* is regarded as the most damaging pathogen of temperate climates, infecting most cool season species, causing Pink Snow Mould and *Microdochium* Patch (Vargas, 2005).

Chemical protectants represent the foremost tool used to control both pathogens. The numerous available chemistries of protectants vary in their uptake and biochemical mode of action. Pathogen activity is inhibited by interference with fungal mitosis, reduction of respiratory enzyme activity, inhibition of sterol, DNA and RNA synthesis, limitation of amino acid uptake or inhibition of ATP production (Smiley *et al*., 1992; Yang *et al*., 2011). While the efficacy and safety of these plant protectants is not disputed, development of alternative means of reducing susceptibility is desirable. One such means is the use of phosphite as a component of an integrated approach to disease management.

Phosphite (PO_3^{3-}) is a reduced form of phosphorus (P) derived from the alkali metal salts of phosphorous acid (H_3PO_3;(Guest & Grant, 1991). The pH is modified to prevent phytotoxicity, commonly by combining with potassium hydroxide (KOH), forming potassium dihydrogen phosphite (KH_2PO_3) or dipotassium hydrogen phosphite (K_2HPO_3). Phosphite is chemically similar to phosphate (PO_4^{3-}), but the different tetrahedral molecular structure of phosphite ensures that enzymes, which react with phosphate to catalyse metabolic processes, do not bind to phosphite in the same manner. This ensures that it does not supply a metabolically usable form of P (Mcdonald *et al*., 2001). Phosphite does however, have significant properties as an inhibitor of plant pathogens (Fenn & Coffey, 1984), but the mode of suppression remains a subject of debate (Abbasi & Lazarovits, 2006). Research shows phosphite acting directly on the pathogen and indirectly in stimulating host defences (Guest & Grant, 1991).

The majority of studies on phosphite mediated inhibition of pathogens have been on its effects on oomycetes. Suppression of *Pythium* by phosphite under field conditions was reported by Sanders in 1983, but when no *in vitro* inhibition was demonstrated it was concluded that control resulted from enhanced host defences. However, Fenn and Coffey (1984, 1987) demonstrated that phosphite inhibited four *Pythium* spp. and *Phytophthora cinnamomi in vitro*. *P. cinnamomi* exhibited sensitivity to phosphite with EC_{50} values (Effective Concentration which reduces growth by 50% of control growth) ranging from 4 to 148 μg ml^{-1} (Wilkinson *et al*., 2001) and *Pythium* spp were inhibited with EC_{50} values between 38.7 and 220.8 μg/ml^{-1} (Cook *et al*., 2009). The direct mode of inhibition seems to involve disruption of the pathogen's metabolism. For example, a study with three *Phytophthora* species showed that phosphite interfered with phosphate metabolism in pathogen cells by causing an accumulation of polyphosphate and pyrophosphate, diverting ATP from other metabolic pathways, and resulting in a decrease in growth (Niere *et al*., 1994). A direct mode of suppression was also indicated by a study showing that phosphite inhibited enzymes of the glycolytic and phosphogluconate pathways, disrupting metabolism in *P. palmivora* by competing with phosphate as an allosteric regulator on several enzymes (Stehmann & Grant, 2000). Evidence of indirect inhibition of pathogens by phosphite has been demonstrated by Daniel and Guest (2006). They concluded that phosphite induced host defence responses, including release of superoxide, localised cell death and increased phenolic compounds in *P. palmivora* challenged *Arabidopsis thaliana*. It has also been suggested that host defences were stimulated when phosphite accumulation was low, but that accumulation to greater concentrations led to direct inhibition of pathogens (Jackson *et al*., 2000).

Phosphite inhibition of ascomycete pathogens has also been reported. Reuveni *et al*. (2003) showed inhibition of *Alternaria alternata* mycelial growth and conidial germination, while Burpee (2005) reported suppression of *in vitro* growth of *Colletotrichum graminicola*. Mills *et al*. (2004) demonstrated that H_2PO_3 not only reduced mycelial growth but caused complete inhibition of sporulation of *A. alternata, Botrytis cinerea* and *Fusarium solani*. Growth of *F. culmorum* and *F. graminearum* was reduced on KH_2PO_3 amended PDA (Hofgaard *et al*., 2010). The same study included the effects of phosphite on *M. majus*, and found that mycelial

growth was reduced by more than 90%at the lowest KH_2PO_3 concentration used (10 µl ml^{-1}), with full inhibition at concentrations of 100 µl ml^{-1}.

Data from field trials conducted to evaluate *M. nivale* suppression by KH_2PO_3, applied alone and in combination with a fungicide (iprodione), demonstrated that phosphite significantly suppressed *M. nivale* infection ($p<0.01$), compared to controls. Iprodione produced significantly higher levels of suppression than phosphite alone ($p<0.01$), but the addition of phosphite significantly enhanced the iprodione control ($p<0.01$). Enhanced sward density and quality as a result of phosphite treatment was also reported (Dempsey *et al*., 2012). The results of these trials have led to research to determine the mode of suppression. The aims include determination of the assimilation, translocation, accumulation and fate of phosphite in treated graminaceous tissues, and the *in vitro* effects of phosphite on the growth and reproduction of *M. nivale* and *M. majus*. The possibility that phosphite can stimulate defence responses in host plants and induce systemic acquired resistance (SAR) is also being investigated.

MATERIALS AND METHODS

Inhibition of mycelial growth of *M. nivale* and *M. majus* isolates were determined by amending PDA (19 g/l) with H_3PO_3, H_3PO_4, KH_2PO_3, and KH_2PO_4 , ranging from 10 to 250 µg/ml^{-1} (n=6). Agar plugs, 5 mm diameter, cut from actively growing colonies, were transferred to the centre of amended and control PDA and incubated in darkness at 18°C. Radial growth measurements at 24 h, 48 h, 72 h and 96 h post inoculation (p.i.) were used to calculate mean daily growth rates and percent relative growth (PRG). The EC_{50} values were determined by probit transforming the PRG and regressing against the Log_{10} of amendments.

The assimilation, translocation and accumulation of phosphite in three graminacious species, *Agrostis stolonifera*, *A. canina* ssp. *canina* and *Poa annua*, were determined using High Performance Ion Chromatography (HPIC), modifying a previously published technique (Roos *et al*., 1999). Foliar treatments at 0.35 g PO_3^{3-}/m^{-1} were applied, and then leaf, crown and root tissues were harvested at 1, 6, 12, 24, 48 h and 1, 2, 3, 4, 5, and 6 weeks post-application (p.a.). The dried and ground samples (0.5 g) were extracted in 10 ml deionised water overnight and subject to HPIC analysis. A Dionex ICS100 ion chromatograph was used with an IonPac AG9-HC Guard Tube (4 x 50 mm), IonPac AS9-HC Analytical Column (unheated 4 x 250 mm), ASRS300 Suppressor (4 mm), DS6 Heated Conductivity Cell and 25 µl injection loop. The eluent was 9 mM sodium carbonate (degassed and pressurised to 1 bar), flowing at 1 ml/min, with a single back pressure loop. Method run time was 18 min. Samples were injected manually through 0.47 micron syringe filters. Calibration was by 12.5, 25. 25, 50, 100, 200, 500 and 1000 ppm mixed phosphate/phosphite standards, prepared from sodium phosphate monobasic anhydrous (H_2NaO_4P) and sodium phosphite dibasic pentahydrate ($Na_2(PHO_3).5H_20$, respectively. Results were adjusted for the weights of extracted sample and reported as ppm dried tissue weight.

RESULTS

Figure 1 shows phosphite accumulation in turfgrass tissues at 6 weeks p.a. This demonstrates that phosphite treated tissues accumulate phosphite rapidly and that some is translocated to the roots, suggesting symplastic mobility. The data also indicate that sequential applications applied on a 3 week cycle would maintain leaf tissue amounts of approximately 2000 ppm. PO_4^{3-} amounts in both treated and control tissues were not significantly different, confirming no *in planta* conversion of PO_3^{3-} to PO_4^{3-}.

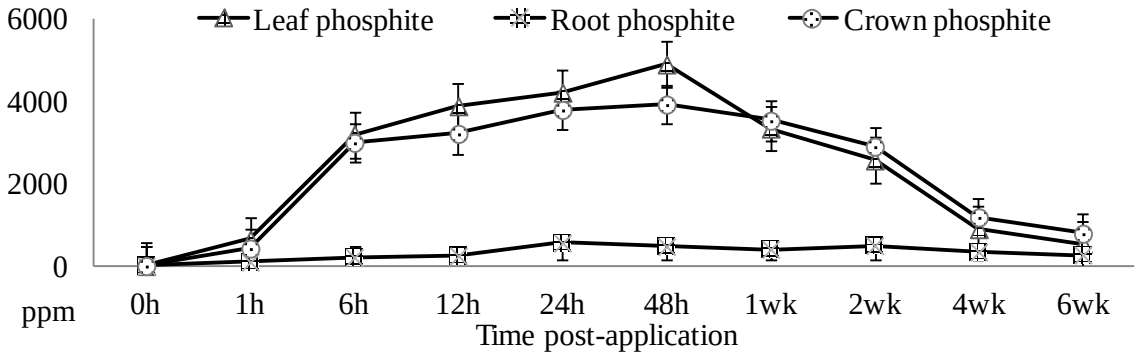

Figure 1. PO_3^{3-} accumulation in treated tissues 6 weeks post application (n=6, bars indicate SE).

In vitro experiments showed that H_3PO_3 and KH_2PO_3 have a direct inhibitory effect on mycelial growth of *M. nivale* and *M. majus* (Figure 2), with total growth inhibition at amounts of 100 µg/ml and above. EC_{50} values for all fungal isolates ranged between 38 and 76 µg/ml. H_3PO_4 and KH_2PO_4 had no significant inhibitory effect. Microscopic analysis of mycelia showed morphological deformities in hyphae growing on phosphite amended PDA, whilst hyphal growth was normal on phosphate amended PDA.

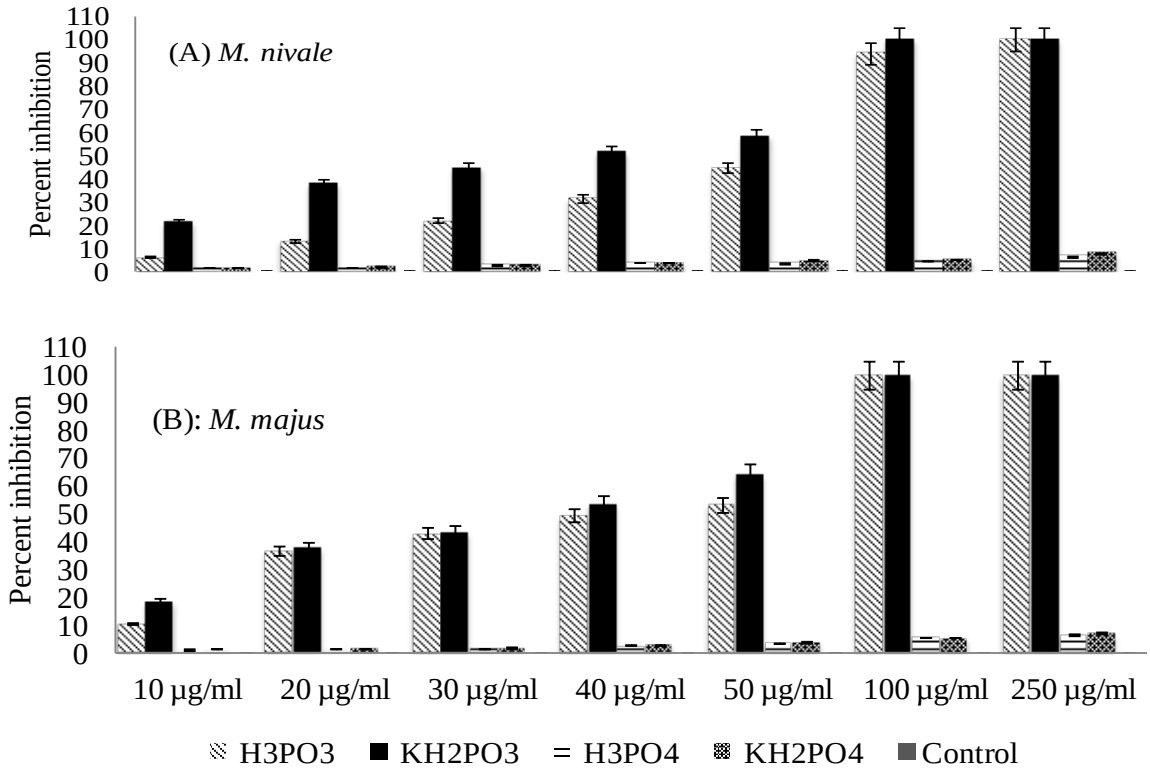

Figure 2. Inhibition of mycelial growth *in vitro* on amended PDA:
(A) *Microdochium nivale,* (B) *M. majus* (n=6; bars indicate SE).

CONCLUSIONS AND FURTHER RESEARCH

Our results shows that phosphite is rapidly assimilated and translocated in the gramineae, and that treated plants are significantly less susceptible to *M. nivale* infection in the field. *In vitro* experiments have demonstrated that phosphite has a direct inhibitory effect on the mycelial growth of both *M. nivale* and *M. majus.* These data compliment the results of Hofgaard *et al.*, 2010, who concluded that development of *M. majus* in winter wheat was reduced by application of foliar fertilisers containing potassium phosphite. Inhibition of mycelial growth *in planta* would allow increased time for initiation of host defences, and the combination of both these direct and indirect effects is likely to contribute to the reduced susceptibility observed. Further research using fluorescent microscopy and spectrophotometry is evaluating secondary metabolic processes to determine the role of phosphite in activating or enhancing inducible defence mechanisms.

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RAMULARIA COLLO-CYGNI – A RAPIDLY DEVELOPING PROBLEM

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Summary: The fungal pathogen *Ramularia collo-cygni* is the main biotic agent involved in the formation of Ramularia leaf spot in barley. The fungus has been shown to move asymptomatically in the host barley plant prior to symptom expression post-flowering. The wet summer of 2012 produced waterlogging at one trial site which induced earlier symptoms of disease. Risk factors associated with controlling the disease in winter and spring crops are being identified. The availability of new fungicide products offers more flexibility in control programmes for growers. However, although control is still reliant on fungicides, varietal resistance ratings have now been produced for UK spring barley varieties. The implications for barley growers are discussed.

INTRODUCTION

Barley (*Hordeum vulgare*) is one of the major world crops and constitutes a major component of UK arable production. In 2013, over 800,000 ha of spring barley was estimated to have been sown in England alone, the highest for over a decade (DEFRA, 2013a). UK Production is estimated to be 7.102 million tonnes (DEFRA, 2013b). Ramularia leaf spot (RLS) has been shown to reduce yield by up to 1.0 t/ha in susceptible varieties (Oxley & Havis, 2004). Symptoms appear post-flowering in the crop. Small rectangular pepper spots appear on upper leaves. The spots often have a chlorotic halo and are bound by the leaf veins. Over time the symptoms coalesce to form large areas of necrotic tissue. Varieties have been shown to differ in their susceptibility to RLS (Havis *et al.,* 2012a) but no official ratings have been published until 2013 (HGCA, 2013). Control of RLS has relied on the timely application of effective fungicides late in the growing season (Havis *et al.,* 2012 b) Although Rcc infection has been shown to be high in certain seed lots, the use of seed treatments to control RLS has not proved more effective than foliar sprays (Havis *et al.,* 2008). A number of highly effective fungicides are available for RLS control, although the potential build-up of resistance is being monitored on an annual basis (Havis *et al.,* 2012b), Fountaine *et al.,* 2012). A recent BBSRC-LINK project has been examining the life cycle of the fungus, the risk factors associated with RLS and the effect of infected seed on disease development. A series of field trials were used to examine the influence of seed treatment and controlled environment experiments were carried out to study the movement of the fungus.

MATERIALS AND METHODS

Field Trials

Seed samples from three varieties (Cocktail, Optic and Decanter) grown in 2010 spring barley trials were treated with experimental seed treatments. The treated seed was sown in field trials (10 m x 2 m plots) at the Bush Estate, Midlothian in March 2012 in a randomised block design. The seed treatments used were prothioconazole + tebuconazole + triazoxide (pro + teb + triaz) (Raxil Pro®), Hot water 1 (2 h at 52 °C followed by 72 h at 25 °C), Hot water 2 (21 °C for 1 h, then 10 min at 52 °C, microwaving at 800 watts for 25 s and dry heat 60 °C for 3 d. In both trials the plots received one foliar fungicide application at GS 25-30 (75 g/l metrofenone (Flexity®) and 80 g/l pyraclostrobin (Insignia®). These fungicides were selected as they had no efficacy against RLS but controlled mildew or rhynchosporium respectively. Leaf layers were assessed for the severity of RLS throughout the growing season and area under disease progress curves (AUDPC) values were calculated using the trapezoidal rule (Whittaker & Robinson, 1967). The plots were taken to yield and treatment means and least significant differences calculated using Genstat Version11.1, (VSN International Ltd, Hemel Hempstead, UK).

Controlled environment experiments

Six spring barley varieties were sown into Fisons Levington compost. Six seeds were sown per 12 cm pot and the pots grown in a glasshouse with supplementary lighting to give a 16 hour day. When plants reached growth stage 37, (flag leaf emerging) (Zadoks *et al.*, 1974) the inoculations were carried out. Mycelial suspensions of a green fluorescent protein transformed isolate of Rcc (Thirugnanasambandam *et al.,* 2011) were drop inoculated onto the F-1 leaf of six spring barley varieties. The plants were covered for 48 h to ensure high humidity and encourage fungal growth. The colonisation of the barley plant by fungus was monitored using confocal microscopy. The inoculation was repeated after ear emergence and the barley head was also drop inoculated. The inoculated seed was saved and germinated in petri dishes and movement of the fungus into new leaves visualised with confocal microscopy.

Risk factors

RLS symptoms in spring barley varietal trials were assessed over a number of years and information sent to HGCA. The data was incorporated into the algorithm to calculate resistance ratings for RLS. Information from spore samplers, winter and spring barley disease risk warnings and trials over the last ten years was incorporated into a series of risk factors relating to each barley crop.

RESULTS

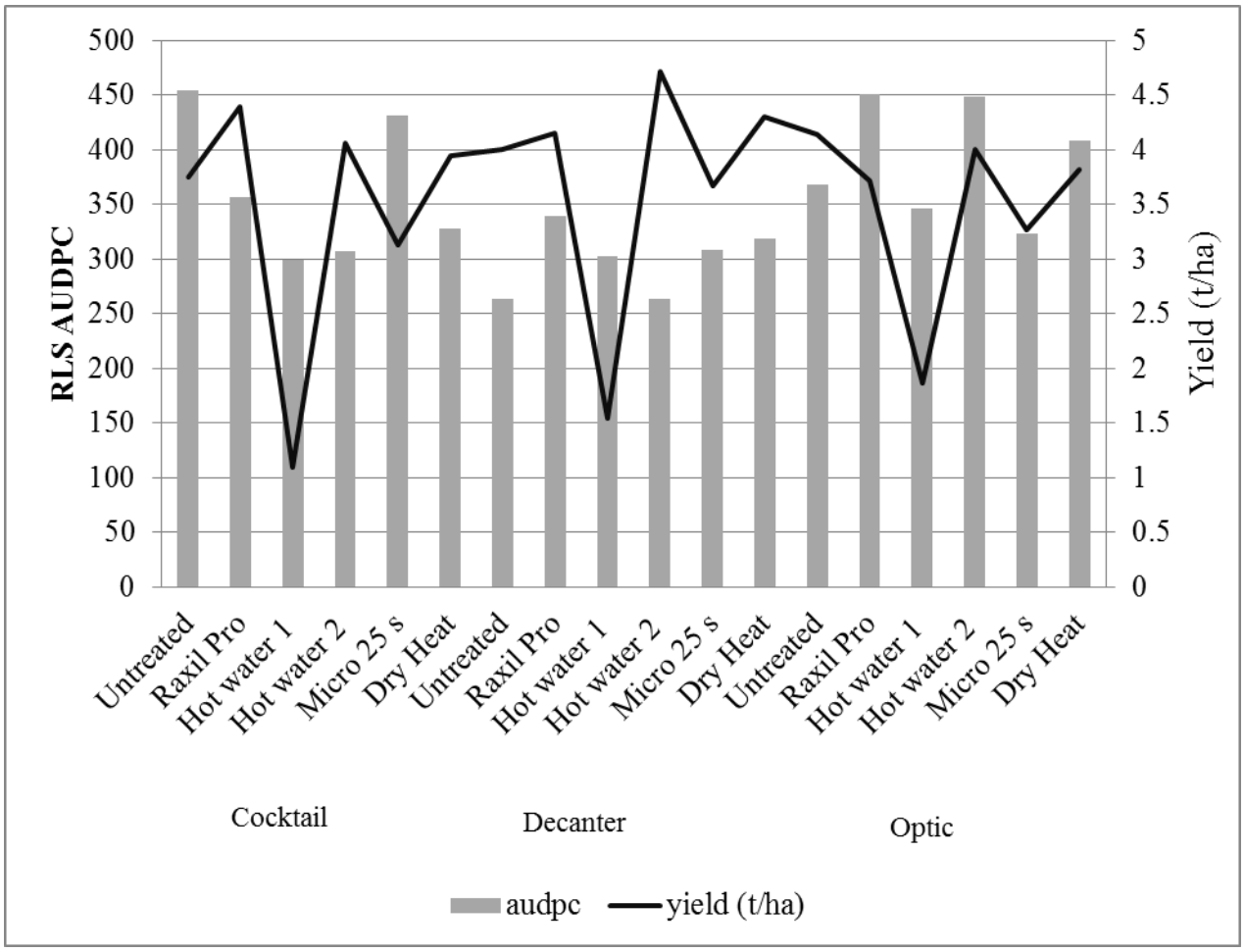

Figure 1. RLS AUDPC and yield data from Bush trial 2012. For treatment x variety AUDPC (F.pr = 0.002), Yield (F.pr = 0.043), LSD (P=0.05) AUDPC 86.4, Yield 0.54.

Significant reductions in RLS AUDPC were observed in the susceptible variety Cocktail with only the microwave treatment not proving effective (Fig. 1). For the same variety, the only significant increase in yield was produced by the fungicidal seed treatment (4.4 t/ha compared to 3.74 t/ha for the untreated). The longer hot water treatment had a detrimental effect on seed germination and a severe effect on yield in this trial. In the resistant variety, Decanter RLS levels were more variable but the Hot water treatment 2 did produce a significant increase in yield, although reductions in other foliar diseases were only modest (data not shown). In the variety Optic no significant differences in RLS AUDPC or yield were observed. In general, RLS levels were high in this trial as the site became waterlogged and symptoms appeared in the crop canopy prior to ear emergence.

Table 1. Risk factors associated with RLS epidemics

Risk Factor	**Winter Barley**	**Spring Barley**
Previous season epidemic		
High disease levels and spore release	+	+
Low disease levels and spore release	–	–
Varietal choice		
Tolerant	Neutral	Neutral
Susceptible	+	+
Cultivation system		
Minimum Tillage	+	+
Ploughed	–	–
Sowing date		
Early (pre spring barley harvest)	+	N/A
Late (post spring barley harvest)	-	N/A
Surface wetness at GS 31		
Prolonged periods of 100% wetness in the crop	+	+
Few periods of crop wetness	-	-
Winter barley epidemics		
High disease levels and spore release	N/A	+
Low disease levels and spore release	N/A	-

Many of the risk factors (Table 1) were common to both crops but symptoms in winter barley do lead to higher levels in neighbouring spring crops.

Table 2. Varietal Resistance ratings for UK spring barley varieties (HGCA, 2013)

Variety	Rating	Variety	Rating	Variety	Rating
Sanette	7.6	Concerto	5.9	Kelim	6.6
Odyssey	5.5	Moonshine	5.1	Natasia	7.0
Chronicle	6.6	NFC-Tipple	6.1	Montoya	6.1
Propino	5.9	Belgravia	6.6	Rhynchostar	3.4
Overture	6.3	Optic	5.2	Garner	4.5
Glassel	5.4	Tesla	6.9	Waggon	6.8
Quench	5.1	Crooner	6.3	Westminster	6.8
Shuffle	6.3	KWS Orphelia	4.7	LSD (P=0.05)	1.7

The highest rating (Table 2) is the new variety Sanette (7.6) and the lowest is Rhynchostar (3.4)

DISCUSSION

The life cycle of the fungal pathogen and the interaction between fungus and host are still being investigated at the physiological and molecular level. The fungus has been shown to

move from infected seed into developing seedlings (Havis *et al.,* 2013). Results from the seed treatment trial confirm previous work that indicated higher control levels could be achieved by foliar sprays rather than seed treatments (Havis *et al.,* 2012b). Fungicidal seed treatments have been shown to more effective against RLS on susceptible varieties (Havis & Oxley, 2008) The negative effect of the 2 hour hot water treatment (Hot water 1) may be due to a poor germination in the seed stocks used in the experiment. In previous trials, this treatment had reduced RLS significantly in cv. Optic (Havis *et al.,* 2012b). Assessments from this trial and others from the same site produced the first report of appearance of symptoms pre-ear emergence in spring barley trials in Scotland. Physical seed treatments may be more effective in years were the disease risk and incidence is low and environmental conditions do not favour the movement and growth of the fungus. RLS symptoms expression is known to be related to stress in the host plant. Waterlogging may be sufficient stress to induce symptoms lower in the canopy in the field. The interaction between the host plant genetics and the fungus are being established (McGrann *et al*., 2013).

Detailed studies with a gfp transformed isolate of Rcc have revealed that the fungus colonises the mesophyll layer and sub-stomatal cavity of the leaf by forming an intricate network of hyphae around the plant cells. (Kaczmarek, unpublished). No penetration of leaf cells has been observed. In controlled experiments, high humidity and the availability of moisture on the leaf surface increased the rate and extent of colonisation. This increased rate of colonisation could be the biological process underpinning the risk warning. Following ear inoculations, the gfp signal was observed in a thick layer of hyphae under the seed coat, outside the aleurone layer. However it was also observed in the pericarp, embryo and endosperm. This confirms the findings of Matušinský *et al*. (2011). When the gfp infected seed was germinated the fungus was seen to colonize developing leaf layers and move into vascular bundles (Kaczmarek, unpublished). These results point strongly towards an endophytic lifestyle of the fungus, particularly during the vegetative growth stage in the crop.

Initial trials on control of RLS with strobilurins indicated optimal control could be achieved by a GS59 spray (Havis *et al*., 2002). However, at that time the active fungicides against RLS could not be sprayed safely onto a crop after ear emergence (Havis *et al.,* 2004) Results from more recent trials in Germany indicate better control by fungicide treatments at applied at GS 55 (Heβ *et al.,* 2009). Advances in fungicide chemistry mean that a wide range of products is now available to control RLS and they have much later safety limits for latest spray timing. For example the SDHI fungicides, bixafen (Siltra X Pro®), xemium (Adexar®) and isopyrazam (Bontima®) can be applied up to full ear emergence (GS59). Trials investigating the efficacy of delayed spraying in the UK are ongoing. The high level of Fusarium Head Blight in cereal crops in 2012 also raises the importance of timing of T2 sprays in spring barley. SRUC trials showed a post-flowering spray to be ineffective in 2012 (Burnett *et al*., unpublished). The production of official resistance ratings for RLS for UK spring barley varieties offers growers the chance to lower the risk of yield loss due to RLS by non-chemical means. Although fully resistant varieties are not yet available the introduction of official ratings will help raise the profile of RLS to barley breeders.

The generation of defined risk factors pertaining to growing barley and the effect of RLS offers the potential of a more detailed and comprehensive algorithm, including factor weighting, being established to assist growers in their crop management in future.

ACKNOWLEDGEMENTS

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FUNGICIDE RESISTANCE IN *RAMULARIA COLLO-CYGNI*

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Summary: *Ramularia collo-cygni* is a dynamic plant pathogen which has the ability to evolve quickly when placed under a strong selection pressure. This paper will outline the past, current and future resistance issues in barley crops infected with *R. collo-cygni* by highlighting the potential resistance mechanisms and their effect on future risks to disease control. This research also highlights the importance of independent testing of fungicide performance against new and emerging pathogens which might not be the primary focus of the Agro-chemical industry. However, declines in efficacy that have been observed in *R. collo-cygni* show that this relatively new, up and coming disease can adapt to the limited treatments currently available, although the evidence obtained to date shows that changes in *R. collo-cygni* populations have undergone selection for a number years following all fungicide treatments and these are only just starting to be realised.

INTRODUCTION

Ramularia collo-cygni is a pathogen in barley of increasing important, first described in northern Italy (Cavara, 1893) as *Ophiocladium hordei* observed in *Hordeum*. Although *R. collo-cygni* has been known for over 100 years, its economical importance has increased in the last couple of decades, when the disease started to be widely reported in Europe and in other continents world-wide (Sachs, 2006)

Fungicide use is the main strategy to control the pathogen within the crop as major cultivar resistance has not yet been deployed successfully in many of the current commercial cultivars. Therefore, the selection of suitable fungicides is required for the successful control of this increasingly important disease.

The development of fungicide resistance in pathogens is directly influenced by the biology and variability of the pathogen as well as the target site and mode of action of the fungicide (Brent & Hollomon 2007). Fungicides, by their very nature, can be highly specific chemicals that target fungal pathogens. This specificity can often result in rapid pathogen evolution. The target site of the fungicide is one of the most important aspects driving pathogen evolution, as fungicides that have a single target site and often tend to evolve fungicide resistance quickly e.g. in the case of methyl benzimidazole carbamates (MBC) and (QoI) fungicides. This phenomenon is termed 'qualitative' resistance and can extend to all compounds with the same mode of action. This resistance can not be readily reversed, even if treatments are withdrawn. However, other fungicide groups such as the sterol demethylation inhibiting (DMI) fungicides tend to develop 'quantitative' resistance, which builds up over time giving gradual shifts in the fungal population that can decline when fungicide is withdrawn. The occurrence of either type

of fungicide resistance can have a significant impact on sustainable food production (Brent & Hollomon 2007).

A REVIEW OF FUNGICIDE RESISTANCE IN *RAMULARIA COLLO-CYGNI*

The name *Ramularia collo-cygni* was introduced by Sutton & Waller (1988) after the S-shape swan neck of its conidiophores (*collum*- neck, *cygnus*- swan). The present taxonomy of the fungus was confirmed by molecular studies conducted by Crous *et al.* (2000, 2009). It currently belongs to *Mycosphaerellacea* family and genus *Ramularia.* The taxonomy of pathogen is important and can help define the level of risk for potential fungicide resistance development when related to other closely related pathogens.

During the late 1990's Ramularia Leaf Spot (RLS) disease was was initially controlled by QoI fungicides, however during 2002 there was a marked decline in activity to QoI fungicides in comparison with previous years (Oxley & Hunter 2005). This was due to the development of QoI resistance among populations of *R. collo-cygnii* in the UK that developed between 2001 and 2002 (Fountaine & Fraaije 2009). A single amino acid substitution, changing glycine into alanine at the position 143 in cytochrome *b* gene, was responsible for this situation. This change was only detected using samples from the Rothamsted Hoosfield archive and is a very valuable source of material to study the evolution of fungicide resistance over time. This archive was also able to show that the pathogen had exisited in low levels in barley plants during the 19th century. However, the levels of *R. collo-cygni* dramatically increased from 1996 (Fountaine & Fraaije 2009) (Fig. 1).

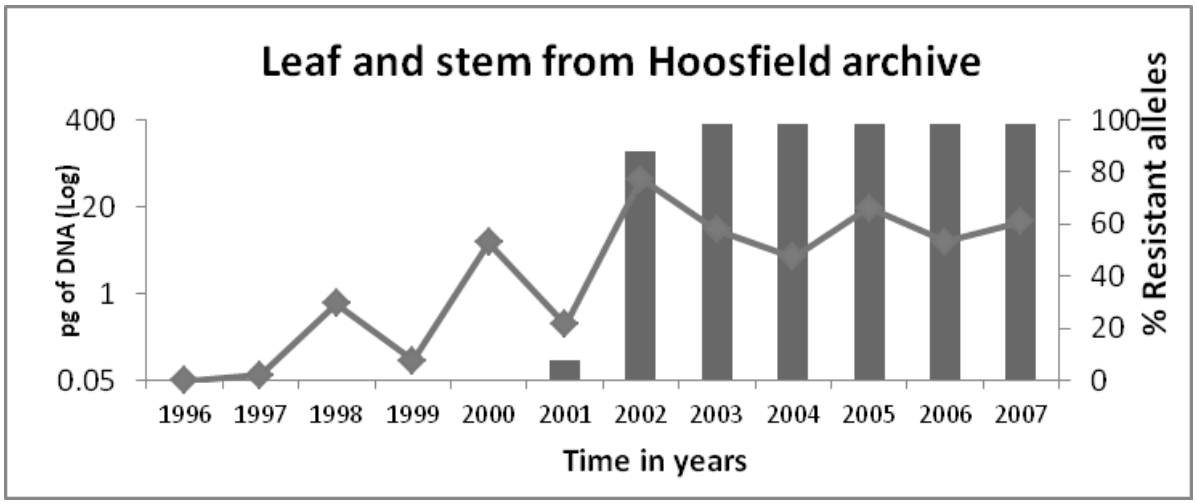

Figure 1. *Ramularia collo-cygni* DNA levels and percentage of A143 alleles present in archived samples using allele-specific real-time PCR.

The rate of QoI fungicide resistance development was also similar to that observered in the genetically related wheat pathogen *Zymoseptoria tritici* (formally known as *Mycosphaerella graminicola).* Since this work was carried out, SRUC has been actively working to understand the potential and molecular mechanisms of fungicide efficacy decline in all major fungicide groups that have been used in barley disease control with a particular focus on *R. collo-cygni.*

One such fungicide group is that of the methyl benzimidazole carbamates (MBC) that have never been used to control RLS at least directly. However, it appears that *R. collo-cygni* has developed resistance in the majority of European populations follow indirect exposure, which has lead to full resistance to compounds such as Carbendazim. As a direct result this has also caused carbendazim resistant populations to become sensitive to another MBC fungicide Zoxamide (Fig. 2) highlighting a negative cross-resistance mechanisms. Moreover, a small percentage of isolates of *R. collo-cygni* where found to be resistant to both compounds.

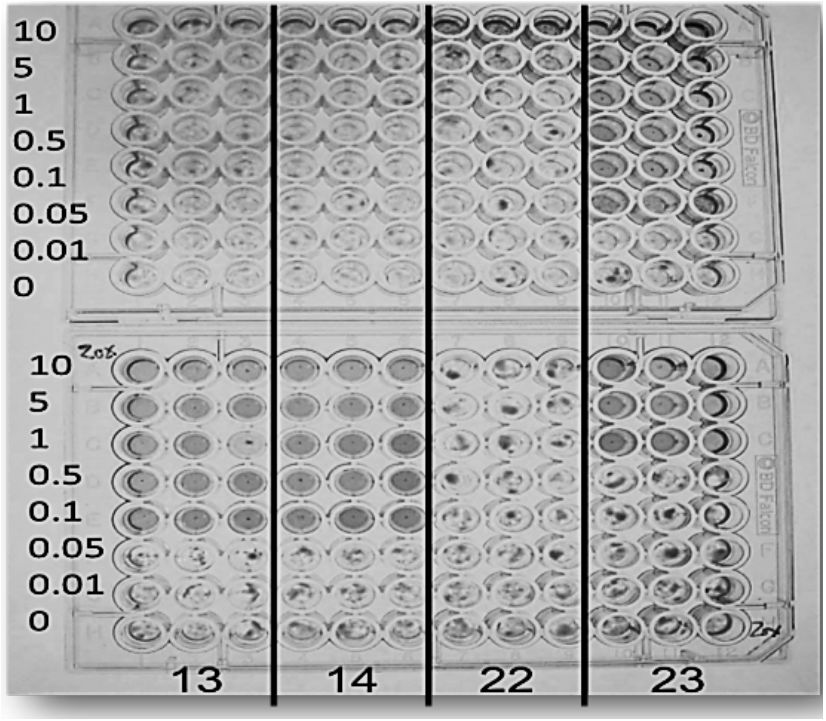

Figure 2. Four isolates of *R. collo-cygni* tested against carbendazim (top plate) and zoxmide (lower plate). Isolate 13 and 14 contain a single mutation at codon 198 and 22 a single mutation at codon 200 in the beta-tubulin gene. Whilst, isolate 23 has the wild type gene with no mutations found in the whole beta-tubulin gene.

The gene encoding the target site protein was sequenced and confirmed the occurrence of two mutations at either codon 198 or 200 in the beta-tubulin gene conferring resistance to MBC fungicides.

As a result of the rapid evolution of fungicide resistance it is currently recomended that different modes of action are used in the control of RLS. This ensures the best control of the disease and minimises the risk of a fungicide resistance development. Sterol demethylation inhibiting (DMI) fungicides or succinate dehydrogenase inhibitors (SDHI) or a mixture of these as a main fungicide and chlorothalonil as an additional mixture partner is recommended to control RLS (HGCA, 2013).

However, the development of fungicide resistance is now putting pressure on the remaining used fungicides for RLS control. The DMI fungicides have been shown to be declining in efficacy in the related pathogen *Z. tritici* due to the development of specific mutation in the CYP51 gene encoding the target site protein following the work by Fraaije et al., (2007).

Ramularia is now seeing some initial changes in efficacy, when tested in the laboratory. The data obtained is showing that EC_{50} values obtained from older isolates are very different different when compared to more recent isolates for a range of DMI fungicides (Table 1). These declines in efficacy can also be linked to the occurrence of specific mutations that have developed in the CYP51 gene of *R. collo-cygni* with a particular focus on the areas around codons 136 and 465 to 467 that are highly variable in *Z. tritici.*

Table 1. The range of EC_{50} calculated using older isolates and more recent isolates of *R. collo-cygni* tested against five different DMI fungicides that have been widely used in barley disease control in the past or in more recent years. Statistical analysis could not be carried out due to the unbalanced nature of the data.

Fungicide	Older Isolates (worldwide) 2002-2005 (7 isolates) Range of EC_{50} values	Newer Isolates Scotland (Bush Estate) 2012 (20 isolates) Range of EC_{50} values
Tebuconazole	0.075 – 0.345	0.760 – 2.671
Metconazole	0.0374 – 0.0692	0.012 – 1.190
Epoxiconazole	0.0094 – 0.057	0.044 – 1.05
Prothioconazole	0.0092 – 0.0097	0.007 – 0.057
Flusilazole	0.0009 – 0.0467	0.103 – 2.164

At present we have been unable to find a *R. collo-cygni* isolates without mutations suggesting that selections has been underway for some time.

Another group of fungicides that is very active in the control of *R. collo-cygni* is the SDHI's and new fungicides from this group have been recently introduced (Havis *et al.,* 2012). However, many pathogens world-wide have developed resistance to these compounds within a few years of there introduction. Currently, the UK has a fully sensitive population of *R. collo-cygni*. However, the risk of resistance development in *R. collo-cygni* is quite high, due in part to the high population diversity (Piotrowska *et al.,* 2013) and also the history of rapid fungicide resistance development. As part of this risk assessment SRUC has developed laboratory mutants that are resistant to SDHI fungicides following UV mutagenesis. The rationale behind this is to predict what mutation or mutations might develop in field isolates of Ramularia and how these specific mutations might affect the active SDHI's used in RLS control. A range of specific mutations have been characterised and the impact of these have been compared in different SDHI bioassays demonstrating that not all mutations affect all SDHI's in the same way (Table 2). However, as these results are only obtained from laboratory mutants alone, we are currently unable to say whether these mutations in the target site gene may occur in the field.

Table 2. Indicates the EC_{50} range for a standard collection of UK isolates of *R. collo-cygni* for each SDHI fungicide. The table also lists the EC_{50} values for the mutant parental strain DK05 for each SDHI active ingredient and the resistance factors following mutagenesis.

EC_{50} values of parental strain and UK population (ppm)					
	IZM	BIX	BOS	FLU	CBX
DK05	0.04	0.04	0.09	0.08	1.49
UK pop. range	0.0004-0.06	0.00001-0.06	0.006-0.48	0.02-0.55	0.11-3.98
RFs (EC_{50} of mutant/ EC_{50} of parental strain)					
DK05	1	1	1	1	1
Mut1	9.24	2.25	9.00	49.90	1.48
Mut2	31.55	24.51	1.53	1.18	2.75
Mut7	44.10	55.30	1113.98	16.77	32.80
Mut8	0.69	10.69	1113.98	15.91	19.19
Mut11	6.76	12.65	37.28	21.82	13.22
Highest EC_{50} from UK pop.	1.27	1.56	5.29	7.32	2.67

DISCUSSION

Fungicide resistance is likely to continue to develop in *R. collo-cygni* as long as the selection pressure, due to fungicide use, continues to be applied to the population. Therefore, integrated management systems to include seed treatments coupled with a risk forecasting to tailor fungicide applications, mixing of active ingredients and alternative modes of action are required to be implemented in RLS control in order to slow down the loss of active ingredients. The most effective methods currently adopted are i) the application of the appropriate dose at the correct timing ii) mixing different chemical with different modes of action in combination and iii) the use of resistant cultivars. However, in the current absence of full varietal resistance, the continued maintenance of effective fungicides is of critical importance. It is also extremely important that independent fungicide resistance testing is maintained in order to give important impartial advice on the performance of these products. The testing of some pathogens such as *R. collo-cygni* can be difficult and the sample sizes too low or the precise mode of action unknown before a new product is launched, allowing the possible risk of resistance developing without appropriate risk reduction strategies. The decline in DMI fungicides is still a cause for concern, as these are one of the most important chemical classes for RLS control such as prothioconazole. However, recently introduced members of the SDHI fungicide group are also potentially at a high risk of resistance development due to the target gene and its high level of activity. As a result, SRUC has an active research interest in this area and has developed a number of rapid screening tools in *R. collo-cygni* to detect for any changes in the resistant status of UK populations which range from bioassays through to high throughput molecular screening assays.

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THE OCCURRENCE AND POPULATION DYNAMICS OF *RHIZOCTONIA SOLANI* IN SOIL OF WINTER WHEAT

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Summary: There is limited information on the occurrence of *Rhizoctonia solani* in UK wheat fields. Ninety wheat fields were surveyed over two years with soil samples collected at pre-sowing and GS 21-31. DNA concentrations of *R. solani* from soil were quantified using Real-time PCR assays. The predominant anastomosis group (AG) of *R. solani* was AG 2-1 found pre-sowing and at GS 21-31 in 69% and 59% of fields respectively for both years. The North and West Midlands had significantly higher concentrations of AG 2-1 DNA in soil than the South West. Previous crop had a significant effect on concentration of AG 2-1 DNA in soil at GS 21-31 irrespective of sampling year or region. In soil where oilseed rape was the previous crop, AG 2-1 DNA was significantly higher than in soils where continuous wheat was grown. This is the first survey to show that the predominant AG of *R. solani* in English wheat fields is AG 2-1.

INTRODUCTION

The genus *Rhizoctonia* comprises a ubiquitous group of soil inhabiting fungi causing a range of destructive diseases on many of crop species. The most studied *Rhizoctonia* pathogen is *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*). *Rhizoctonia solani* causes damping-off, root rot and stem rot in many crops, including wheat (Ogoshi *et al.*, 1990).

Isolates of *R. solani* are classified into anastomosis groups (AGs) based on the ability of hyphae to fuse and exchange genetic material (Ogoshi, 1987). To date 13 AGs (1-13) belonging to *R. solani* have been identified with several of these containing subgroups (Carling *et al.*, 2002).

The host range of the various AGs of *R. solani* is considerable and contains many economically important crops including potatoes, sugar beet, oilseed rape and cereals (Ogoshi 1987; Schillinger & Paulitz, 2006; Babiker *et al.*, 2013). This wide host range makes rotation ineffective in controlling *R. solani*. Furthermore, in the absence of a host *R. solani* can survive for long periods of time on soil organic matter (Ogoshi, 1987).

Rhizoctonia solani AGs 2-1, 3, 4, 5, 8 and 11 have been reported as a pathogen of wheat in Australia Turkey and the USA (Ogoshi *et al.*, 1990; MacNish & Neate, 1996; Demirci, 1998). A recent European wide soil survey of arable fields (n = 282) using a soil baiting method, revealed that in the UK (n = 32) the predominant AG of *R. solani* was AG 9 (Goll *et al.*, 2013). In addition AGs 2-1, 3 (TB), 5, 8, and 11 were isolated from UK soils (Goll *et al.*, 2013).

However, samples were taken prior to planting, only in one season (2009/10) and at one time point from uncultivated fields.

Rhizoctonia solani on wheat in the UK has received little attention and is rarely considered by growers and/or agronomists to cause significant disease on the crop. The aim of this study was to determine the incidence and population dynamics of *R. solani* in English wheat fields using molecular methods in relation to agronomy practices (e.g. cultivation, previous crop).

MATERIALS AND METHODS

Soil and plant sampling

Soil samples were collected from 90 randomly selected winter wheat fields in England during 2011/12 and 2012/13 (Figure 1). In each field 36 points were sampled in a gridline design from the same one hectare area of the field throughout the survey. Soil cores were removed from the top 10-15cm. Soil samples were taken post cultivation, but within two weeks of sowing (pre-sowing), and at growth stage (GS) 21-31 of the wheat crop (Zadoks *et al.*, 1974). Agronomy information was recorded including previous crop, soil texture, cultivation and sowing date.

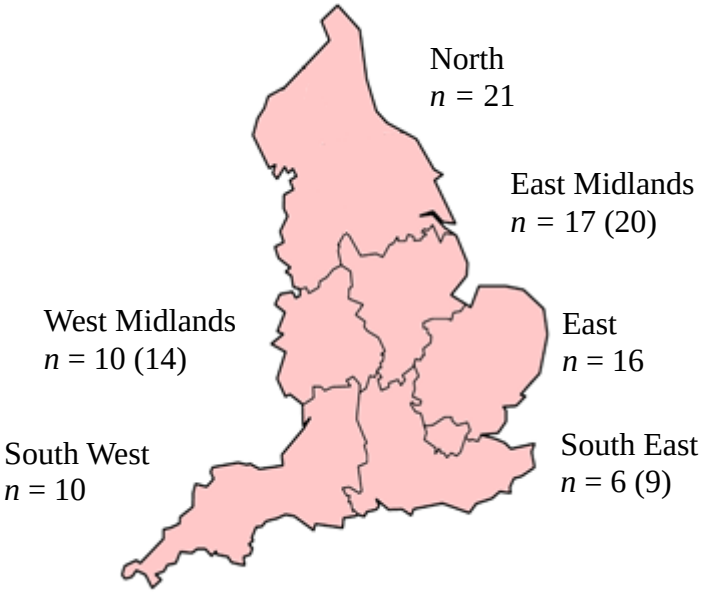

Figure 1. Regional distribution of fields surveyed in England. Pre-sowing *n* = 80 fields and GS 21-31 *n* = 90 fields. Numbers in parentheses are fields sampled at GS-21-31.

Soil DNA extraction

Soil DNA was extracted using the method developed by Woodhall *et al.* (2012). DNA was purified using the Wizard® Magnetic DNA Purification System (Promega UK Ltd) and eluted using a Kingfisher® ML magnetic particle processor (Thermo Electron Corporation).

Quantitative PCR assays

Pathogen DNA was quantified using Real-time PCR (TaqMan®) assays, performed using the Applied Biosystems 7500 real-time PCR system. Targeted *R. solani* AGs were 2-1, 3 (PT), 4 (I, II, II), 5, 8, and 9. Primer and probe sequences are described in Budge *et al*. (2009).

Statistical analysis

All data was analysed using Genstat® 15th edition (VSN International Ltd., UK). DNA data was transformed using log 10 transformation to normalise residuals before analysis using ANOVA unbalanced design.

RESULTS

Incidence of *Rhizoctonia solani* in soil pre-sowing and at GS 21-31 of winter wheat

Rhizoctonia solani AG 2-1 was found pre-sowing and at GS 21-31 in 69% and 59% of fields respectively for both years of sampling (Table 1). There were no significant differences in concentrations of AG 2-1 DNA between years or sampling periods (Table 1). Anastomosis groups 5 and 8 occurred in only 5% of fields sampled at pre-sowing in 2011/12 season (data not shown). AG 3 (PT), 4 and 9 were not detected in soil.

Table 1. Incidence and quantification of AG 2-1 DNA at pre-sowing and GS 21-31. DNA is log10 transformed.

	Pre-sowing*		GS 21-31**		Mean (Year)	
	Incidence (%)	DNA pg g^{-1} of soil	Incidence (%)	DNA pg g^{-1} of soil	Incidence (%)	DNA pg g^{-1} of soil
2011/12	69	0.12 (1.31)	56	-0.05 (0.90)	62	0.03 (1.08)
2012/13	68	-0.08 (0.83)	63	-0.10 (0.8)	66	-0.09 (0.8)
Mean (GS)	69	0.03 (1.07)	59	-0.07 (0.85)		
SED				***P*-value**		
Year	0.390			0.751		
GS	0.388			0.815		
Year.GS	0.552			0.85		

Back-transformed means are in parenthesis
Pre-sowing * 2011/12 42 fields, 2012/13 38 fields
GS21-31 **2011/12 52 fields, 2012/13 38 fields

Effect of agronomy on *R. solani* AG 2-1 DNA in soil pre-sowing and at GS 21-31 of winter wheat

There were no significant interactions between agronomy factors (i.e. previous crop, soil texture, cultivation and sowing date) and year of sampling on concentrations of AG 2-1 DNA

in soil quantified at pre-sowing or at GS 21-31 of wheat. Region and previous crop were the only agronomy factors significantly affecting the DNA of AG 2-1 in soil (Figures 2 and 3).

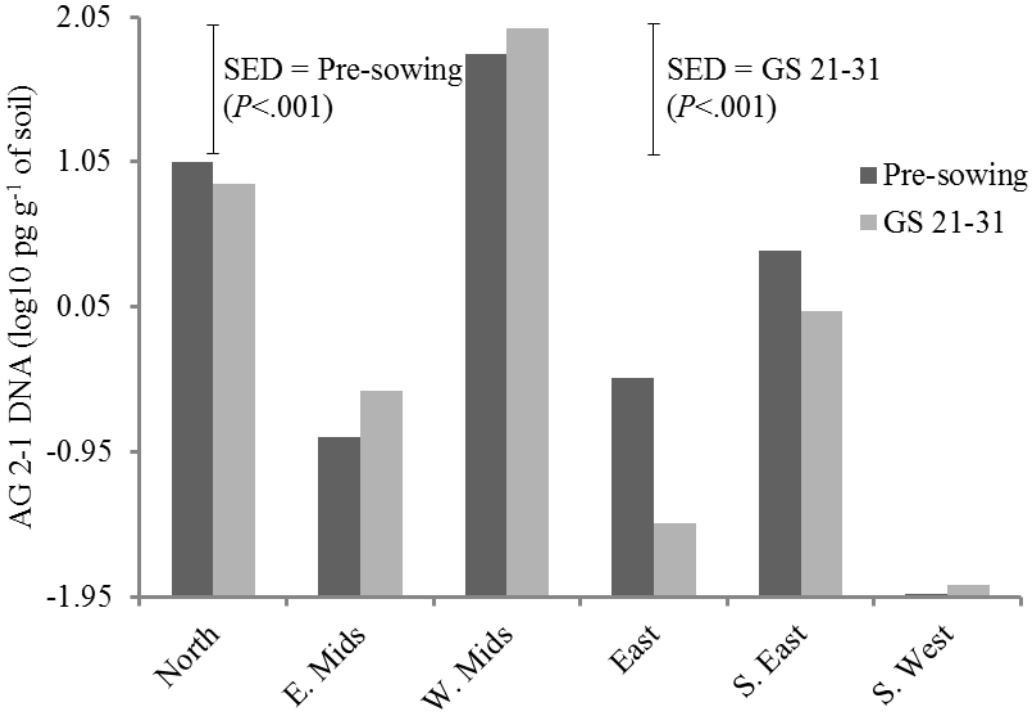

Figure 2. Effect of region on *R. solani* AG 2-1 DNA concentrations at pre-sowing and GS21-31. DNA is log10 transformed.

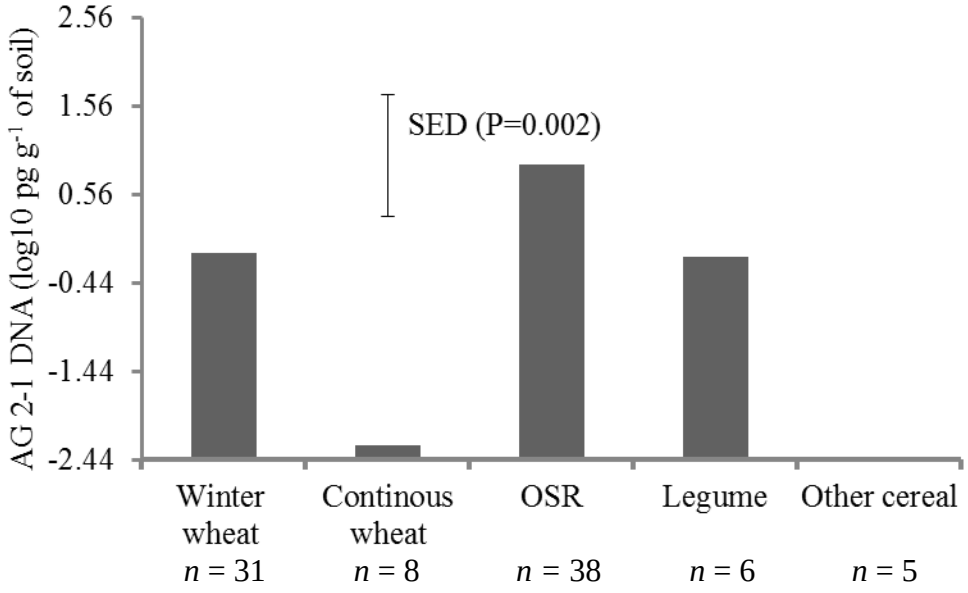

Figure 3. Effect of previous crop on *R. solani* AG 2-1 DNA concentrations at GS21-31. DNA is log10 transformed.

Soils in the West Midlands and the North had significantly higher concentrations of AG 2-1 DNA than soils in the South West region at pre-sowing (P<.001) and at GS 21-31 (P<.001) (Figure 2). Concentrations of AG 2-1 DNA in soil were low on the East side of England, with the South West having the lowest concentrations at both sampling periods.

Previous crop had a significant (P=0.005) effect on concentrations of AG 2-1 in soil at GS 21-31 but not at pre-sowing. When OSR was the previous crop concentrations of AG 2-1 DNA were more than 1000-fold higher than when previous crop was continuous wheat or other cereals (maize and oats) (Figure 3).

DISCUSSION

This survey of English wheat fields showed that the predominant AG of *R. solani* was AG 2-1 found in 69% and 59% of fields at pre-sowing and at GS 21-31 respectively. This is in contrast with the survey carried out by Goll *et al.* (2013), which reported AG 2-1 occurrence in only 13% of locations while the predominant AG was identified as AG 9 occurring in 34% of locations. The difference in results is possibly due to different methods employed to detect *R. solani.* We used AG specific quantitative PCR assays whilst Goll *et al.* (2013) isolated *R. solani* using a soil baiting method which is non-target specific. The low incidence of AG 2-1 occurring in Goll *et al.* (2013) survey suggests soil baiting is not an effective method to detect and isolate AG 2-1. Furthermore, we failed to detect AG 9 in wheat growing fields at pre-sowing (2011/12) and it is possible that differences in geographical locations and/or cropping history of the sampled fields in the individual surveys may be a factor.

Rhizoctonia solani AGs 5 and 8 were detected in only 5% of fields. Both AG 5 and 8 are known pathogens of wheat (MacNish & Neate, 1996; Demirci, 1998; Woodhall *et al.*, 2012). However, their low incidence in soil suggests that the distribution of the two pathogens in England may be restricted to individual fields.

The only two agronomy factors affecting AG 2-1 DNA levels in soil were region and previous crop. The lack of significant interactions between region, previous crop and year of sampling indicates that the effect of these factors is consistent across different seasons and the effect of previous crop is also similar across regions. Thus, there was a general trend of decline of AG2-1 DNA from the North in a South Eastern direction. It is likely that climatic conditions associated with each region and/or frequency of cropping may help to explain the observed differences.

At GS 21-31 when the previous crop was OSR, concentrations of AG 2-1 DNA were significantly higher than when the previous crop was continuous wheat or other cereals (maize and oats). AG 2-1 is known to be an aggressive pathogen of OSR seedlings causing pre- and post-germination damping-off diseases (Babiker *et al.,* 2013). It is likely that OSR plays an important role in selecting for AG 2-1 in intensive OSR rotations. The increase of pathogen DNA following OSR may be due to volunteer plants or crop residue providing a host for the survival of the pathogen.

This survey has found AG 2-1 to be the predominant *R. solani* in wheat fields in England. However, the final effect on wheat yields by this pathogen is unknown. Further data will be

added to this survey to model the risks factors for *R. solani* and additionally for *R. cerealis* in wheat rotations to allow appropriate management strategies to be implemented.

ACKNOWLEDGEMENTS
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UPDATE ON THE EFFECT ON ESTABLISHMENT OF SPRING BARLEY AND OATS BY *MICRODOCHIUM NIVALE* AND *M. MAJUS*

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Summary: Data was obtained from field experiments where spring barley and oats with a range of infection levels of *Microdochium nivale* and/or *M. majus*, were sown on three dates (S1, S2, S3) during March, April and May in 2012 & 2013. No relationship was observed between seed infection and seedling loss at emergence for barley except in sowing S3 in 2013. For oat seed there was a significant correlation for sowing S1 in 2013. All seedling losses in 2012 were very small for both barley and oats. This may have been due to the period of warm soil temperatures and low rainfall directly after sowing. Analysis of *Microdochium* isolates from 2013 seed lots showed an *M. nivale* host preference for oats.

INTRODUCTION

Prior to 2008 in Scotland, spring seed bed conditions were considered less conducive for transfer of *Microdochium nivale* and *Microdochium majus* (*Microdochium*) from infected seed to seedlings, causing *Microdochium* seedling blight in spring cereals. Since then average seed infection levels have increased significantly and there have been suggestions of poor establishment in some spring barley crops due to *Microdochium*.

Cockerell *et al.* (2009), based on limited data sets, suggested that spring wheat and oats are at risk from high levels of *Microdochium* infection, and spring barley is also at risk but at levels exceeding 30% seed infection. This is contrary to field experiments in 2011 by McNeil *et al*. (2012) who found no significant losses on spring barley due to *Microdochium*, despite seed having infection levels as high as 91%. However, some seedling loss was reported for spring oats.

Barley and oat seed lots in previous experiments showed a clear difference in host preference of *Microdochium* species (McNeil *et al*. 2012). Almost all the colonies identified from the oat seed lots were *M. nivale*, whereas an approximate 80 (*M. majus)*:20 (*M. nivale*) split was reported for barley. It is known that *Microdochium* affects seedlings at temperatures as low as 3^{o}C (Haigh *et al*. 2009). In an inoculation experiment at 10^{o}C, Simpson *et al* (2000) found that both *Microdochium* species were pathogenic to wheat and rye but only *M. nivale* caused significant disease in oats, agreeing with the findings of McNeil *et al* (2012). There was no evidence from experiments in 2011 to suggest which *Microdochium* sp. might cause lower seedling establishment in barley.

This paper reports results from a further 2 years of field experiments since the results reported by McNeil *et al*. (2012), to determine what *Microdochium* seed infection level will cause poor emergence in spring barley and oats. The paper also looks again at the host preference of *M. nivale* and *M. majus* on seed lots used in the field experiments.

MATERIALS AND METHODS

Seed lots

Untreated spring barley and oat seed lots with a range of *Microdochium* infection levels were selected from samples submitted to the Official Seed Testing Station (OSTS), SASA for testing. Samples originated from various areas of Scotland. Details of seed lots are given in Table 1.

Table 1. Year, seed treatment, total sample numbers and percentage *M. nivale* and *M. majus* in 2012 & 2013.

Year	*Seed treatment*	*Total sample numbers*	*No of barley samples*	*No of oat samples*	*Barley % infection*	*Oats % infection*
2012	Anchor	15	10	5	4.5 - 91	18 - 65
2013	Redigo	12	7	5	63 - 90	13 - 80

Agar plate and Germination tests

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

Germination tests were conducted in accordance with ISTA Rules 2011, using the rolled-paper towel method or organic growing medium if appropriate.

Field experiments

Ten metre square plots were sown, with a target rate of 400 seeds/m^2 for both barley and oats. A randomised block design was selected for the trial, with 4 blocks sown over 3 dates. Sowing dates were 2 weeks apart. Seed lots were sown both untreated, and treated with recommended seed treatments (Table 1) at the manufacturers recommended rate. Percentage seedling loss was calculated as a percentage of treated seed plant populations.

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

Colonies of *M. nivale* or *M. majus* from 2013 seed lots were identified using PCR following a simple DNA extraction in TrisEDTA buffer. To discriminate between these two species, the primers of Glynn *et al.* (2005)) were used for identification.

RESULTS

Field experiment

The 2012 field experiment showed no significant correlation between seedling loss and seed infection for spring barley or spring oats (Table 2). Over all sowings, the highest seedling loss for spring barley was 35% in a seed lot with 94% seed infection, and for spring oats the 2 highest losses were in seed lots with 64% seed infection (22% loss) and 42% seed infection (31% loss). Both these seed lots were cultivar Firth and laboratory germinations were 96 and 89% respectively.

In 2013, for spring oats, there was a significant increase in seedling loss with increasing seed infection for sowing S1, $R^2 = 0.911$ (Table 2). Seedling losses ranged from 8% – 21% (Figure 1). The largest seedling loss for oats was 26%, observed in sowing S2, on a seed lot with 72% seed infection. Most seed lots showed a low level of loss, with most less than 10% in sowings S2 and S3.

In 2013, no pattern was observed between the three sowing dates for spring barley. A positive correlation (R^2) was found in sowing S3, with seedling loss increasing as seed infection increased ($p<0.01$), Table 2. The greatest losses were observed in S2, a 17% loss with 63.5% seed infection and a 22% loss with 78.5% seed infection (Figure 2). Maximum seedling loss at the highest seed infection 90% was 15%.

Table 2. Correlations (R^2 value) between seed infection levels and seedling loss for 3 sowings (S1, S2, S3) in each of 2 years 2012 and 2013.

Year	Crop	S1		S2		S3	
		R^2	p	R^2	p	R^2	p
2012	Barley	0.462	NS	0.378	NS	0.156	NS
	Oats	0.152	NS	0.012	NS	0.398	NS
2013	Barley	0.131	NS	0.142	NS	0.911	$p<0.01$
	Oats	0.911	$p<0.1$	0.339	NS	0.110	NS

Microdochium species identification

The identification of *Microdochium* species from the colonies isolated from agar plates, showed for the five samples of oats tested, that *M. nivale* was predominant with a small percentage of *M. majus*. For six of the seven barley seed lots tested, there is a greater proportion of *M. majus*. Seed lot 7 has a greater proportion of *M. nivale* (Figure 3).

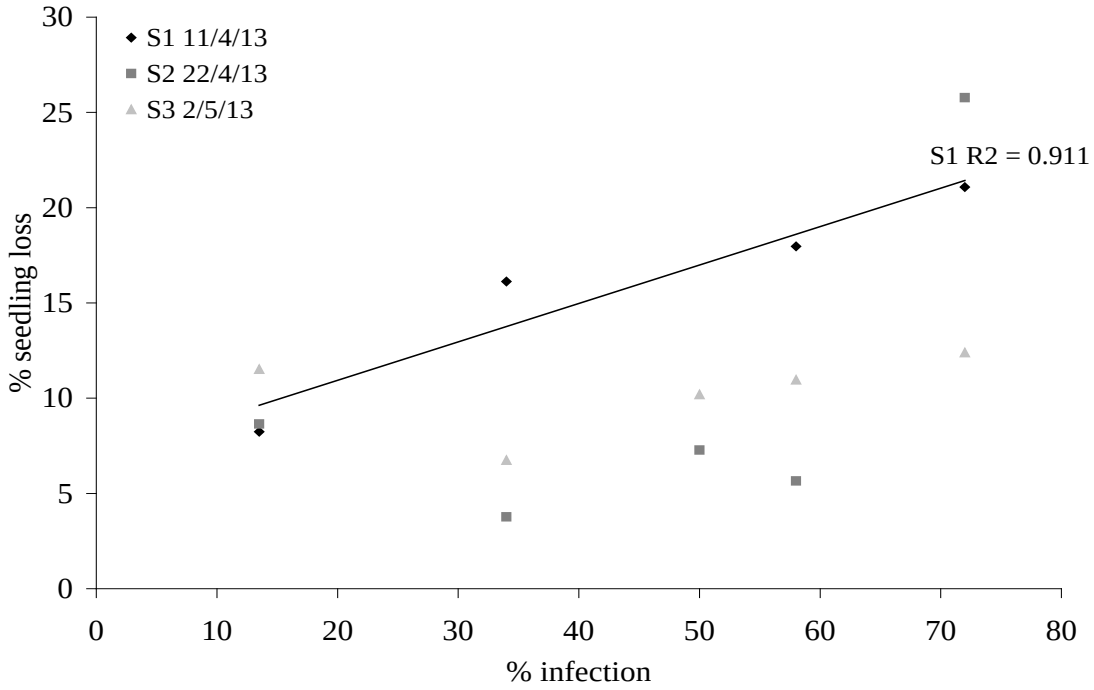

Figure 1. Percentage untreated germination and mean seedling loss against percentage infection for 3 sowings of spring oats sown March to May 2013.

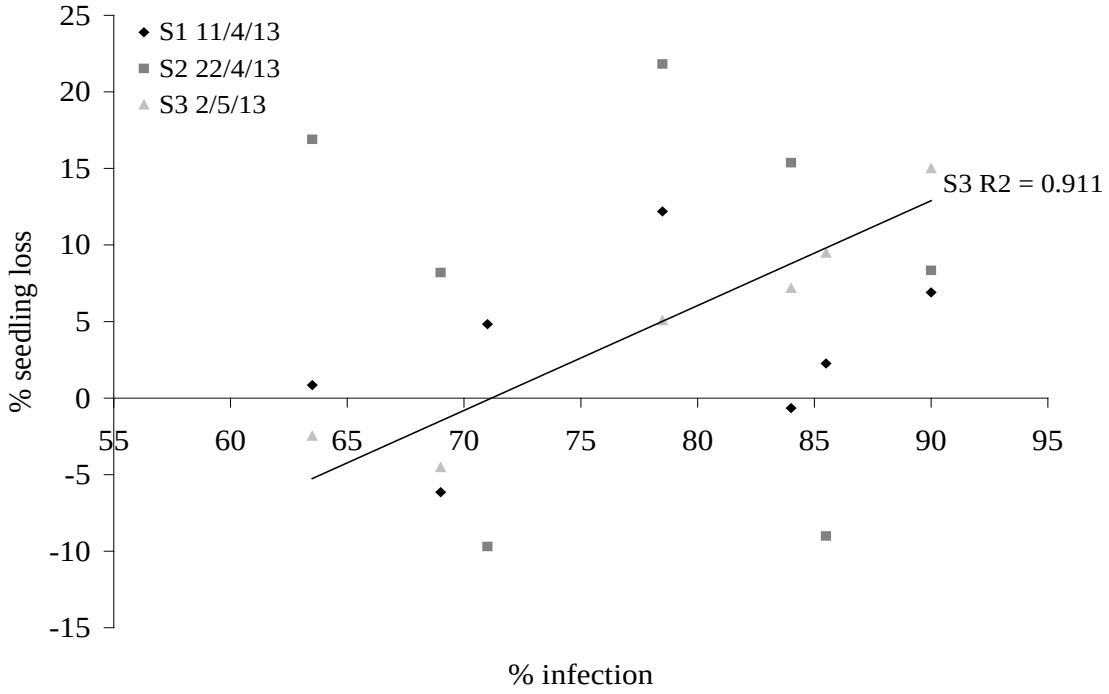

Figure 2. Percentage untreated germination and mean seedling loss against percentage infection for 3 sowings of spring barley sown March to May 2013.

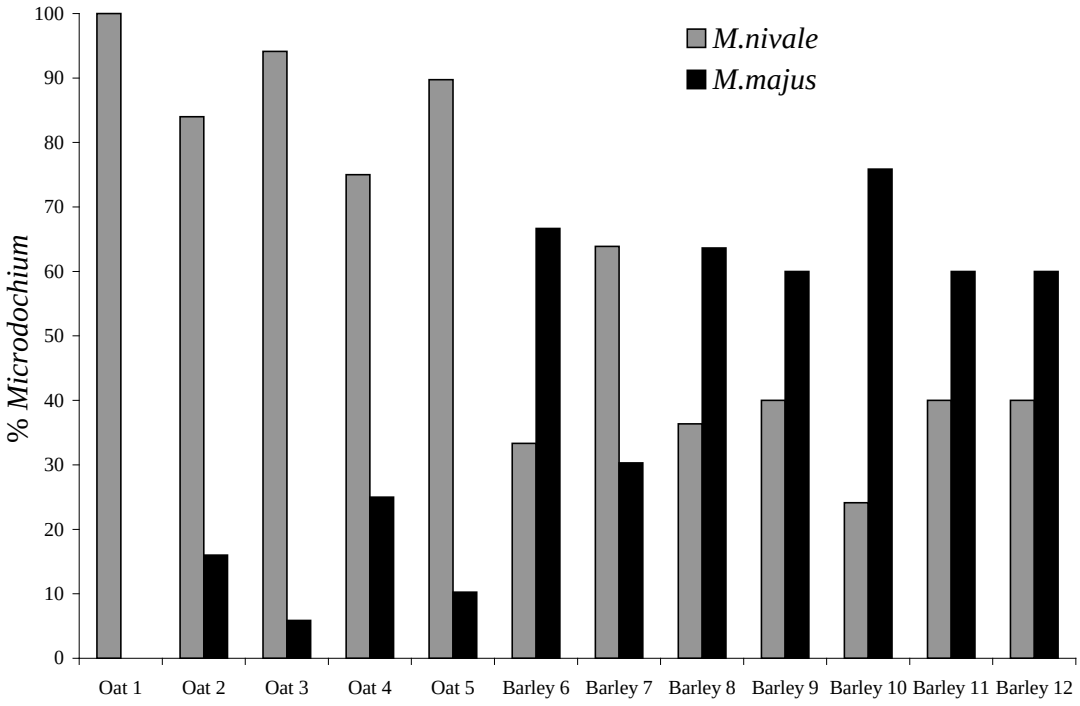

Figure 3. Percentage of each *Microdochium* species present on each seed lot sown in 2013.

DISCUSSION

Over three years of data and nine sowings the highest seedling loss in spring barley was 22% in a sample with 78.5% seed infection. There was some evidence in 2013 that seedling losses increase as seed infection increases, but this was not evident in most sowings despite seed infection levels ranging from 2% to 91% across all experiments.

Seedling losses were generally higher in spring oats, with the highest seedling loss of 31% in a seed lot with 42.5% infection. There was some evidence over the three years that seedling losses increased along with seed infection, with three sowings from nine showing a significant correlation. It is worth noting however that despite poor relationships between seed infection and seedling loss in 2013 for sowings S2 and S3, losses of between 4% and 26% occurred.

The weather in 2012 for 2 weeks after each sowing was dry and warm with a mean rainfall and temperature of 3.7mm and 9.1^{o}C respectively. Analysis of the weather data after each sowing date for both 2012 and 2013 has revealed soil temperatures at 30cm depth of between 7 and 10^{o}C, and relatively low average daily rainfall of 2 to 5mm. The unusually cold spring of 2013 delayed sowing of S1 until well into April and soil temperatures remained below 10^{o}C until into late May. This cold seed bed may have delayed seedling growth, leading to better transmission of *Microdochium* from the seed to seedling in sowing S1 for oats, and decreasing transmission in subsequent sowings as soil temperature rose.
In both 2011 and 2013, *Microdochium nivale* colonies were predominantly isolated from the oat seed and mostly *Microdochium majus* from the barley seed (although more mixed in 2013), showing a clear difference in host preference of each species. Recently Nielsen *et al* (2013)

found on Danish cereal samples that *M. majus* showed a higher prevalence compared to *M. nivale* on all species (including oats) except rye.

In 2013 the seed bed temperature for sowing S1 remained below 10^{o}C for some days after sowing. If Simpson *et al*'s (2000) findings that *M. nivale* was more pathogenic at this temperature than *M. majus* are correct then this could explain the loss seen in sowing S1 for oats but not for barley. However, this is not true for all seed lots as more *M. nivale* isolates than *M. majus* were detected on 'Barley 7'. Even though the overall *Microdochium* seed infection level was 69%, this seed lot consistently gave negative seedling losses.

In conclusion, for spring barley there is a risk of seedling losses, with highest losses seen above 30%. Three years of field experiments have shown no clear relationship between seed infection and seedling loss.

For spring oats there is a higher risk of seedling loss particularly when seed bed temperatures are low. Under these conditions there is a good relationship between seed infection and seedling loss with an average of 0.6% loss for every 1% *Microdochium* seed infection (based on 2011 data). *M. nivale* is the predominant pathogen and most likely to cause seedling losses in oats.

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THE DEVELOPMENT OF A NOVEL INSECTICIDE SEED TREATMENT FOR USE IN NORTHERN AND CENTRAL EUROPE FOR PROTECTION AGAINST SOIL DWELLING AND FOLIAR PESTS IN CEREAL CROPS

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Summary: SP*16006 466FS is an insecticide seed treatment that delivers 25g clothianidin + 25g imidacloprid when applied to 100 kg of winter cereal seed. Both components are insecticides belonging to the neonicotinoid class but vary in their spectra of activity. The combination exploits these differences in order to deliver effective protection against a number of economically damaging cereal pests in the UK and continental Europe. This paper summarises the pan-European development of the product and demonstrates the benefits it will bring to growing cereal crops in northern and central Europe and discusses the responsibilities for good stewardship.

INTRODUCTION

Neonicotinoid insecticide seed treatments for winter sown cereal crops first became available in the last years of the 20th century with imidacloprid applied at 35 g a.i. per 100 kg seed in the UK (Raxil Secur® and Sibutol Secur®) to give protection against the aphid vectors of barley yellows dwarf virus (BYDV). At about the same time, imidacloprid 70 g a.i. per 100 kg seed was launched under the more challenging pest conditions in France for protection against aphids / BYDV and wireworm damage (Gaucho Orge® and Gaucho Blé®). In 2006, clothianidin based seed treatments (Redigo Deter® and Raxil Deter®) were approved in the UK at a clothianidin rate of 50g a.i. per 100 kg seed for protection against wider range of pests particularly aphids vectors of BYDV and crops establishment pests wireworm and slugs. Following commercial usage it was observed that these seed treatments also gave reductions in damage caused by more sporadic insect pests such as gout fly, frit fly and cereal flea beetle. During and subsequent to the development of these seed treatments, it became apparent that whilst both imidacloprid and clothianidin shared many attributes, they did demonstrate a number of subtle differences in their spectra of activity. It was decided therefore to develop a formulation coded SP*16006 that contained both active substances (25g clothianidin + 25g imidacloprid per 100 kg) that could be suitable for use as a seed treatment to give protection against a wider range of pests of winter sown cereal crops in many countries across northern and central Europe.

MATERIALS AND METHODS

Small plot replicated trials were conducted to EPPO Guidelines primarily in the UK, France and the Czech Republic with additional trials in Germany, Poland, Slovakia and Italy. In total 150 trials were successfully performed in Europe. All trials were conducted by or on contract to Bayer CropScience using officially approved trials teams and organisations. Experimental plot sizes ranged from 18 to 36 m² (with some early stage trials having smaller plot sizes and

later stage trials having much larger plot sizes) and treatments were replicated 4 times in randomised block designs.

The experimental treatment coded SP*16006 was applied to deliver 25g imidacloprid + 25g clothianidin per 100kg seed and was compared with treatments of imidacloprid and or clothianidin as approved in the countries where the trials were conducted. Seed treatments were applied in Monheim, Germany using a commercial scale seed treatment applicator and in Cambridge, UK using a laboratory scale applicator. Treated seed was analysed for chemical loading and effects on germination. Field efficacy and crop safety evaluations were performed according to EPPO Guidelines. A minimum of 5 x 1m of row per plot was counted to determine plant establishment and aphids were assessed by counting their numbers on 25 – 100 plants per plot. BYDV infection was assessed by determining the area infected per plot of chlorotic / necrotic plants visually or by using 0.5 m² quadrats when leaf symptoms were obvious in the spring but before GS50. Wireworm and slug damage was assessed in terms of the reduction in crop establishment by counting plant numbers having first established the cause of plant loss. Most assessments were statistically analysed with angular transformations being used where appropriate. A number of the efficacy and crop safety trials were harvested using plot combine harvesters and the resulting grain yields were corrected to 86% d.m. No artificial infestation of pests took place and efficacy trials were place in areas where pests were known to be present or likely to develop. Conversely, crop safety trials were sited in areas where pests were less likely to infest the trials.

RESULTS

Due to the numbers of trials performed in Europe, only a representative number can be reported in this paper for each pest and indication. All results in the same columns are from the same trials.

Pests affecting crop establishment

Wireworm (*Agriotes* spp.):
Wireworm is a pest that is present throughout Europe but is particularly severe in cereal crops in northern France. The results in Table 1 demonstrate that as time progresses after crop emergence, the crop damage continues in unprotected plots and crop density reduces. Both seed treatments give effective protection reflected in increases in relative crop stand. Similar results were recorded in winter barley trials.

Table 1. Crop establishment (plants / m row) from 4 wireworm infested trials in France, winter wheat (harvest year 2011), relative to untreated.

Treatment	Rate g a.i./ 100 kg	Days after sowing no. trials:	14-18 4	31-41 4	84-185 4
Untreated			100 *(22.1)*	100 *(18.0)*	100 *(15.1)*
SP*16006	50		117.8	124.6	156.9
imidacloprid	70		119.4	125.2	140.9

In one of the four trials, the imidacloprid 70g rate was significantly more effective than SP*16006, but there were no significant differences in the remaining three trials.

Slugs (mainly *Deroceras reticulatum* and *Arion* spp.):
These pests are ubiquitous in northern Europe causing post emergence leaf shredding damage but in the UK they can also cause significant reductions in crop establishment due to pre-crop emergence grain hollowing. Crop establishment data is shown in Table 2.

Table 2. Crop establishment (plants / m row) from 14 slug infested trials in the UK, winter wheat (harvest years 2010-13), relative to untreated.

Treatment	Rate g a.i./ 100 kg	Final crop stand no. trials:	Mean 14	Min 14	Max 14
Untreated			100 *(38.8)*	*(24 -*	*67)*
SP*16006	50		115.3	102.8	156.5
clothianidin	50		110.0	91.5	143.2

Whilst there were rarely any significant differences in crop stand between either of the seed treatments (SP*16006 was significantly greater than clothianidin in one trial), there was a consistent trend that indicated that SP*16006 resulted in greater crop establishment than clothianidin (50g a.i./100kg seeds). The protection afforded by the seed treatments in reducing subterranean grain hollowing, did not extend to reducing damage caused by slugs grazing on emerged crops.

Cereal Ground Beetle:
The main species of Zabrus that causes economic damage (in the larval phase) to cereal crops is *Z. tenebrioides* . This is a pest that is present at low levels in many countries across Europe and can become locally important. However, in the Mediterranean coastal areas it has become a major pest having a significant effect on crop establishment and yield. In the UK, damage caused by Zabrus was once confined to areas of ‘chalky’ soils but is now found in many counties of southern England. Crop establishment data is shown in Table 3.

Table 3. Crop establishment from 7 Zabrus infested trials in southern France, winter wheat (harvest years 2010-11), relative to untreated.

Treatment	Rate g a.i./ 100 kg	Days after sowing no. trials:	9-30 7	21-48 7	37-92 7
Untreated			100 *(22.8)*	*(21.8)*	*(17.2)*
SP*16006	50		128.4	160.3	184.4
imidacloprid	70		129.0	161.8	181.4

In one out of seven trials the imidacloprid 70g treatment gave significantly higher crop stand than SP*16006 but there were no significant differences in the remaining six trials. Six of these trials were harvested and SP*16006 increased yield by 48.6% and imidacloprid increased yield by 47.2% (relative to untreated which gave a mean yield of 4.2 t/ha) but there were no significant differences in yield between the seed treatments.

Foliar pests

Cereal aphid vectors of BYDV, primarily *Rhopalosiphum padi*:
With the exception of slugs, pests which attack the foliage of cereals in the autumn and winter following sowing generally do not cause significant physical damage to the crops. They do, however, often carry viral diseases that can seriously reduce the yield potential of the crops. Aphid species which are vectors of BYDV are the most widespread of these foliar pests and trials were conducted throughout north, western and central Europe. The % reduction in infected plants is shown in tables 4 and 5.

Table 4. Reduction in % plants with aphid infestation in north and west Europe (UK, France Germany) and central Europe (Czech Republic) on winter barley (harvest years 2008 to 2012).

Treatment	Rate g a.i./100 kg no. trials:	North Europe 9	West	Central Europe 4
Untreated		*(43.5% infected plants)*		*(0.3 aphids/leaf)*
SP*16006	50	98.5 (range 92-100)		93.9 (range 88-100)
clothianidin	50			93.8 (range 87-100)
imidacloprid	70	98.2 (range 94-100)		

Table 5. Reduction in% plants with aphid infestation in north and west Europe (France) and central Europe (Czech Republic) on winter wheat (harvest years 2008 to 2011).

Treatment	Rate g a.i./100 kg no. trials:	North Europe 10	West	Central Europe 5
Untreated		*(15.4% infected plants)*		*(0.2 aphids/leaf)*
SP*16006	50	95.7 (range 85-100)		87.8 (range 73-98)
clothianidin	50			90.3 (range 80-98)
imidacloprid	70	94.8 (range 84-100)		

There were no significant differences in aphid control between any of the seed treatments in the winter barley and winter wheat trials.

BYDV and yield

BYDV infections caused substantial reductions in yield potential in both wheat and barley as demonstrated in the Tables 6 & 7 below.

There were no significant differences between SP*16006 and the commercial comparison seed treatments in terms of BYDV control and yield responses in both winter wheat and winter barley.

Table 6. Reduction in BYDV % infected area per plot in north and west Europe (UK, France, Germany) and % relative yield on winter barley (harvest years 2008 to 2011).

Treatment	Rate g a.i./100 kg no. trials:	BYDV % red. 8	Yield % rel. 6	BYDV % red. 6	Yield % rel. 6
Untreated		*(49.6% infected)*	*(5.6 t/ha)*	*(84.3% infected)*	*(2.7 t/ha)*
SP*16006	50	92.9 (83-100)	171.5 (100-372)	92.2 (83-100)	185.8 (115-372)
clothianidin	50	95.6 (87-100)	170.0 (94-378)		
imidacloprid	70			88.6 (67-100)	182.5 (94-378)

Table 7. % Reduction in BYDV infected area per plot in north and west Europe (UK, France, Germany) and % relative yield on winter wheat (harvest years 2008 to 2011).

Treatment	Rate g a.i./100 kg no. trials:	BYDV % red. 8	Yield % rel. 5	BYDV % red. 9	Yield % rel. 7
Untreated		*(27.1% infected)*	*(5.6 t/ha)*	*(25.6% infected)*	*(6.1 t/ha)*
SP*16006	50	81.8 (43-100)	121.9 (98-153)	90.0 (70-100)	123.6 (98-170)
clothianidin	50	83.7 (50-100)	122.1 (97-162)		
imidacloprid	70			86.9 (72-100)	123.2 (95-169)

Crop Safety

A number of trials (8 w. barley and 9 w. wheat) were conducted in UK, France and Germany in situations of low to no apparent pest attack in which SP*16006 was tested at the proposed commercial rate of 50g a.i./100kg seed (1N) and at 70g a.i./100kg seed (1.4N) to evaluate the effect on crop stand and yield. Results are given below in Table 8.

DISCUSSION

From the moment cereal seeds are planted, they become potential food sources for a range of subterranean insects with further insects attacking the emerge seedlings. Cultural control measures can play an important role in reducing the impact of pest attack such as the date of drilling, the nature of soil cultivations, varietal choice and increasing environmental stewardship to encourage natural predation on insect pests. These measures alone, however, are not sufficiently reliable to allow the crops reach their yield potential and the use of targeted insecticide seed treatments such as imidacloprid and clothianidin not only give very effective pest control but have less of an environmental impact on non-target organisms and can be used close to waterways unlike many foliar insecticide sprays.

Table 8. Relative final crop stand (as a % of plants per m^2 at GS11-13) and % relative yield on winter barley and winter barley in the absence of significant pest pressure (harvest years 2010 to 2011).

		winter barley		winter wheat	
Treatment	Rate g a.i./100 kg no. trials:	stand % rel. 8	Yield % rel. 4	stand % rel. 9	Yield % rel. 2
Untreated		*(208.5 / m^2)*	*(6.5 t/ha)*	*(189.8 / m^2)*	*(7.1 t/ha)*
SP*16006	50	98.2 (92-105)	-	104.5 (99-115)	101.9 (101-103)
SP*16006	70	97.1 (92-102)	104.1 (101-110)	104.8 (98-120)	102.4 (101-104)
imidacloprid	70	100.9 (94-109)	105.1 (98-117)	105.0 (94-124)	102.6 (101-104)

The integrated use of seed treatments has made them an important management tool on many farms (Adam & Hopkinson, 2006) and the increased use of such seed treatments on commercial farms in the UK demonstrates the value placed in these treatments by professional growers. With currently available treatments, yield losses in cereal crops are usually very low (Clarke *et al*, 2009).

The combination of imidacloprid with clothianidin (both at reduced rates compared to the individual commercial treatments) gave similar efficacy to the single a.i. seed treatments against a range of important insect pests in the UK and continental Europe (and in the case of slug protection, greater efficacy). The use of insecticide seed treatments brings with it responsibilities to all involved in the production chain to reduce potential environmental contamination. Chemical manufactures are improving formulations and providing coatings to reduce release of dust and seed suppliers need to ensure that they clean the seed stocks before accurately applying the chemicals. Farmers need to employ good farm stewardship by ensuring accurate calibration of seed drills and careful drilling. Any excess dust needs to be contained in the seed bags and spilt seed must be collected up or buried.

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SADDLE GALL MIDGE (*Haplodiplosis marginata*) IN WINTER WHEAT

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Summary: Recent outbreaks of saddle gall midge (*Haplodiplosis marginata*) in winter wheat crops raise many questions about potential yield losses and available control measures. A survey conducted with farmers and agronomists early in 2012 suggests the frequency of outbreaks is increasing and significant populations can be found across many counties, including in Northern Britain. It is not uncommon for saddle gall midge populations to be present in sufficient numbers to cause significant yield losses, but no available research pinpoints a practical treatment threshold beyond which damage is likely to be economically important. Currently, no insecticides are approved in the UK for the control of saddle gall midge, but recent research does prove the effectiveness of appropriately-timed treatment with Dursban WG insecticide (chlorpyrifos). Further research needs to be conducted to establish the environmental conditions that promote adult emergence, the criteria for triggering an epidemic, and the population threshold leading to economic losses.

INTRODUCTION

Saddle gall midge (*Haplodiplosis marginata*) is an insect pest that was until recently thought to be only a sporadic problem in wheat. Known to cause isolated, serious damage to wheat yields in the UK in the 1960's and 1970's, it seemed to have disappeared until recently. During the 2010 and 2011 wheat growing seasons there have been numerous reports of saddle gall midge appearing in localized epidemics and causing yield losses to varying degrees.

The ecology of saddle gall midge is such that it meets many of the criteria to become a successful economic pest in the UK. This paper outlines historical and recent infestations, the biology and life cycle of the pest, results of a recent survey of pest incidence and damage levels, and trials results assessing possible future control methods.

Historically, saddle gall midge was a known problem to UK agriculture during the 1960's and 1970's. After a period of disappearing from notably causing serious injury to wheat it has once again begun to appear in economically damaging numbers. No specific link is documented between this resurgence and a specific set of criteria, although speculation about closer cereal rotations has been suggested as a possible cause. The wheat most severely affected in 2010 and 2011 were typically grown as continuous wheat crops. However, historical work in Europe by Basedow (1986) showed that despite a field growing wheat continuously for 11 years, saddle gall midge outbreaks causing economic damage only occurred twice during the assessment period. This result suggests that more or different factors are responsible for the routine development of damaging population levels of saddle gall midge.

One theory for saddle gall midge only causing damage to crops sporadically, irrespective of an optimal supply of host plants (such as continuous wheat plantings), is that their emergence as adults needs to coincide with the growth period of the crop that is most susceptible to feeding, i.e. stem elongation. If the midge larvae appear after this, it is thought that the stem has hardened sufficiently to limit the ability of the larvae to feed and survive through to overwintering.

BIOLOGY AND LIFE CYCLE

Saddle gall midge, most widely accepted as *Haplodiplosis marginata* (von Roser), but also known as *Haplodiplosis equestris* (Wagner), belongs to a large family of flies (Order: *Diptera*) collectively referred to as 'gall midges' (Family: *Cecidomyiidae)* (Encyclopaedia of Life, 2010). Saddle gall midge have only one generation per year (HYPP Zoology, 1997), which begins when mature larvae, which are about 5 mm long and orange-red to red in colour, break diapause and emerge from soil burrows. The trigger to break diapause and emerge onto the soil surface is not fully understood, but theories include when the upper layers of soil reach a temperature of around 18°C (HYPP Zoology, 1997) and a certain (as yet undefined) day length is achieved (Skuhravy *et al.*, 1983). Larvae can appear as early as late April (Dewar, 2012) but are not known to attack plants at this time. The larvae then pupate on the soil surface; a process that lasts about 7 days.
After emerging, adults mate and females fly to a suitable host to lay their eggs. Saddle gall midge has a range of cereal and grass hosts; primarily couch grass (*Elytrigia repens*), wheat, barley and rarely oats (Skuhravy *et al.*, 1983; Dewar, 2012). Eggs are usually laid in a line or grid shape on the upper surface of the leaf, but often on the underside of the leaves as well.

The larvae emerge within 1-2 weeks after eggs are laid, then move down the leaf and take refuge underneath the leaf sheath. It is here they begin feeding on the stem. These young larvae are initially a whitish-green, turning orangish-red as they grow (Dewar, 2012). The feeding on the stem, while not easily apparent on the outer surface of the leaf sheath, causes saddle shaped depressions that are swollen at either end, giving the pest its colloquial name. After the larvae have achieved full size they fall to the ground to overwinter in small burrows made in the soil, ready to begin the cycle again. Skuhravy *et al.* (1983) states between 5% and 20% of the larvae may remain in diapause for another year, and Dewar (2012) added that in some years as many as 75% may not break diapause.

The damage caused to the stem by the larvae feeding can vary in severity. More severe attacks may cause the occurrence of 'whiteheads', which could be confused with other conditions, such as those caused by cereal diseases. In severe cases the feeding can weaken the stem to a point where it can no longer support the grain ear and the stem will snap, potentially resulting in a total loss of the grain from that stem. The most vulnerable growth stage for the wheat plant is during stem elongation (Zadoks GS 31-39), where the stem is still soft and actively growing.

RESULTS

New information about saddle gall midge is available from two recent areas of research. Early in 2012 a consortium of interested parties worked together to conduct a survey on the current

status of saddle gall midge infestations in UK wheat crops. The consortium members were ADAS, AICC, Dow AgroSciences Ltd, HGCA and NIAB TAG. In total, 140 surveys were completed by farmers and agronomists across the UK. All surveys were analysed and a summary of the findings released in May 2012 (FarmingUK website, 2012).

The other research conducted during 2012 was a completely randomized and replicated field trial, at two separate locations, to assess the levels of control possible, and the reduction in plant damage achieved, from different application timings of chlorpyrifos as a 75% w/w WG. The application rates and treatment timings were chosen to reflect the currently approved label for use in winter wheat, i.e. 1.0 kg/ha before flag leaf sheath extending and 0.6 kg/ha after flag leaf sheath extending but before flowering. Applications were made using LERAP '3 star' low-drift nozzles in accordance with the 'Chlorpyrifos: Say No to Drift' campaign, with extended 20 metre buffer zones. All trials work was conducted by Dewar Crop Protection at two sites in Suffolk.

Survey Results

Completed saddle gall midge surveys were received from farmers and agronomists covering wheat crops grown in many counties of England, plus East Lothian and Aberdeenshire in Scotland. Over half of the survey respondents reported evidence of saddle gall midge infestations in their wheat crops, with 70% of these finding larvae in the soil and/or eggs laid on crop leaves. Nearly half of respondents noted whiteheads in their wheat crops, which could not be attributed to fungal disease, with 42% finding the characteristic galls on wheat stems as a result of larval feeding. Of those farmers and agronomists who had observed signs of saddle gall midge infestation, 52% recorded yield losses at harvest. Survey responses are shown in Figure 1.

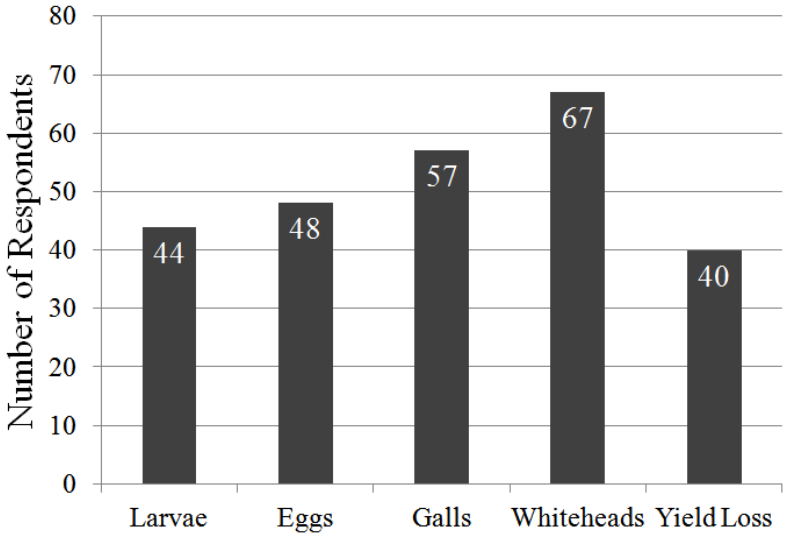

Figure 1. Symptoms of saddle gall midge infestations observed in wheat crops.

The majority of cases of yield loss, 48% of those experiencing it, were suffered in continuous wheat situations. However, 24% of saddle gall midge infestations leading to loss in yield were seen in first wheats following a non-cereal break crop, signifying the pest is not exclusively a problem of continuous cereals.

Trials Results

The efficacy of chlorpyrifos in reducing saddle gall midge larval numbers, and subsequent stem galls, was assessed by application of two different product rates at single or repeated treatment timings. The treatment list is shown in Table 1.

Table 1. Treatment list for saddle gall midge efficacy trials.

Application Code	Treatment	Rate	Application Timing
	Untreated		
A	Chlorpyrifos	1.0 kg/ha (750 g a.i./ha)	Flag leaf visible (Zadoks GS 37)
B	Chlorpyrifos	0.6 kg/ha (450 g a.i./ha)	Flag leaf sheath extending (Zadoks GS 41)
A	Chlorpyrifos	1.0 kg/ha (750 g a.i./ha)	Flag leaf visible (Zadoks GS 37)
	followed by		
B	Chlorpyrifos	0.6 kg/ha (450 g a.i./ha)	Flag leaf sheath extending (Zadoks GS 41)

Trials were assessed on the 2nd July 2012 (Chedburgh) and 4th July 2012 (Stradishall) for the presence of saddle gall midge larvae and stem galls. Results are shown in Figures 2 and 3.

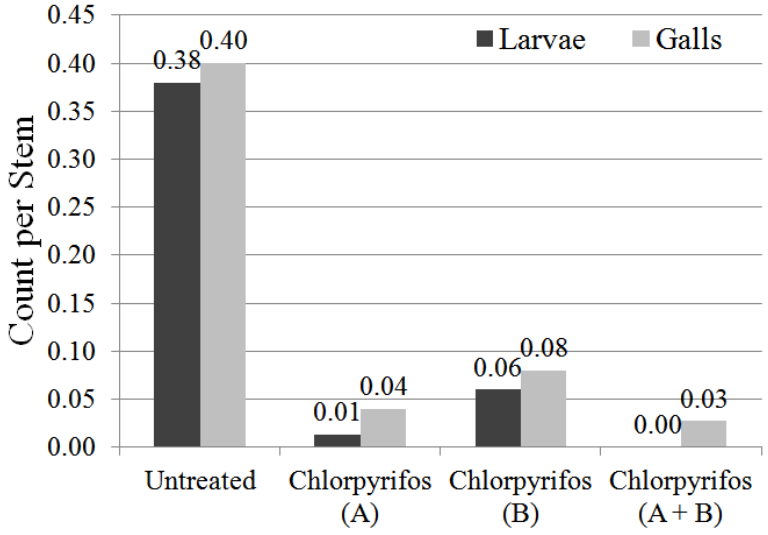

Figure 2. Chlorpyrifos effect on saddle gall midge larvae and stem galls (Chedburgh).

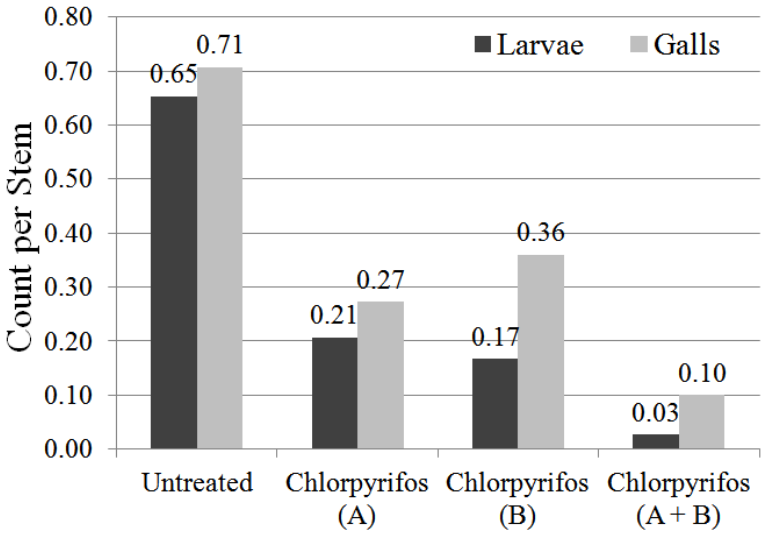

Figure 3. Chlorpyrifos effect on saddle gall midge larvae and stem galls (Stradishall).

The data show a single application of chlorpyrifos, applied either at 1.0 kg/ha to wheat at the flag leaf visible growth stage (Zadoks GS 37) or 0.6 kg/ha at flag leaf sheath extending (Zadoks GS 41), achieved a significant reduction in the numbers of larvae per stem at both trial sites when compared with the untreated control (p=0.05). Count data also showed these treatments caused a numerical decrease in the number of galls per stem. However, at the Stradishall site this was not a statistically significant reduction (p=0.05). A sequenced application of chlorpyrifos, 1.0 kg/ha at GS 37 followed by 0.6 kg/ha at GS 41, resulted in very high levels of control of larvae and subsequent galls. This was the most effective treatment at both sites, but not significantly better than a single application of chlorpyrifos at GS 37 (p=0.05).

CONCLUSIONS

Although traditionally considered a sporadic pest, recent in-field observations and responses to the survey indicate the potential for severe damage to be caused by saddle gall midge infestations. There is a trend for this damage to be more prevalent where continuous wheat predominates in arable rotations, but the occurrences noted in first wheat crops after a break indicate they can also be at risk.

While the physiological life cycle of the pest is well-documented, there remains a lack of understanding about the conditions required to trigger an epidemic, as well as the environmental factors that promote adult emergence. Also, the population threshold that triggers economic damage is yet to be determined.

Research work confirms treatment with chlorpyrifos at 1.0 kg/ha is an effective means of control when applied to wheat crops at the flag leaf visible growth stage (Zadoks GS 37). However, there is currently no UK approval for any insecticide for the control of saddle gall midge (CRD, 2012). Further research work needs to be conducted into yield effects, treatment

thresholds and approved, effective control measures. Until then, rotation into a non-host crop, e.g. oilseed rape, potatoes, remains the best approach in areas with high historical infestations.

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ALTERNATIVE INSECTICIDES TO CONTROL CEREAL APHIDS, *SITOBION AVENAE*, THAT ARE RESISTANT TO PYRETHROIDS

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Summary: Cereal aphids, *Sitobion avenae*, have developed resistance to pyrethroids in the last few years, culminating in control failure at some locations in England 2011 and 2012, associated in the latter year with local epidemics of barley yellow dwarf virus (BYDV). Studies by Rothamsted Research and Syngenta have shown this to be due to target site resistance, which can be detected in populations by using a vial test, and in individual aphids using a DNA diagnostic test. Field trials in autumn 2012 using inoculations of a clone of *S. avenae* that was resistant to pyrethroids showed that cypermethrin at 25 g a.i./ha and deltamethrin at 7.5 g a.i./ha gave significantly poorer control than chlorpyrifos at 450 g a.i./ha, but not lambda-cyhalothrin at 7.5 g a.i./ha. In two trials to test alternative products, the carbamate pirimicarb at 120 g a.i./ha gave moderate control at one site, but good control at another; with neonicotinoids, thiacloprid gave good control at both sites, while acetamiprid at 50g a.i./ha was relatively poor. Pymetrozine at 100 g a.i./ha plus adjuvant oil gave good control at the one site it was tested. Chlorpyrifos gave best control. None of these alternatives are currently approved for use against aphids in cereals in the autumn, but these results will give regulators some evidence for their activity against resistant grain aphids, should alternatives be required.

INTRODUCTION

Control of the grain aphid, *Sitobion avenae* has relied heavily upon the use of pyrethroids in recent years, largely because they are cheap, and readily mixable with fungicides in disease control programmes. As a consequence, the majority of cereals are treated every year in the autumn to control aphids carrying barley yellow dwarf virus, often as an insurance, and sometimes twice a year when cereals are also treated with pyrethroids in the summer to control grain aphids. The most recent survey done on pesticide use in the UK showed that 71% of winter barley and 76% of wheat was treated by one of five pyrethroids in 2012, although no distinction was made between summer and autumn use (Table 1; Garthwaite *et al*., 2013), and this was largely unchanged since the previous survey in 2010 (Garthwaite *et al*., 2011).

Table 1. Proportion of cereals (% of census area) treated with pyrethroids in the UK in 2012 mostly to control aphids (Garthwaite *et al*., 2013).

Insecticide AI	Winter barley	Spring barley	Wheat
Cypermethrin	31	4	33
Lambda-cyhalothrin	19	6	24
Esvenvalerate	10	3	8
Alpha-cypermethrin	6		5
Zeta-cypermethrin	5	2	6
Total	**71**	**15**	**76**

Not surprisingly, this usage, whether or not it is needed, has resulted in selection of resistant aphids. Their occurrence was first described in 2012 (Foster *et al*., 2013), but have since been recorded in many sites across the UK following a survey funded by Syngenta, backed up by further tests using a DNA-based diagnostic assay developed at Rothamsted (Williamson & Foster, In Preparation). In 2012, of 17 samples collected from East Anglia, and tested using a vial test, which requires live aphids, 35% of the samples collected were classified as resistant, after less than 60% of the individuals in vial tests died at a discriminatory dose of insecticide (Table 2). In 2013, 38% of sixteen samples tested in vials were classed as resistant; of these and a further 14 that were collected that year, but tested only using the DNA assay, 50% contained some individuals that carried the *kdr* mutation conferring resistance to pyrethroids. These results correlate well with similar testing of dead *S avenae* caught in Rothamsted Insect Survey suction traps operating in the same areas, namely the Broom's Barn, Rothamsted and Kirton traps in east and central England (Williamson & Foster, In Preparation).

Table 2. Resistance status of *S. avenae* collected from cereals in the UK in 2012 and 2013.

Year	Test method			
	Insecticide-coated vials		DNA-based assay	
	No of samples	% resistant (mortality <60%)	No of samples	% resistant (>50% heterozygotes)
2012	17	35	20	80*
2013	16	38	30	50

*in 17 of these samples tests were done on survivors of the vial test, hence the higher percentage

Thus, resistance has now become a feature of cereal aphid control, but perhaps has not been seen on a catastrophic scale yet because summer epidemics of aphids are relatively rare these days compared to the 1970's, due to the activities of predators. However, there is some circumstantial evidence that the mini-epidemic of BYDV seen in England in the spring of 2012, following a mild winter that would have allowed overwintering aphids to survive quite well, may have been exacerbated by resistant *S. avenae* that survived the traditional autumn

sprays of pyrethroids used to control another aphid species, the bird-cherry aphid, *Rhopalosiphum padi,* which is regarded as the main vector of BYDV.

In response to this situation, experiments were set up to investigate the efficacy of three commonly used pyrethroids against resistant *S avenae* in winter sown cereals, and to test alternative insecticides that might be useful for controlling them if they become a serious problem in the future.

MATERIALS AND METHODS

Comparison of aphid clones

Two clones of *S. avenae,* one susceptible to insecticides (SS, homozygous susceptible), and one containing a single copy of the kdr gene (i.e. it was heterozygous for the *kdr* mutation-SR), were inoculated into plots of winter barley, cv Cassia not treated with an insecticidal seed treatment, sown on 21 September 2012 at Brooms Barn Research Centre in Suffolk. These were compared to a clone of susceptible *R. padi.* Each 2 x 6 m plot was inoculated with each of the three clones in a randomised position down the centres of the plot. Inoculation was achieved by placing a tiller of barley infested with 50-100 aphids along a short length of row in which at least 8 plants were present. As the tiller dried up, aphids moved from these tillers onto the young seedlings. Treatments were applied on 5 November using a Trials Equipment 'Lunchbox' sprayer with a 2 m offset boom sprayer delivering 200 L/ha through Tjet flat fan nozzles.

Alternatives to pyrethroids

In two larger trials only the resistant clone of *S. avenae* was used for inoculation. Trials were carried out at two sites, one in the same field at Broom's Barn as the clone trial, and the other at Stetchworth Estates, Cambridgeshire (sown on 29 September), using the same winter barley seed. In these trials there were 6 inoculation points in each plot, 3m from either end and one in the centre, 1 m in from each side – i.e. two rows of three inoculation points. Insecticides were applied as before, but two passes were made with the sprayer, one up each side of the plot, treating half the plot with each pass. Plots at Broom's Barn were infested on 17 October at GS 13 and sprayed on 20 October; plots at Stetchworth were infested on 19 October at GS12 and sprayed on 23 October.

Treatments

In the clone trial, three pyrethroids, lambda-cyhalothrin (Hallmark Zeon from Syngenta), cypermethrin (Toppel 10 from United Phosphorus Ltd) and deltamethrin (Decis Protech from Bayer) were compared with the organophosphorus product, chlorpyrifos (Dursban WG from Dow Agrosciences). In the efficacy trials comparing alternatives to pyrethroids, seven treatments including an untreated control were tested at Broom's Barn, but an additional treatment was included at Stetchworth that had not been available when the trial at Broom's Barn was sprayed. Treatments included lambda-cyhalothrin and cypermethrin, the carbamate pirimicarb (Aphox from Syngenta), chlorpyrifos, two neonicotinoids, thiacloprid (Biscaya from Bayer) and acetamiprid (InSyst from Certis), and, at Stetchworth only, pymetrozine (Plenum from Syngenta). Rates of application are shown in Table 4.

Assessments

In all three trials, aphids were counted on 8 plants at each inoculation point, on three occasions 3, 6-8 and 13-15 days after sprays were applied. Only selected but representative data are presented here.

Data analyses

Data were analysed by analysis of variance using Genstat XV. Aphid numbers were transformed (log N+1) prior to analysis.

RESULTS

Comparison of aphid clones

All treatments reduced aphid numbers in each clone 3 days after spraying, but this was surprisingly not significant. However, 8 days after application, all treatments gave significant reductions compared to untreated (Table 3). In the susceptible clone of *S. avenae* control was over 85% three days after sprays were applied, rising to over 95% 8 days after application, with no significant differences between treatments. Similar results were observed with the susceptible clone of *R padi*, although deltamethrin gave slightly poorer control at the first assessment. However, with resistant *S. avenae*, , control by cypermethrin was significantly poorer than lambda-cyhalothrin and chlorpyrifos eight days after application; the performance of deltamethrin was intermediate between these treatments.

Efficacy of alternative treatments

In the other two trials where only the resistant *S avenae* clone was used for inoculation, best control after 6-8 days at Broom's Barn and Stetchworth was achieved with chlorpyrifos (99%), followed by lambda-cyhalothrin (85 and 94% resp.) and pymetrozine at the latter site (92%). Pirimicarb and thiacloprid gave moderate control at Broom's Barn (73-74%), but good control at Stetchworth (97 and 99% resp.) (Table 4). Poor control was given by acetamiprid (62%) at Broom's Barn, a little better at Stetchworth (82%), but poorest control was given by cypermethrin (47 and 66%) at Broom's Barn and Stetchworth respectively.

DISCUSSION

Indications from the clone trial showed that, for two pyrethroids at least, control of resistant *S. avenae* was significantly poorer than for the susceptible clones of both *S.avenae* and *R. padi*. The fact that lambda-cyhalothrin did not show the same effect as cypermethrin and deltamethrin might be due to the relatively high rate used – 7.5 g a.i./ha is the rate recommended for aphid control in winter oilseed rape, while 5 g a.i./ha is the rate suggested for wheat.

Of the alternatives tested as potential replacements for pyrethroids, chlorpyrifos was

consistently the best, considerably better than thiacloprid. Pirimicarb worked well at one site but not so well at the other; acetamiprid was poor at both. Pymetrozine gave relatively poor control 3 days after spraying at Stetchworth, but eventually gave complete control there, suggesting that it is a slow starter, but comes good later.

Although chlorpyrifos and thiacloprid are approved for use in cereals, neither is currently approved for use against aphids in the autumn. This is also the case for acetamiprid and pymetrozine. These results will therefore give regulators some useful information about the comparative efficacy of these alternative compounds for potential future use.

Table 3. Effect of pyrethroids on the number of aphids per plant of susceptible and resistant *S. avenae*, and susceptible *R. padi*.on winter barley in 2012.

Clone	rate a.i./ha	3 DAA Log (n+1)	Back-trans	% control	8 DAA Log (n+1)	Back-trans	% control
Susceptible *S avenae*							
Untreated	-	0.650	3.47		0.487 a	2.07	
L-cyhalothrin	7.5	0.132	0.4	90	0.030 b	0.1	97
Cypermethrin	25	0.182	0.5	85	0.021 b	0.1	98
Deltamethrin	7.5	0.150	0.4	88	0.039 b	0.1	96
Chlorpyrifos	450	0.067	0.2	95	0.042 b	0.1	95
Resistant *S avenae*							
Untreated	-	0.595	2.94		0.484 a	2.05	
L-cyhalothrin	7.5	0.199	0.6	80	0.032 c	0.1	96
Cypermethrin	25	0.303	1.0	66	0.206 b	0.6	70
Deltamethrin	7.5	0.306	1.0	65	0.131 bc	0.4	83
Chlorpyrifos	450	0.141	0.4	87	0.021 c	0.1	98
Susceptible *R. padi*							
Untreated	-	0.852	6.12		0.782 a	5.05	
L-cyhalothrin	7.5	0.070	0.2	97	0.030 b	0.1	99
Cypermethrin	25	0.222	0.7	89	0.087 b	0.2	96
Deltamethrin	7.5	0.406	1.5	75	0.019 b	0.0	99
Chlorpyrifos	450	0.096	0.2	96	0.051 b	0.1	98
F Probability		0.458			0.018		
Significance		NS			*		
SED (68 d.f.)		0.116			0.081		
LSD (5%)		0.233			0.162		

DAA = days after application;
NS = not significant, * = significantly different at P<0.05
Data followed by different letters are significantly different at P<0.05

Table 4. Efficacy of alternative insecticides against pyrethroid-resistant *S. avenae* 6-8 days after application of sprays in autumn 2012

Treatment	Rate a.i.ha	Broom's Barn Log (N+1)	Back -trans	% control	Stetchworth Log (N+1)	Back-trans	% control
Untreated	-	0.412 a	1.58		0.213 a	0.63	
L-cyhalothrin	7.5	0.093 c	0.24	85	0.015 b	0.04	94
Cypermethrin	25	0.262 b	0.15	48	0.085 b	0.22	66
Pirimicarb	120	0.156 bc	0.43	73	0.008 b	0.02	97
Chlorpyrifos	450	0.004 d	0.01	99	0.002 b	0.01	99
Thiacloprid	72	0.149 bc	0.41	74	0.002 b	0.01	99
Acetamiprid	50	0.202 b	0.59	62	0.037 b	0.09	86
Pymetrozine + oil	100				0.023 b	0.05	92
F Pr.		< 0.001			< 0.001		
Significance		***			***		
SED 21 df		0.063			0.039		
LSD (5%)		0.131			0.815		

*** = significantly different at P< 0.001
Data followed by different letters are significantly different at P<0.05

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INVESTIGATING TOLERANCE OF OILSEED RAPE TO SLUG DAMAGE

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Summary: In the UK, application rates of molluscicide slug pellets continue to increase, however with the future of metaldehyde, the most commonly used active ingredient for slug control, under threat there is a need to consider alternative measures. To understand the ability of crops to tolerate damage caused by slugs, two experiments were done where slug feeding was simulated by manipulating seed rate and leaf pruning treatments in oilseed rape in North Yorkshire. Crop tolerance to both low plant population number and leaf pruning was found to be determined by environmental conditions which regulate compensatory growth. Removal of a single cotyledon or the first true leaf had no significant effect on yield. Therefore, if a super-optimal plant population has been established then there may be scope to tolerate a modest level of slug damage during early growth.

INTRODUCTION

Currently, decisions on whether or not to apply a molluscicide do not take account of the ability of crops to tolerate slug damage. In oilseed rape slug damage can result in the reduction of plant populations due to plants being bitten off at or below soil level. In addition, leaf grazing can reduce green leaf area. The effect of changes in plant population on the yield of winter wheat is well understood (Spink *et al.*, 2000; Spink *et al*., 2004), whereas for oilseed rape the understanding is poor. Limited experimental data has shown that oilseed rape has a high tolerance to low plant numbers.

The effect of leaf grazing on crop yield of oilseed rape is poorly understood and no data in the literature exists. Photosynthesis from the first formed leaves of oilseed rape does not contribute directly to yield because these leaves have senesced by the time the seeds begin to fill. However, it is possible that loss of early leaf function may have an impact on the crop's ability to compensate for low plant numbers through additional branching. To improve decisions about whether crops require slug control it is necessary to provide evidence about the degree of tolerance crops have to damage. This needs to take account of the minimum plant population required to achieve maximum potential yield and how slug damage on early leaves affects yield and whether or not this is influenced by plant population.

MATERIALS AND METHODS

Two experiments were done in winter oilseed rape near Malton in North Yorkshire, one in 2010-2011 and one in 2011-2012. An open pollinated variety, Castille and a hybrid variety,

Excalibur were used. A range of seed rates were used to mimic the loss of plants and leaf pruning treatments were used to mimic the effect of slug grazing. The experimental sites were chosen as they rarely suffer from slug attack. Each experiment was arranged as a split-plot design using seed rate and variety as the main plot factors and leaf pruning treatments as the sub-plot factors with four replicates. Seed rate treatments were 10, 20, 40, 80 and 160 seeds/m^2. These seed rates were chosen to span those currently used in the UK and also to give a sufficiently wide range in order to fit a statistically robust linear plus exponential response curve from which the economic optimum was calculated. Leaf pruning was carried out on a 1.2m by 1.2m quadrat area. Pruning treatments are shown in Table 1.

Table 1. Leaf pruning treatments for oilseed rape.

Treatment number	Leaf pruning
1	No leaf damage
2	Remove one cotyledon
3	Remove 1st leaf as the 2nd leaf is emerging
4	Remove 1st leaf as the 2nd leaf is emerging and 2nd leaf as 3rd leaf is emerging
5	Remove 1st, 2nd, 3rd and 4th leaves as each successive leaf is emerging

Assessments

Plant population
Once five true leaves had emerged, the number of plants in each of the pruned areas and in an unpruned area of each plot were assessed. The number of plants was counted within one 0.5m x 0.5m quadrat placed diagonally to the rows within each pruned area and in an unpruned area.

Quadrat yield
About two weeks before harvest a 1m x 1m area of crop from each of the pruned areas and one unpruned area close to the pruned areas was sampled. Care was taken to sample from the centre of each 1.2m x 1.2m pruned areas. The fresh weight of the whole sample was recorded and a 20% sub-sample was then taken by fresh weight. The pods were removed from the sub-sample, dried to 0% moisture, the seeds threshed out and their weight recorded.

Harvest
All plots were direct combined with a small plot combine. The seed weight and moisture content was measured and the yield at 91% dry matter (DM) was calculated.

Statistical analysis

Analysis of variance was used to calculate whether treatments were significantly different using Genstat version 12 (VSN International Ltd, Harpenden, UK). Linear and exponential seed rate response curves were fitted to the seed yield for each seed rate treatment. Full details of the methods are described in DEFRA project report PS2805. The equation for the best-fit curve was then used to calculate the economically optimum seed rate for each trial by assuming oilseed rape seed costs of £10/kg for a conventional variety and £20/kg for a hybrid variety and an oilseed rape seed price of £360/t. The cost of each seed rate treatment (seeds/m^2) was calculated by using the seed costs described above and the thousand seed weight of 5g of seed.

RESULTS

2010-2011

Seed rate treatments

The optimum seed rate was 140 seeds/m^2 for Castille and 112 seeds/m^2 for Excalibur (Figure 1). The optimum seed rate for Castille resulted in a plant population of approximately 98 plants/m^2 and for Excalibur 91 plants/m^2.

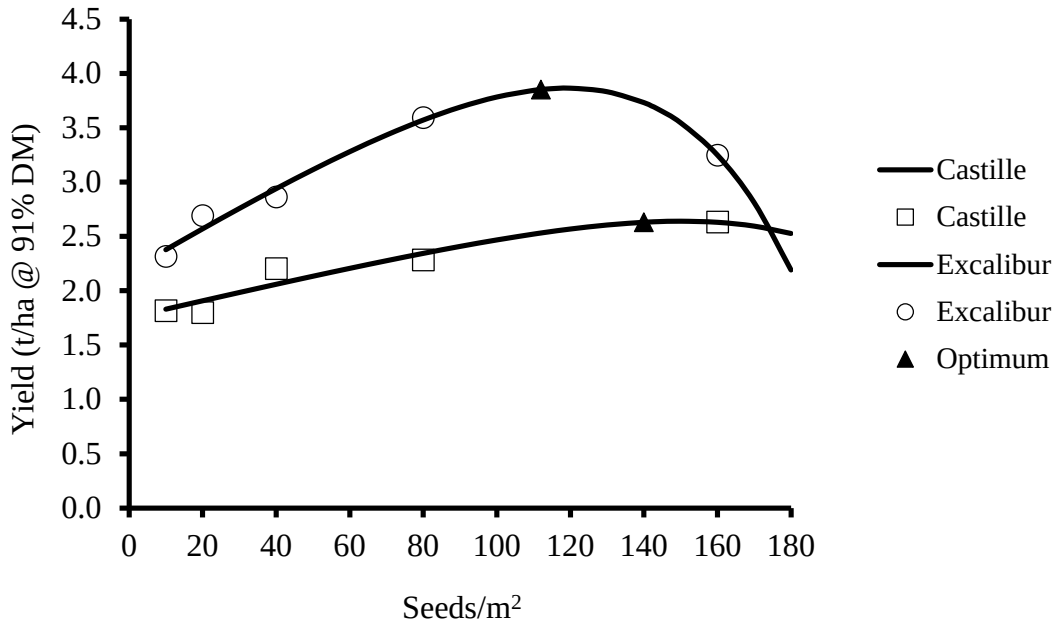

Figure 1. Effect of seed rate on the combine yield of oilseed rape in 2011.

Leaf pruning treatments

There was a significant effect of leaf pruning on yield ($P<0.001$, Table 2). Removal of a single cotyledon or the first true leaf did not significantly reduce yield, whereas removal of the first two leaves or the first four leaves significantly reduced yield by 1.37 and 2.03 t/ha respectively compared to the unpruned treatment (Table 2). Although seed rate had a significant effect on yield there was no evidence to suggest that the impact of pruning on yield was greater at lower seed rates (Table 2). The hybrid variety Excalibur produced significantly greater yields than the open pollinated variety Castille, but there was no difference between the two varieties in terms of their tolerance to leaf pruning.

2012

Seed rate treatments

The optimum seed rate was 32 seeds/m^2 for Castille (Figure 2), which resulted in a plant population of approximately 21 plants/m^2. It was not possible to fit a yield response curve for Excalibur because seed rate did not significantly affect yield. The highest yielding seed rate for Excalibur was 40 seeds/m^2 which yielded 3.57 t/ha compared with 3.42 t/ha for 20 seeds/m^2 and 3.28 t/ha for 10 seeds/m^2. A yield increase of only 0.03 t/ha is required to offset the cost of

increasing the seed rate for a hybrid variety by 10 seeds/m^2. Therefore the most profitable seed rate treatment was at 40 seeds/m^2 which equated to 30 plants/m^2.

Table 2. Quadrat yield of oilseed rape at 91% dry matter (t/ha) in 2011. Data presented is the average of two varieties, Castille and Excalibur. (ANOVA P values, associated standard errors of the difference (SED), degrees of freedom (df) and least significant difference values (LSD) are also provided).

Seed rate	Pruning Treatments					Grand Mean
	1	2	3	4	5	
10	2.60	2.34	2.06	1.21	0.69	1.78
20	3.01	2.43	2.28	1.11	0.65	1.89
40	2.77	2.48	2.16	1.39	0.78	1.92
80	3.02	3.21	3.02	1.86	1.04	2.41
160	2.89	3.33	3.20	2.04	1.16	2.52
Grand Mean	2.85	2.76	2.54	1.52	0.86	2.10
		P	SED	df	LSD	
Pruning treatment		<.001	0.175	12	0.381	
Seed rate		<.001	0.102	12	0.222	
Variety		<.001	0.098	74	0.195	
Pruning x seed rate		0.704	0.325	64		
Pruning x variety		0.659	0.233	28		
Seed rate x variety		0.056	0.185	22		
Pruning x seed rate x variety		0.955	0.474	120		

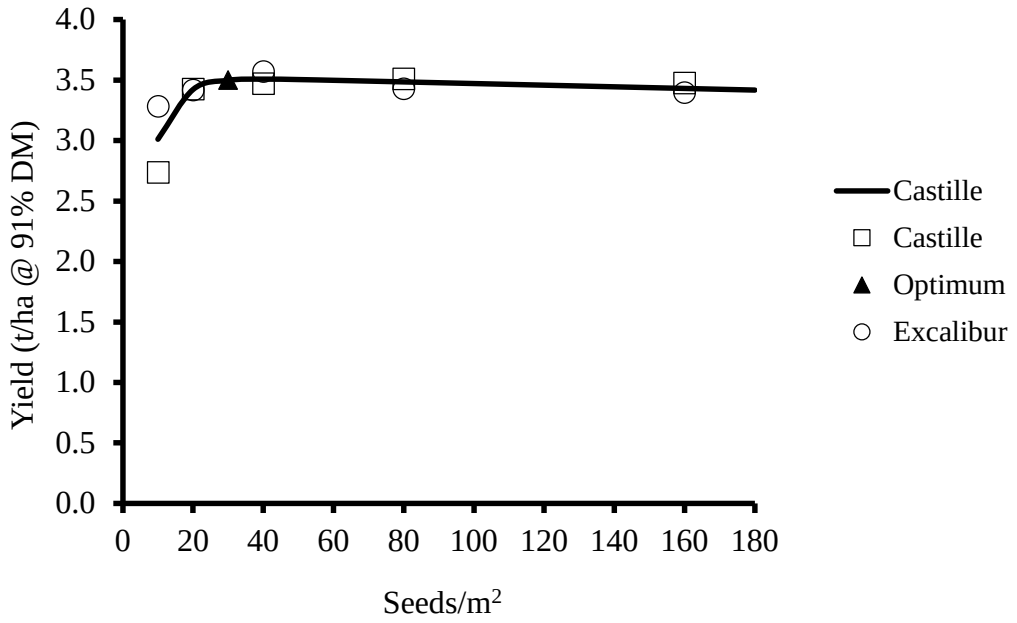

Figure 2. Effect of seed rate on the combine yield of oilseed rape in 2012.

Table 3. Quadrat yield of oilseed rape at 91% dry matter (t/ha) at High Mowthorpe in 2012. Data presented is the average of two varieties, Castille and Excalibur. (ANOVA P values, associated standard errors of the difference (SED), degrees of freedom (df) and least significant difference values (LSD) are also provided).

Seed rate	Pruning Treatments					Grand Mean
	1	2	3	4	5	
10	2.03	2.70	2.65	2.25	2.78	2.48
20	2.44	2.12	2.54	2.58	2.62	2.46
40	2.78	3.40	2.97	2.68	3.00	2.96
80	3.12	3.73	3.58	2.91	2.71	3.21
160	3.03	3.00	3.23	2.68	3.61	3.11
Grand Mean	2.68	2.96	2.98	2.63	2.98	2.85
		P	SED	df	LSD	
Pruning treatment		0.478	0.261	12		
Seed rate		0.125	0.332	12		
Variety		0.050	0.125	74	0.249	
Pruning x seed rate		0.947	0.611	64		
Pruning x variety		0.055	0.328	28	0.671	
Seed rate x variety		0.084	0.386	22		
Pruning x seed rate x variety		0.032	0.754	120	1.492	

Leaf pruning treatments

There was no significant effect of pruning treatment on yield (Table 3). Notably, each pruning treatment, with the exception of the removal of the first and second true leaves, led to a small (0.3t/ha) but insignificant increase in yield. Seed rate did not have a significant effect on yield and there was no interaction with leaf pruning (Table 3), thus again highlighting that lower plant populations are no less tolerant to slug damage. Excalibur produced significantly greater yields than Castille, but again there was no difference between the two varieties in terms of their tolerance to leaf pruning.

DISCUSSION

Optimum plant population

There were large differences in optimum plant population number between the two experimental seasons. In 2010-2011, optimum plant population for Castille was 98 plants/m^2 whilst in 2011-2012 it was much lower at just 21 plants/m^2. Similarly, for Excalibur, there was a reduction in the optimum plant population of 61 plants/m^2 between 2010-2011 and 2011-2012. The growing season of 2010-2011 had an exceptionally dry spring during March, April and May. The experimental site was shallow soil overlying chalk which had a low water holding capacity. It is probable that severe water stress reduced the capacity of the plants to produce compensatory branches which meant that a high density of plants was required to achieve potential yield at this site in 2010-2011. It is also interesting to note that the optimum plant populations did not differ greatly for the conventional and hybrid varieties. A wide range of seed rates are used in commercial practice. Famers sowing in the normal window for oilseed

rape between the end of August and beginning of September may typically sow conventional varieties at between 50 and 120 seeds/m^2 and hybrids at between 40 and 100 seeds/m^2. Therefore, plant populations of 30 to 80 plants/m^2 may be typical. This would indicate that a proportion of commercial crops cannot tolerate further plant losses to slugs.

Tolerance to leaf pruning

Severe leaf pruning was found to have a significant effect on yield in the 2010-2011 experiment but not in the 2011-2012 experiment (Tables 2 and 3). Interestingly removal of a single cotyledon or the first true leaf had no significant effect on yield in either experiment. It was not surprising that yield was significantly negatively affected by pruning in 2010-2011 and not in 2011-2012 given that the dry conditions restricted compensatory growth in 2010-2011. The leaf pruning treatments imposed in the experiments were relatively severe and further work should address the effect of removal of smaller areas of leaves and later leaves only.

Implications for slug control

Oilseed rape was shown to have a great ability to compensate for low plant populations when environmental conditions allow compensatory growth. Therefore crops with super-optimal plant populations would in theory be able to tolerate loss of plants due to slug attack. However, farmers have been encouraged to sow the crop at lower seed rates as this has a yield benefit and lower seed costs (e.g. HGCA Oilseed rape guide 2012). In the 1980's recommended seed rates were commonly 120 to 140 seeds/m^2 whereas currently recommendations of 40-80 seeds/m^2 are more normal. These populations are close to optimum plant populations so the inherent tolerance of the crop has been effectively reduced by the trend for lower seed rates. Consequently there is relatively little scope for oilseed rape crops to tolerate plant loss due to slug attack. Given the use of lower seed rates and the uncertainty associated with oilseed rape plant establishment it would seem prudent for growers to use some sort of slug control at drilling if slug thresholds have been exceeded.

Although there was a general trend for severe leaf pruning to reduce seed yield, results suggest that there was little significant impact from the loss of a single cotyledon or the first true leaf. Potentially therefore, if a super-optimal plant population has been established at the one leaf stage then there could be scope to delay slug pellet applications aimed at the one true leaf stage until slugs are active on the soil surface.

ACKNOWLEDGMENTS

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THE USE OF FLORASULAM BASED PRODUCTS FOR THE CONTROL OF ALS RESISTANT WEEDS IN CEREAL CROPS

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Summary: The use of ALS mode of action chemistry is a key option for the control of broad-leaved weeds in cereal crops. Resistance of *Stellaria media* (common chickweed) within the UK to metsulfuron-methyl was first identified in 2000 in Scotland, *Papaver rhoeas* (common poppy) in 2001 and reports of a decline in Mayweed species control throughout the United Kingdom in recent years. Metsulfuron-methyl, tribenuron-methyl and thifensulfuron-methyl (sulfonylurea class, SU) and florasulam (a triazolopyrimidine) are ALS-inhibitors and the resistance mechanism to these herbicides in broadleaf weeds is typically a result of target site mutation. Two ALS gene point mutations which confer target site resistance have been confirmed; $Proline_{197}$ conveys resistance to SU chemistry and moderate or no resistance to florasulam whilst $Tryptophan_{574}$ conveys resistance to both SU chemistry and florasulam (Delye *et al*, 2011). As a result the resistance profile of florasulam differs from that of the SU's. Where $Proline_{197}$ populations have been identified, florasulam containing products provide an opportunity to achieve higher control of ALS resistant broad-leaved weeds than that of the sulfonylurea chemistry, though chemical solutions should not be solely relied upon to control herbicide resistant weeds. The adoption of an integrated management approach involving cultural techniques (cultivation type, sowing dates, changes in crop type) and the use of alternative modes of action within the rotation will lessen the selection pressure to ALS products.

INTRODUCTION

ALS mode of action chemistry has been and remains an essential management tool for the control of broad-leaved weeds in a range of crops throughout Europe. A consequence of the widespread use of any herbicide is the potential of plant species developing mutations to convey resistance to the chemical mode of action. Heap (2013) reported 403 unique cases, with 218 species (129 monocots and 89 dicots) as resistant. Weeds have evolved resistance to 21 of the 25 known herbicide sites of action. Herbicide resistance to the grassweed species *Alopecurus myosuroides*, *Avena spp.* and *Lolium multiflorum* is widely documented whilst resistance to broad-leaved species *Stellaria media*, mayweed species and *Papaver rhoeas* are receiving greater recognition of their distribution throughout the UK. The two ALS gene point mutations that have been confirmed to affect the ALS chemistry are target site resistance mechanisms and are not dose rate dependent. $Proline_{197}$ affects the sulfonylurea (SU) chemistry (metsulfuron-methyl, tribenuron-methyl and thifensulfonyl-methyl), with moderate or no resistance to the broad leaved weed herbicide, florasulam. $Tryptophan_{574}$ conveys resistance to SU and triazolopyrimidine chemistry (Moss *et al.*, 2011). Resistant *S. media* has been

identified in 13 counties in Scotland, Northern Ireland and England, *P. rhoeas* in 7 English counties and populations of scentless mayweed (*Tripleurospermum inodorum*) has been confirmed (HGCA 2009, Moss 2011).

Boxer™ (50 g a.i./litre florasulam, formulation code EF-1343), Spitfire™ (100 g a.e./litre fluroxypyr and 5 g a.i./litre florasulam, formulation code GF-2257) and Galaxy™ (100 g a.e./litre fluroxypyr, 80 g a.e./litre clopyralid and 2.5 g a.i./litre florasulam, formulation code GF-1374) are registered in the UK for control of a range of broad-leaved weeds in winter and spring cereal crops. GF-2257 and GF-1374 utilise additional modes of action in fluroxypyr (Herbicide Resistance Action Committee group O) or clopyralid (group O), in addition to florasulam (group B). Fluroxypyr is highly efficacious against *S. media*, which to-date has no recorded resistance to this species. The addition of clopyralid in GF-1374 provides an alternative mode of action against mayweed spp. where resistance is suspected.

MATERIALS AND METHODS

Laboratory Pot tests

Seed samples for presented glasshouse studies were collected from either trial sites or field failures in Scotland and Germany. Plants were grown in a sandy loam soil and raised under glasshouse conditions until they reached GS 12–18. Once at the required growth stage treatments were applied using an overhead track sprayer calibrated to deliver a total volume of 200 litres/ha. Control plants received a water spray only. For all species and populations there were 5 replicate pots per treatment. Post application all species were kept in a greenhouse under conditions suitable for healthy growth and development of the test species. Plants were assessed for visual control (%) at 7 day intervals up to a maximum of 28 days post treatment.

Field studies

Trial design
Field trials were established in the UK, with all trials utilizing a randomised complete block design consisting of four replicates, with a minimum plot size of 2 x 6 m. Treatment applications were applied using precision small plot sprayers at 200 litres water per hectare through flat fan nozzles at medium spray quality.

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

Efficacy assessment
Weed control was assessed in laboratory and field tests at given intervals after treatment and expressed as % control where 0% represents no kill and 100% represents total kill.

Field Trial application timing
Applications (Table 1) were made to winter cereals at GS 24-30.

Table 1. Application growth stages and densities.

	Growth stage	Weed density/m2	Weed height (cm)
Stellaria media	GS 51-61	80	10
Papaver rhoeas	GS 30	15	4
Mayweed spp	GS 30-51	5	8

RESULTS

Laboratory tests

Data are presented from two studies. Figure 1 summarises *M. chamomilla* sample from a susceptible sample and ALS resistant sample obtained from Germany. Table 2 summarises data from a *S. media* sample obtained from a field in Scotland.

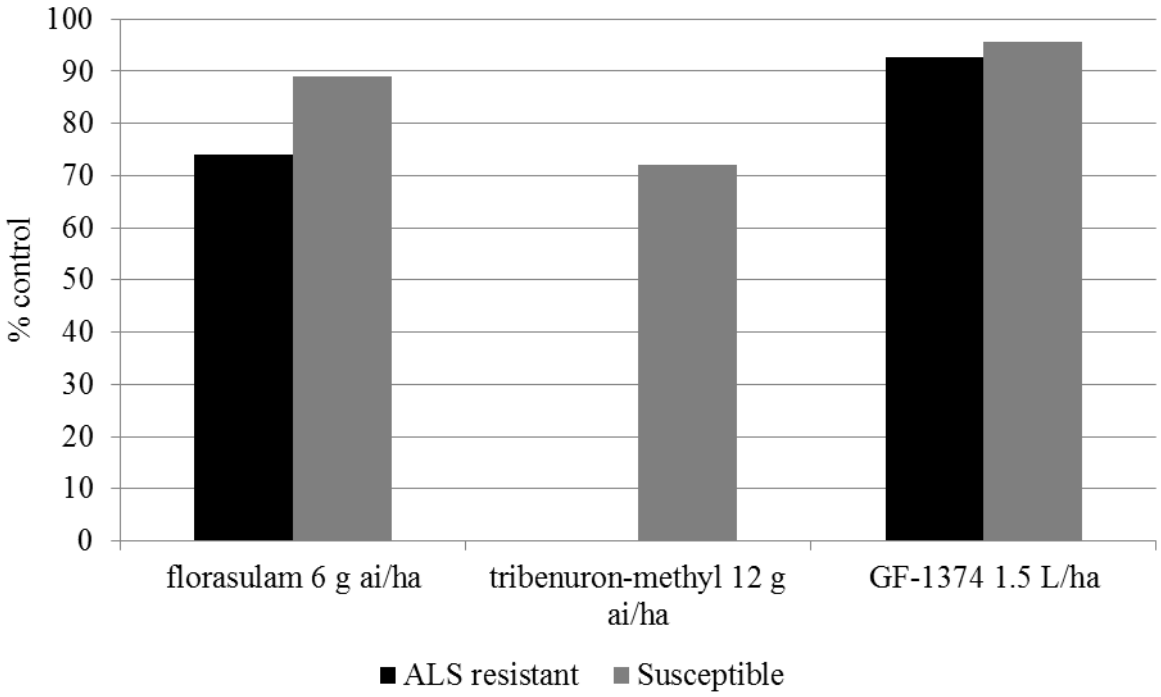

Figure 1. Mean % control of *M. chamomilla* at 21 days after application. Germany 2007

Field data

Data are presented from three replicated field trials conducted in the UK where ALS resistant populations of *S. media*, *P. rhoeas* and *T. inodorum* were present (Table 3, 4, and 5). GF-2257 (florasulam + fluroxypyr) either alone or in tank mixture with a methylated seed oil adjuvant were applied and compared to metsulfuron-methyl. Crop injury assessments were conducted but no injury was observed from any treatment and no data are presented.

Table 2. Mean % reduction of fresh weight ± S.E of *S. media* at 21 days after spraying.

	Metsulfuron-methyl, 6 g a.i./ha	Florasulam, 5 g a.i./ha
Susceptible standard	98.0 ± 0.5	98.0 ± 0.2
Scottish sample	45.6 ± 16.9***	99.3 ± 0.1

*** = RRR resistance confirmed; probably reducing herbicide performance in field

Table 3. Mean % visual control of an ALS resistant *S. media* population, 59 days after application. UK 2013 (P=0.05, LSD=10.89)

	% Visual control
Untreated	0.0
GF-2257 1 litre/ha + adjuvant 1 litre/ha	99
Metsulfuron-methyl 6 g a.i./ha	0.0

Table 4. Mean % visual control of an ALS resistant *P. rhoeas* population, 106 days after application. UK 2013 (P=0.05, LSD=3.64)

	% Visual control
Untreated	0.0
GF-2257 1 litre/ha	98
Metsulfuron-methyl 6 g a.i./ha	0.0

Table 5. Mean % visual control of an ALS resistant *Matricaria spp.* population, 59 days after application. UK 2013 (P=0.05, LSD=61.70)

	% Visual control
Untreated	0.0
GF-2257 1 litre/ha + adjuvant 1 litre/ha	98.0
Metsulfuron-methyl 6 g a.i./ha	25.0

DISCUSSION

The results reported in Table 2 have been confirmed as being of a *S. media* biotype affected by the Proline$_{197}$ mutation (Marshall *et al*, 2010). Florasulam (EF-1343) recorded no difference between the susceptible standard or to the Scottish sample, whilst metsulfuron-methyl was confirmed as resistant, recording a 46% reduction in fresh weight.

ALS resistant *M. chamomilla* sample obtained in Germany was resistant to tribenuron-methyl, whilst florasulam caused a small decline in efficacy compared to the susceptible standard (Figure 1). GF-1374 (florasulam + fluroxypyr + clopyralid) recorded the highest control (>92%) and highlights the value of the alternate mode of action provided by clopyralid.

Three UK replicated field trials demonstrate the impact in control that the ALS target site resistance can exert upon this chemistry. Data presented in Tables 3, 4 & 5 show statistically significant differences in control between florasulam containing products and metsulfuron-methyl. *Stellaria media* control from GF-2257 + adjuvant, was 98.5% whilst metsulfuron-methyl was 0% (Table 3). GF-2257, as well as containing florasulam also contains fluroxypyr, an active ingredient that has activity on *S. media* to which no resistance has been reported. The GF-2257 + adjuvant provided 98% *P.rhoeas* control compared to 0% control from metsulfuron-methyl (Table 4). *Matricaria spp* control provided by GF-2257 + adjuvant (98%) compared to 25% from metsulfuron-methyl, clearly demonstrating the benefit of using products with multiple modes of action (Table 5).

Growers that have ALS resistant *Alopecurus myosuroides* and *Lolium multiflorum* are used to tackling the problem with the use of alternate modes of action applied pre-emergence of the grassweed or post emergence, or normally at both application timings. As a single post emergence application of an ALS graminicide will no longer deliver reliable and high levels of control that were achievable only a few years ago (Moss, 2013). An integrated approach is required utilising changes in cultivation practices, amendments in drilling dates and cropping changes. The application of graminicides with different modes of action is considered the final change.

Where once incidences of lower than expected broad-leaved weed control were discounted as meteorological affects, a greater understanding now exists about key broad-leaved species resistance to ALS mode of action. Fortunately there are a wider number of modes of action available for broad-leaved weed control in cereal crops. If resistance is expected then it is essential to understand the resistance profile in the field so that the most suitable product is selected. Where Proline$_{197}$ is confirmed, products such as GF-2257 and GF-1374 herbicides can offer an effective control option. However it has to be cautioned that where ALS resistance is suspected or confirmed, it is advised to utilise additional modes of action and appropriate cultural control methods.

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DURATION OF CANOPY PROTECTION REQUIRED BY SPRING BARLEY POST-ANTHESIS

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Summary: Experiments were conducted at two sites between 2009 and 2012 to investigate the duration of protection of canopy light interception required to maximise the yield of spring barley. Commencing at 50% ear emergence (Zadoks GS55), shades were erected at weekly intervals over disease-free plots to reduce incident radiation on the canopy by approximately 67% and left in place until harvest. The critical period requiring protection was between 3-5 weeks after GS55 (depending on the site-year) or approximately the first 75% of grain filling. Fungicide timing experiments showed that where disease pressure was low to moderate, a single application of prothioconazole plus pyraclostrobin at T1 gave sufficient canopy protection during grain filling to maximise yield. An additional T2 application at booting was required when disease was severe. Applications after ear emergence gave no further yield increase even though they resulted in a prolonged canopy lifespan, because the effects occurred late in the critical period.

INTRODUCTION

Fungicide applications to spring barley are usually timed at the start of stem extension (T1) and at booting (T2). The aim of the T1 application is to protect the canopy during stem extension and the development of grain sites, whilst the aim of the T2 application is to protect the canopy from disease post-anthesis to ensure an adequate supply of assimilate for grain filling. The optimum timing of these treatments has been determined empirically rather than through a consideration of the physiological requirements of the crop during grain filling. Assimilate for grain filling comes from both concurrent photosynthesis during the grain filling period and from the remobilisation of storage reserves deposited pre-anthesis. There is evidence that under some circumstances, storage reserves may be able to buffer yield against large reductions in photosynthetic activity resulting from late season drought or reductions in incident radiation (Willey & Holliday, 1971; Gallagher *et al*., 1976). There is also some evidence to suggest that the main positive effect of fungicides on mean grain weight in barley is the result of an increase in grain storage capacity rather than assimilate supply for grain filling (Bingham *et al*., 2010). A greater understanding of the duration of canopy protection required would allow greater flexibility in the management of fungicides because there may be opportunities to adjust the timing and dose of fungicide according to the resistance of the variety to late season disease, the disease pressure at the site and the required duration of protection. The aim of

experiments here was to quantify how long the crop must be protected from large reductions in canopy light interception in order to maximise yield. This was achieved using shading to reduce incident radiation thereby mimicking the effects of a sudden disease-induced reduction in light interception by canopy green area. This approach allowed more precise control of the timing of the reduction than could be achieved by manipulating disease epidemics. The results were compared with the effects of different fungicide timings on yield, green leaf area and light interception in non-shaded crops.

MATERIALS AND METHODS

Full details of the experimental methods can be found in Bingham *et al*. (2013). Only a brief summary is given here. Two series of experiments were conducted at two sites, ADAS Rosemaund (Herefordshire) and SRUC Edinburgh between 2009 and 2012. In the first, plots of spring barley cv Westminster were grown and treated with prothioconazole (Proline® @ 0.4 litres/ha) plus pyraclostrobin (Comet 200® @ 0.63 litres/ha) at T1 and T2 to prevent disease development. At weekly intervals commencing at Zadoks GS55 (Tottman, 1987) shade netting was erected at a height of 1.5 above ground level over designated plots and discard areas and left in place until harvest. The shade netting (Haygrove Ltd, Ledbury, UK) was constructed of an open mesh of black polyethylene that allowed rainfall to penetrate whilst restricting transmission of photosynthetically active radiation (PAR) by 64-69% depending on the site. At the ends of the plots (E and W) the netting was secured below canopy height to prevent direct light penetrating under the shading when the solar zenith angle (from the vertical) was large. Along the N and S edges, the netting was secured 1.2 m above ground level to provide adequate ventilation under the shade, whilst preventing ambient light reaching the experimental plot. Discard areas were included to ensure the shades did not cast a shadow over non-shaded plots. Each week from GS55 onwards 20 shoots were sampled at random for dry matter determination of ears and straw. Absolute leaf area, % disease and % green leaf area were measured on a further 10 shoots sampled every two weeks and canopy light interception determined using a Sunscan Canopy Analysis System (Delta T Devices Ltd, Cambridge, UK). Plots were combine harvested for measurement of yield and mean grain weight.

In the second series of experiments three varieties of spring barley with relatively high susceptibility to disease were grown (Optic, Forensic and Waggon). Each was treated with prothioconazole plus pyraclostrobin (rates and products as above) at T1 only, or T1 plus T2 at either 2, 4 or 6 weeks after T1 (the T2 timings approximated to GS37, GS49 or GS71 respectively); untreated plots served as controls. Measurements of absolute leaf area, % disease, % green leaf area and canopy light interception were made every two weeks from GS55 onwards as described above.

RESULTS

The effect of the time of shading on yield in three site-years is shown in Fig. 1. The shades erected at GS55 and left in place until harvest caused the greatest reduction in yield. As the shading was imposed progressively later during the grain filling period, its effects on yield diminished. The overall pattern of response was similar in each of the site-years although the magnitude of the effect on yield differed. The time at which there was no further significant reduction in yield by shading was around 3 weeks after GS55 at SRUC in 2010, 4-5 weeks at

ADAS 2009 and 5 weeks at SRUC 2011 (Fig. 1). At these times there was still an appreciable amount of green leaf area on the crop, with the average % GLA across the top 4 leaves in the range 25–50%. Stem and ear % green area was greater (data not shown). These data suggest that the PAR interception by the canopy must be protected for a period of 3–5 weeks after 50% ear emergence (GS55) in order to maximise yield. This corresponds to approximately the first 75% of grain filling; thereafter yield is relatively insensitive to major reductions in PAR interception.

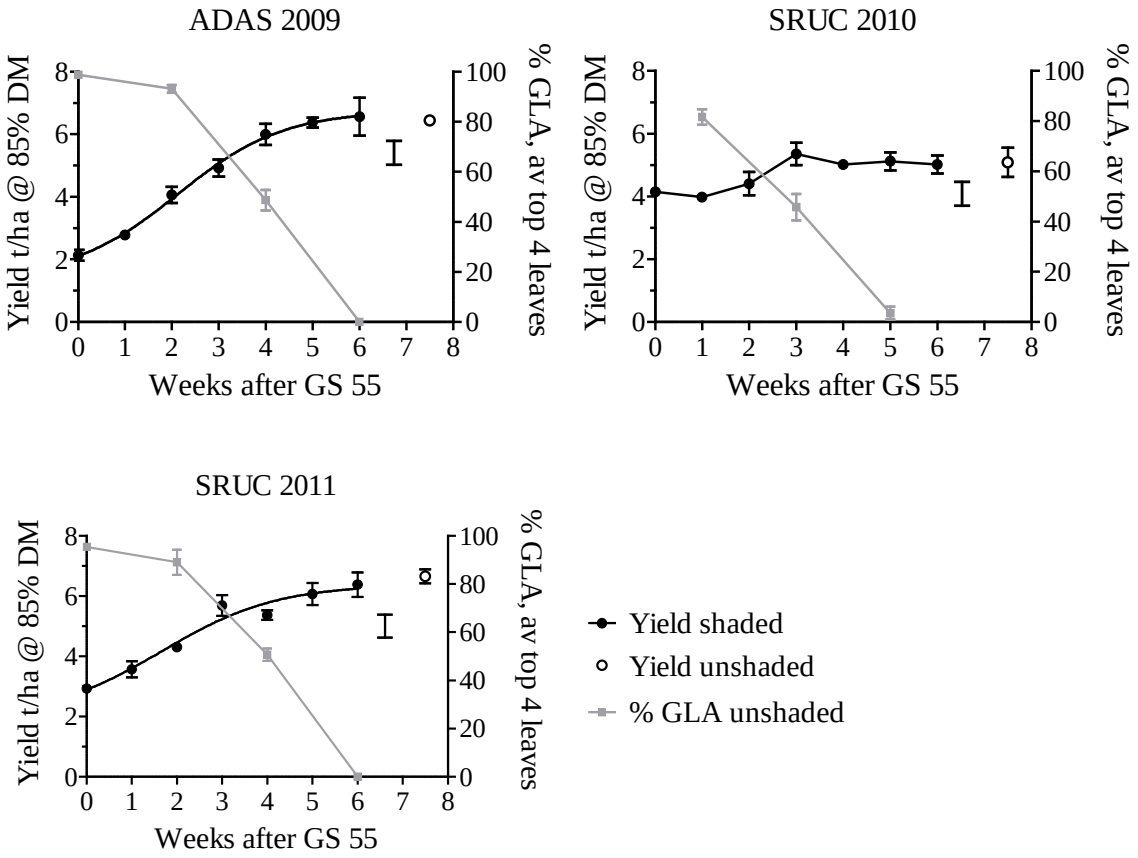

Figure 1. Effects of the time of onset of shading on yield and the % green leaf area (GLA) averaged over the top 4 leaves for unshaded plots. Values are means ± SEM of 3 replicates. The stand-alone vertical bar represents the LSD (5% probability) for the effects of time of shading on yield following a one-way anova. A sigmoid function was fitted to yield data for ADAS 2009 and SRUC 2011. The fit was poor for SRUC 2010, and thus a non-fitted line is shown.

Fungicide timing experiments were conducted at ADAS in 2011 and SRUC in 2011 and 2012. Powdery mildew (*Blumeria graminis*) and rhynchosporium (*Rhynchosporium commune*) were the main foliar disease at ADAS and SRUC in 2011 and were present at low to moderate severities (<8.0% averaged over the top three leaves at mid grain filling). There was also some physiological spotting at SRUC in 2011 (up 6-12% severity depending on the variety). At

SRUC in 2012 there was a severe ramularia leaf spot (RLS) (*Ramularia collo-cygni*) epidemic (up to 50% severity averaged over the top three leaves. Significant yield increases to fungicide treatment were found at all sites ranging from circa 0.5-1.0 t/ha @ 85% dry matter. At ADAS and SRUC in 2011 T1 applications on their own gave sufficient protection of the canopy during stem extension and grain filling to maximise yield. There was no significant further yield increase from an additional T2 treatment. At SRUC in 2012, where there was a severe RLS epidemic, an additional yield response occurred following a T2 application at either GS49 or GS71.

DISCUSSION

These experiments provide, for the first time, a benchmark against which the required duration of canopy protection sought from fungicides can be assessed for spring barley. Protection of canopy light interception is needed for approximately the first 75% of grain filling. Presumably after that the remainder of the available grain storage capacity can be filled from storage reserves. Not surprisingly, the requirement for a T2 fungicide application to provide this protection depended on the disease pressure at the site. When disease was low to moderate, a T1 spray was sufficient to give the required protection, but when disease was severe an additional T2 application at booting was required. There was no evidence from these experiments that, even with severe post-anthesis foliar disease, a later T2 application (after ear emergence) provided any yield benefit over a treatment at GS49. Although at all sites late applications of T2 (GS71) gave a prolonged canopy lifespan compared to earlier T2 timings, the effects occurred too late in the grain filling period to have any effect on yield, i.e. the response occurred late in, or outside, the critical period requiring protection as determined by the shading experiments.

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THE BENEFITS OF USING RESISTANT CULTIVARS AND FUNGICIDES TO MANAGE LIGHT LEAF SPOT (*PYRENOPEZIZA BRASSICAE*) IN WINTER OILSEED RAPE

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Summary: Light leaf spot is often an intractable problem in winter oilseed rape crops. The raising of varietal standards has helped to manage the disease but levels remain stable in the north and continue to increase in the south. Fungicides provide a partial solution but control is focused on DMI fungicides and there are concerns that the activity and persistence of products is sometimes compromised by variations in pathogen sensitivity. Standard fungicide timings are autumn and spring and dose and yield response data suggests optimal products which are discussed. Varietal resistance ratings for previously resistant varieties have been eroded in recent seasons and the paper concludes that varietal resistance and chemistry should be used together in integrated programmes to maximise the long-term efficacy of both strategies.

INTRODUCTION

Light leaf spot, caused by the pathogen *Pyrenopeziza brassicae*, is the most common disease reported on oilseed rape in Scotland in Scottish Government Plant Health monitoring (www.sruc.ac.uk/crops) and has remained relatively stable in incidence and severity over recent seasons. CropMonitor data (www.cropmonitor.co.uk) for England shows that the disease has increased in incidence and severity since 2007 and affected 30% of plants in England in 2012 and 2013. The disease can lead to a significant loss in green leaf area, with commensurate losses in photosynthetic ability and yield, but it can also infect pods and cause premature ripening pod splitting and further losses. Control is normally through the use of fungicides applied in the autumn and spring although timings are often compromised by the ability to travel on land. Varietal resistance has improved in recent years, with a minimum rating of 5 (recently revised upwards) on the Recommended List (HGCA RL list, www.hgca.com/varieties) for the North and 4 for the East / West region. The pathogen is highly adaptable however, and previous studies have shown rapid onsets of fungicide resistance, for example to MBC fungicides (Sutherland & Walker, 1984). Current control is reliant on demethylation inhibitor (DMI) azole fungicides where a wide range in sensitivity with the pathogen population is already known (and variations in sensitivity to azole fungicides (Burnett 2003; 2004), Carter *et al*. (2013) have recently confirmed this can be related to mutations in the Cyp51 protein at sites implicated in reduced sensitivity to other pathogens. Declines in field performance of varieties with previously effective varietal resistance have

also been noted – for example the variety Cracker was downgraded from a 9 to an 8 in the 2013 (HGCA RL list, www.hgca.com/varieties).
This paper sets out work from trials at SRUC and ADAS and discusses fungicide products, optimum timings and the value of cultivar resistance. Sensitivity data is also presented and the effect of varietal resistance on disease levels and fungicide efficacy shown.

MATERIALS AND METHODS

Fungicide x variety trial

A cultivar x fungicide experiment was sown on 31 August 2009 at Towthorpe, E. Yorks. The cultivars Castille and Cuillin were sown at 100 seeds/m^2 and Krypton at 70 seeds/m^2. Plot size was 3.5 x 24.0 m. Each treatment had three-fold replication, laid out in a randomised block design. Three fungicide spray treatments with prothioconazole (0.35 l/ha applied as Proline 250 which has a full label dose of 0.72 l/ha and 250 gai/l) were applied in autumn, spring or autumn + spring (Table 1). Light leaf spot resistance ratings were Cuillin 9, Krypton 7 and Castille 5. Disease assessments were done using a whole plot % leaf area infected in the autumn, (7 December) winter (12 February) and spring (16 March) and on 25 plants per plot pre-harvest (21 July 2010, GS 6,4). Yields were adjusted to 91% dry matter.

Table 1. Fungicide treatments applied to variety x fungicide trial in 2009.

Treat-ment	Active ingredient and rate applied	Cultivar	Growth stage applied	Date applied
1	untreated	Castille	-	-
2	prothioconazole 0.35	Castille	1,8	27 Nov
3	prothioconazole 0.35	Castille	3,1	4 Mar
4	prothioconazole 0.35	Castille	1,8 and 3,1	27 Nov and 4 Mar
5	untreated	Cuillin	-	-
6	prothioconazole 0.35	Cuillin	1,8	27 Nov
7	prothioconazole 0.35	Cuillin	3,1	4 Mar
8	prothioconazole 0.35	Cuillin	1,8 and 3,1	27 Nov and 4 Mar
9	untreated	Krypton	-	-
10	prothioconazole 0.35	Krypton	1,8	27 Nov
11	prothioconazole 0.35	Krypton	3,1	4 Mar
12	prothioconazole 0.35	Krypton	1,8 and 3,1	27 Nov and 4 Mar

Fungicide timing trial

Table 2. Fungicide treatments applied to timing trials in 2011/12 and 2011/12

Treat-ment	Active ingredient	Dose	Growth stage applied	Month applied
1	untreated	-	-	-
2	prothioconazole	0.35	2,1	Nov
3	prothioconazole	0.35	3,1	Mar
4	prothioconazole	0.35	2,3 and 3,1	Jan and March

5	prothioconazole	0.35	2,1 and 3,1	Nov and March

Fungicide timing trials were sown on 28 August 2010 and 15 September 2011 at MacRobert Farm, Aberdeenshire. The cultivar Castille was used and the plot size was 2 x 12 m. There were four replicates of each treatment, laid out in a randomised block design. Three fungicide spray treatments with prothioconazole (0.35 l/ha applied as Proline 250) were applied in autumn, spring, autumn + spring and winter (termed delayed autumn) + spring (see Table 2). Disease assessments were done using a whole plot % severity score in the autumn, winter (February) and spring (March) and on 25 plants per plot. Yields were adjusted to 91% dry matter.

Fungicide sensitivity testing

Samples were collected from field trials at the sites noted in Table 5. Fifty leaves were taken from untreated plots in May or June (i.e. post second spray timing). Isolates were taken by incubating leaves in damp chambers at 18°C for 48 hours. A sterile needle was then used to pick visible spores from the leaf surface which were plated onto antibiotic amended Potato Dextrose Agar. Spore suspensions (20 µl) of test isolates were added to each well of 96-well Petri plates. Alkyl Ester Broth in 1 litre of distilled water, amended with fungicide to achieve final concentrations of 100, 50, 10, 5, 1, 0.5, 0.1 and 0 mg of active ingredient of the test fungicide/litre. The fungicides tested were prothioconazole (as Proline 250), tebuconazole (as Folicur), fluopyram (technical grade), bixafen (technical grade), flusilazole (as Sanction) and prochloraz (as Poraz). Each isolate was tested in three replicate wells. Plates were measured for optical density (absorbance at 450 nm) on a BMG Omega plate reader initially and again after incubation (14 days in dark, 19°C with gentle rocking). From the subsequent differences the ED_{50} values were calculated on the MARS data analysis software.

RESULTS

Fungicide x variety trial

Light leaf spot had started to appear on 7 December only on Krypton and affected 10% of plants of this variety on 12 February. Light leaf spot subsequently increased rapidly between mid-February and mid-March so that by 16 March (Table 3), 100% of untreated plants of Castille and Krypton were infected. Only a trace of light leaf spot was found on Cuillin, significantly lower than on the other varieties so no significant differences in disease from fungicide treatment were noted on this variety. Control with fungicide was poorer on Castille, compared to Krypton.

Pre-harvest disease assessments of light leaf spot on stems and pods showed significant varietal differences between the untreated controls and continuing fungicidal control from the autumn + spring and some spring treatments (Table 3). All fungicide treatments gave significant control of light leaf spot on stems and pods with no significant differences between timings. Varieties were significantly different from each other for stem light leaf spot severity. Phoma stem canker and phoma stem lesions were present as small lesions at low levels and were controlled by spring and autumn + spring fungicides. Varietal differences were significant for phoma stem lesions (mean incidence was Castille 6%, Cullin 23% and Krypton 11.7 with LSD =9.93), but not for stem canker (Phoma data not shown).

Averaged over all treatments, Cuillin (4.84 t/ha) had a significant yield advantage of 0.65 t/ha over Castille and 0.74 t/ha over Krypton (P <0.001). There were no significant effects from fungicides but positive trends of 0.1-0.2 t/ha were evident for the two-spray treatments and spring sprays on Krypton and Cuillin. Fungicide effects and interactions of variety x fungicide were not significant. The responses to fungicide were small and less than expected for this level of light leaf spot. Krypton gave rather more consistent positive yield trends to fungicide treatment.

Table 3. Light leaf spot disease levels on leaves, stems and pods and yield in variety x fungicide trial, E Yorks. 2010

Treat-ment	Variety and treatment	% leaf area 16 March	% stem area 21 July	% pod area 21 July	Yield t/ha	Yield response t/ha
1	Castille - UT	15.7	16.0	8.0	4.23	
2	Castille - P A	12.7	12.0	5.7	4.20	-0.03
3	Castille - P S	12.7	11.3	6.0	3.99	-0.24
4	Castille - P A+S	11.0	8.50	4.4	4.32	0.09
5	Cuillin - UT	0.00	4.70	3.0	4.76	
6	Cuillin - P A	0.00	2.20	2.2	4.75	-0.01
7	Cuillin - P S	0.67	1.50	1.7	4.90	0.14
8	Cuillin - P A+S	0.00	1.20	1.3	4.94	0.18
9	Krypton - UT	13.0	10.3	5.1	3.94	
10	Krypton - P A	6.00	7.20	4.1	4.11	0.17
11	Krypton - P S	8.67	7.80	3.9	4.18	0.24
12	Krypton - P A+S	6.00	7.70	3.9	4.14	0.20
P		<0.001 V and F NS VxF	<0.001 V and F NS VxF	<0.001 V and F NS VxF	<0.001 V NS F, NS FxV	
LSD		1.87 V 2.17 F	1.55 V 1.80 F	0.74 V 0.86 F	0.212 V 0.425 FxV	

UT = untreated, PA fungicide in autumn, PS fungicide in spring, PA+S fungicide autumn and spring

NS = no significant interaction, F = fungicide, V = variety, VxF = interaction between variety and fungicide

Fungicide timing trial

Light leaf spot had started to appear at trace levels in trials in late November each season but significant differences in disease levels were only noted in April assessments (reported in Table 4). No pod infection was noted. Control with all the fungicide treatments was significant compared to the untreated controls but there were no significant differences between the spray timing treatments. There was a trend, however, for control to be poorer from the spring and the delayed double treatment compared the autumn and the conventionally timed double treatment. As with the variety x fungicide trial control was far from complete – both trial series failed to show more than 20-30% reductions in light leaf spot on this variety.

Table 4. Light leaf spot disease levels on leaves and yield in fungicide timing trials, Aberdeen 2011 and 2012

Treat-ment	Timing	Disease severity April (% leaf surface infected)	Yield t/ha 91%DM	Yield response t/ha
1	-	13.0	2.20	-
2	November	8.55	2.41	0.21
3	March	9.28	2.27	0.07
4	January + March	8.74	2.23	0.03
5	November + March	8.51	2.64	0.44
P		0.047	0.039	
LSD		2.063	0.327	

Yields in the trials were poor as they suffered grazing damage (pigeons and slugs). There was a significant response to treatment from the November and March two spray programme but not from the delayed application two spray programme or from either single spray programme although there was a trend for the single autumn application to perform better than the delayed two spray programme.

Table 5. Influence of site on mean sensitivity of isolates ED_{50} (ppm) (number of isolates tested in brackets)

Site and Year	ED_{50} (ppm) prothioconazole	ED_{50} (ppm) tebuconazole	ED_{50} (ppm) flusilazole	ED_{50} (ppm) prochloraz
Aberdeen 2010	1.56 (3)	2.76 (3)	6.67 (9)	2.19 (3)
High Mowthorpe 2010	7.35 (2)	0.64 (2)	12.1 (5)	1.52 (2)
Aberdeen 2011	0.51 (12)	0.16 (11)	-	-
High Mowthorpe 2011	0.15 (11)	0.34 (9)	-	-
Midlothian 2012	16.0 (5)	15.6(5)	-	-
Cambridge 2012	8.98 (4)	7.21 (6)		
P	<0.001	0.001	0.037	<0.001

There were significant differences in the sensitivity of isolates collected between sites for all the fungicides tested.

DISCUSSION

Cuillin showed very good disease resistance to light leaf spot and Krypton showed less light leaf spot than Castille, particularly when treated with fungicide. Fungicide trends suggested that they gave more effective control of light leaf spot on Krypton than on Castille and this appeared to be reflected in better yield trends on Krypton. Control on Castille was poor and clearly there is concern that fungicides are not giving very effective control. At this site, spring sprays were delayed by the weather and more effective control might have been achieved with a February treatment as infection took off in the February to March period. The cold, dry spring curtailed light leaf spot activity and spread to the upper leaves and pods which may have

decreased fungicide benefits. The yield trends were positive and benefits of 0.1 t/ha would enable fungicide costs to be recovered. In the timing trials in Aberdeen the infection was earlier on Castille and there was a greater benefit to the autumn application compared to the spring treatment. Delaying the autumn application to January reduced control and yield, suggesting protectant use of fungicide is the better strategy. Yield benefits were small in both sets of trials; the Aberdeen sites suffered from high overwintering plant losses and the East Yorkshire site from a late infection of light leaf spot. The small benefits from fungicide treatment were a feature of the 2009/10 season even in Scotland and have been attributed to the prolonged cold conditions and loss of leaves during the winter.

The substantial benefit from growing Cuillin in areas where light leaf spot is a threat was larger than might be expected from Recommended List data for 2010/11. In the North region Cuillin differed from Krypton and Castille by 3% and 8% respectively. This contrasts with a 16% and 13% difference for treated yield in the variety x timing trial. Previous and current data highlights the variable sensitivity of the pathogen to fungicides and the difficulties of controlling infection on susceptible varieties were demonstrated in these trials. The timing of sprays in these trials and the contrasting results as to the relative benefits of autumn or spring sprays at the two sites suggests that optimal timing relates to disease onset and that efficacy could be improved by applications timed in response to warning systems monitoring for the disease. There is a strong case for growing resistant varieties in this challenging light leaf spot environment and using a fungicide spray in the autumn or winter and a second in the spring.

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THE EFFICACY OF LATE FUNGICIDE APPLICATION ON FOLIAR DISEASES AND HEAD BLIGHTS IN WINTER AND SPRING BARLEY

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Summary: *Fusarium* Head blight (FHB) is a cereal disease complex which is becoming an increasing problem due to recent warmer and wetter growing seasons. The use of fungicides at standard timings can protect, control and reduce foliar diseases on barley and enhance yield. By delaying these standard timing applications we can continue to protect, control and reduce earlier foliar fungal infections as well as reducing later *Fusarium* spp. infections. Two field trials studied late season fungicide applications on spring barley in Scotland and their combined effect with weather on late season *Fusarium* levels.

INTRODUCTION

Cereal production in Scotland in 2013 is estimated at 2.8million tonnes with barley production contributing about 70% of this total. There are many disease complexes which affect cereal production, one of which is FHB. Many species contribute to the range of symptoms observed. The most common species are *Fusarium graminearum* and *Fusarium culmorum*. The ears of cereal crops are also infected by *Microdochium nivale. Microdochium nivale* has been shown to have a negative effect on seed germination (Cockerell *et al.,* 2009). FHB infection during early flowering in warm and wet conditions can result in the whole or part of the ear becoming bleached. Later infections to the grain can result in yield loss and it is at this stage that natural toxic substances known as mycotoxins are produced.

Table 1. Legal maximum mycotoxin levels in cereals and processed foods

Product	Mycotoxin (ppb)	
	deoxynivalenol	zearalenone
Unprocessed cereals other than durum wheat, oats and maize	1250	100
Unprocessed durum wheat and oats	1750	100
Unprocessed maize	1750	200
Cereal flour	750	75
Maize flour	750	200
Bread,pastries,biscuits,cereal snacks and breakfast cereals	500	50
Processed cereal based food (Infants, young children, baby food)	200	20

The most common mycotoxins are deoxynivalenol (DON), zearalenone (ZON) and HT-2 and T2. These are produced on cereal crops in the field. Another mycotoxin, ochratoxin A, is produced on grains in store. There are legal maximum levels set for cereals and cereal products for human consumption and even small amounts can be harmful to humans and animals.

The use of fungicides at standard timings can protect, control and reduce foliar and head diseases in barley and enhance yield and quality. In the UK, *Fusarium* spp levels in winter and spring barley can be influenced by fungicide spray timings and weather effects. A number of fungicides are known to be effective against Fusarium and Microdochium on cereal ears.

MATERIALS AND METHODS

Field Trials

Two field trails were set up to measure Fusarium control in spring barley at the Bush Estate, Midlothian in 2012. The trials were a randomized block design with a standard plot size (2m x 10m). In the first trial, the effect of alternating the timing of the T2 spray on disease infection was examined. In the second trial the efficacy of different T2 fungicides was examined. The varieties used in Trial 1 were Forensic, Optic and Waggon and in Trial 2 Westminster, Garner and Quench were used.

Treatments in Trial 1 received prothioconazole (pro) (275 g/l) (Proline®) at 0.36l/ha at GS30/31. The remaining plots received pyraclostrobin (pyr) (200g/l) (Comet®200) at 0.625l/ha either at 0, 2, 4 or 6 weeks after T1 application. An untreated control was included for each variety.

Table 2. Spray Program details for Trial 2

Trts	Variety	Timing GS 30/31	Timing GS 41-49
1	Westminster	chlor 1.0l/ha	chlor 1.0l/ha
2	Westminster	pro 0.36l/ha + pyr 0.625l/ha	
3	Westminster	pro 0.36l/ha	pro 0.36l/ha
4	Westminster	pro 0.36l/ha + pyr 0.625l/ha	pro 0.36l/ha + pyr 0.625l/ha
5	Westminster	Untreated	
6	Westminster		pro 0.36l/ha +pyr 0.625l/ha
7	Westminster	pyr 0.625l/ha	pyr 0.625l/ha
8	Garner	pro 0.36l/ha + pyr 0.625l/ha	
9	Garner	pro 0.36l/ha + pyr 0.625l/ha	pro 0.36l/ha + pyr 0.625l/ha
10	Garner	Untreated	
11	Garner		pro 0.36l/ha + pyr 0.625l/ha
12	Quench	Untreated	
13	Quench	pro 0.36l/ha + pyr 0.625l/ha	pro 0.36l/ha + pyr 0.625l/ha
14	Quench	pro 0.36l/ha + pyr 0.625l/ha	
15	Quench		pro 0.36l/ha + pyr 0.625l/ha

Spray Program – Trial 2

Treatments in trial 2 included chlorothalonil (chlor) (450g/l) Bravo®, prothioconazole (pro) (275 g/l) and pyraclostrobin (pyr) (200g/l). An untreated control was used for each variety. Timing and rates are detailed in table 2 above.

Grain samples were taken from all treatments and both trials at harvest 2012.

Identification of fungi

Potato Dextrose Agar (PDA) plates inoculated with 0.1% penicillin G and 0.1% Streptomycin sulphate (sodium salt) were used for fungal isolation. Barley seeds were sterilised in 8% sodium hypochlorite for 8 minutes to eliminate the possibility of fungal/bacterial growth from the husk. The grain was then rinsed in distilled water containing Tween® 20 (polyoxyethylene sorbitol ester) for three five minute cycles. Following the final rinse five seeds were placed on each 9cm agar plate. Three replicate plates were used per plot. Plates were then incubated without light at 20^{o}C for 7 days. Fungal identification was based on colony growth and colour. The percentage of *Fusarium* spp and *Microdochium nivale* was calculated for each plate.

RESULTS

In Trial 1, the T4 treatment on the variety Optic gave the best results in terms of reducing both *Microdochium nivale* and *Fusarium* spp levels. *Microdochium nivale* levels were generally lower than *Fusarium* spp. Disease levels were higher in Optic in untreated plots. Flowering dates are similar for Optic and Forensic while Waggon is a later flowering variety (HGCA, 2010)

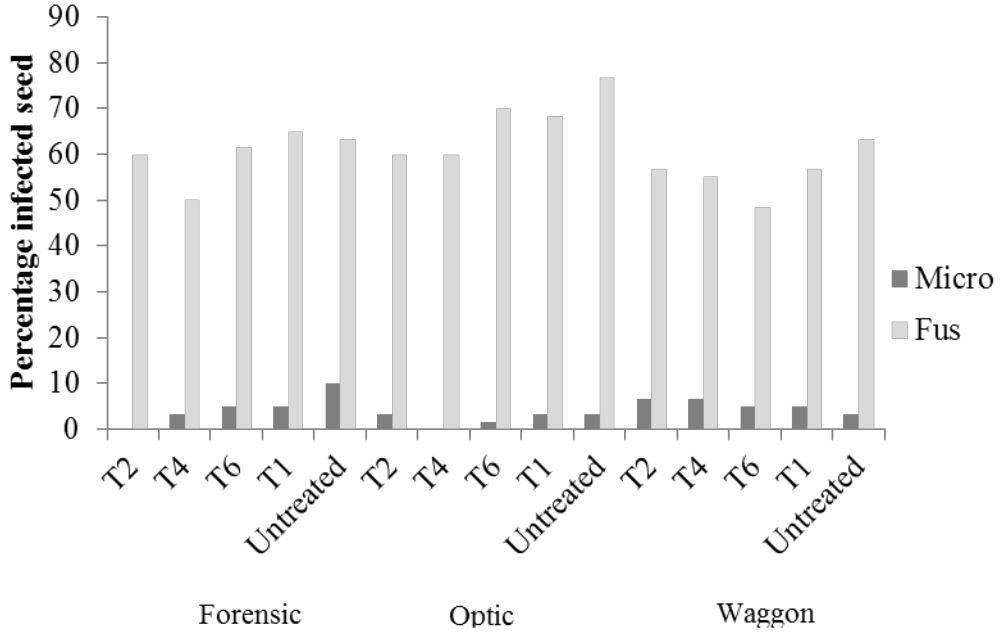

Figure 1. Fungal infection in seed from Trial 1.
LSD (P=0.05) Micro = 6.29, Fus = 22.14

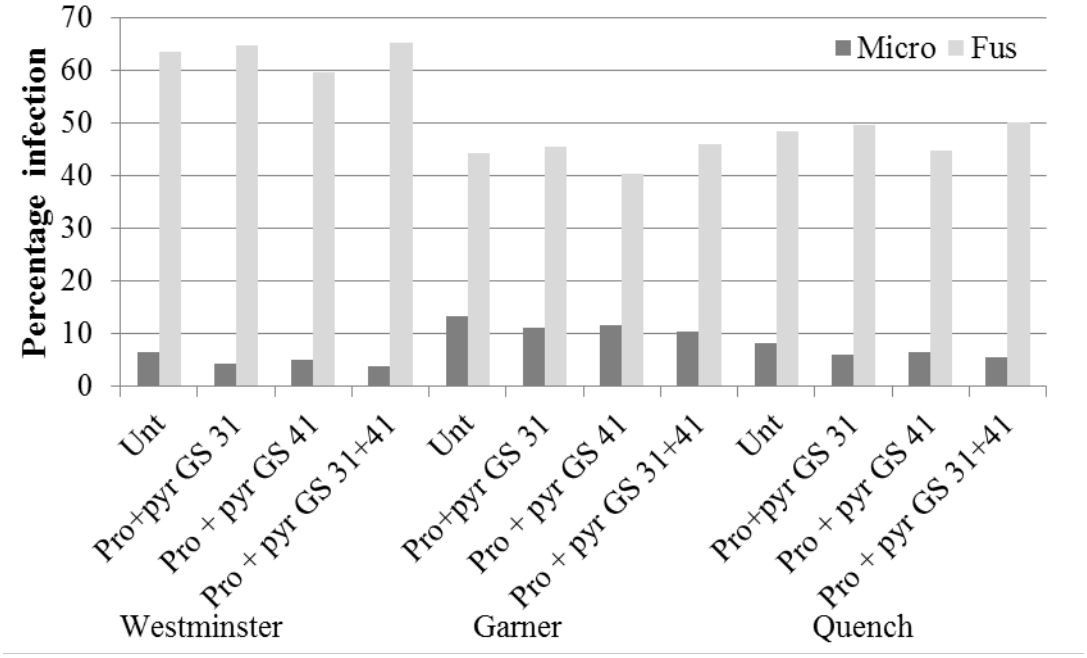

Figure 2. Fungal infection in Trial 2.

LSD (P=0.05), Micro = 6.43, Fus = 17.3

DISCUSSION

A number have methods have been proposed for the movement of FHB from the base of the crop to the ear (Parry *et al.*, 1995). These include contaminated insect vectors, systemic growth within the plant, wind and rain splash of spores. Propagules of *Fusarium avenceum*, *Fusarium. culmorum, Fusarium graminearum* and *Fusarium poae* have been trapped at ear height in cereal crops (Martin, 1988). In addition, ascospore concentration of *Microdochium nivale* 20 cm above the soil has been shown to increase 20 fold after rain (Millar & Colhoun, 1969).

The summer of 2012 was remarkable for rainfall levels, with many areas in Scotland experiencing over 200% of their normal summer rainfall (Met Office). A meteorological station was situated at Bush Estate, close to the trial site. Rainfall data for July showed very few dry periods and one heavy rainfall event (over 80mm) on the 13th of July (Fig. 3). This was still during the spring barley flowering period in the trials. These very wet conditions facilitated the movement of *Fusarium* spp spores up the crop canopy and onto the ear. Many of the trials at the site exhibited FHB symptoms.

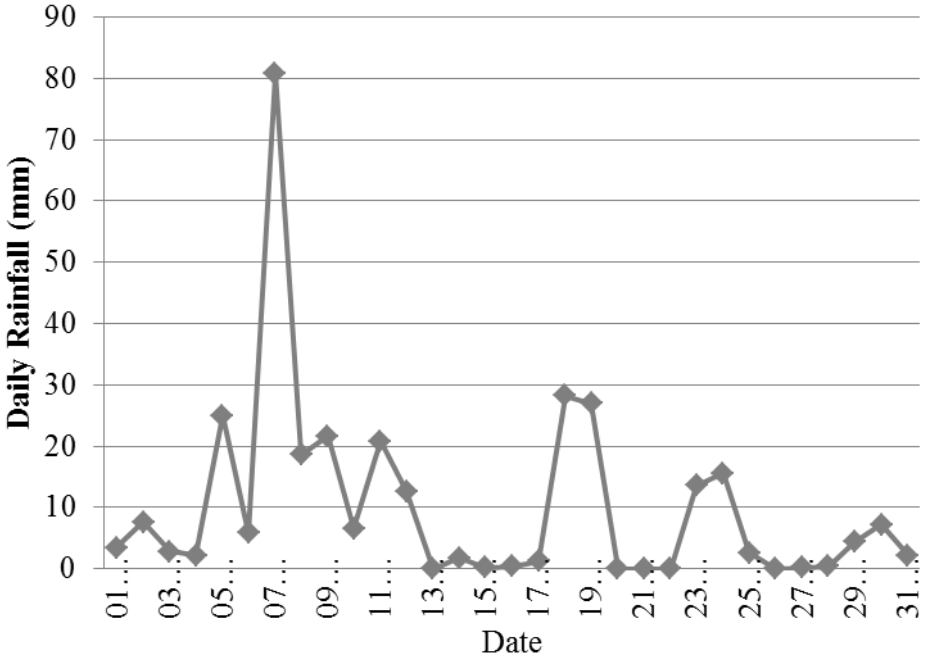

Figure 3. Rainfall at Bush Estate, Midlothian July 2012.

The growing conditions in 2012 gave rise to very high disease levels and the spray programs applied gave no significant decrease in disease levels. In stark contrast, the 2013 season was more normal in terms of rainfall and slightly warmer and this in turn gave rise to very low levels of disease. This trial study is ongoing in order to determine the most effective spray applications to control *Fusarium* spp and *M. nivale*.

ACKNOWLEDGEMENTS

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RHYNCHOSPORIUM COMMUNE – UNDERSTANDING THE EFFECTS OF VARIETY, FUNGICIDE RESISTANCE AND SEED-BORNE INFECTION ON DISEASE LEVELS IN BARLEY

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Summary: *Rhynchosporium commune* is one of the most devastating diseases of barley crops in Northern Britain due to the cool and wet growing conditions. Crop losses of 30-40% have been recorded. This paper will examine the influence of three major factors on disease epidemics, viz the roles of varietal resistance to symptom expression, fungicide resistance exhibited by the pathogen and fungal seed-borne infection are examined. A combination of field trials, laboratory assays and quantitative molecular diagnostics was utilised in this HGCA-funded project. An assessment of the effect of *R. commune* disease levels on yield was also produced from SRUC field data. The implications for future *R. commune* control programmes are discussed.

INTRODUCTION

The fungal pathogen *Rhynchosporium commune* is the major biotic factor involved in the formation of rhynchosporium lesions on barley crops (Zaffarano *et al.,* 2011). Lesions are initially grey/green but eventually darken with a distinct black edge. Rhynchosporium is now considered to be the major economic disease to affect UK winter and spring barley crops with yield losses as high as 30-40% being recorded (Fitt *et al.,* 2012). These losses are accompanied by reductions in grain quality. The primary inoculum for *R. commune* is considered to arise from crop debris and seed-borne infection with secondary infection due to the release of rain splash spores from infected lesions (Zhan *et al.,* 2008, Fountaine *et al.* 2010). The polycyclic nature of *R. commune* and the recent discovery of asymptomatic colonisation of barley by the fungus, suggest that the pathogen can undergo many generations *in planta* before symptoms appear in the crop.

The timescale and length of growing period differences of winter and spring barley mean rhynchosporium control is different in each crop. The presence of *R. commune* in seed initiates disease symptoms in winter crops, which can spread in appropriate conditions over winter. Therefore, control programmes are based on eradication of the pathogen. Spring barley in contrast grows more rapidly in warmer conditions and the pathogen has less time to colonise

the barley plant and produce symptoms, therefore programmes are protectant and aimed at the preventing infection of new crop growth. Management of rhynchosporium in crops therefore requires a balance between genetic resistance and fungicide usage. However, in some cases, e.g. brewing, the market can limit the scope for the use of resistant varieties.

Varietal resistance ratings for some winter barley varieties have been questioned as disease levels on varieties rated as resistant have been shown to be significant in high disease pressure situations. Previous HGCA research suggested timing of assessments may be one reason for this apparently anomaly (Oxley *et al*., 2007). The new research which suggests seed infection is an important source of disease may be the cause of an unpredictable relationship between resistance ratings and field performance. Seed infection may also be responsible for spreading strains of *R. commune* less sensitive to fungicides from one region to another and this observation may help explain why the presence of resistant *R. commune* is not always related to previous fungicide use on a farm. These discoveries highlight the importance to growers of timely and robust fungicide programmes to protect crop yield and of careful stewardship of fungicides to maintain an effective arsenal against rhynchosporium. Fungicide performance data consistently indicates that protectant sprays are more effective than eradicant ones (HGCA, 2013).

In previous research into the optimal fungicide use to control rhynchosporium, treatment with a single fungicide did not achieve the best disease control, yield or margin. Prothioconazole (Proline®) was the key fungicide component in a fungicide mixture for both disease control and yield in winter and spring barley. Cyprodinil (Unix®) was also key for yield in winter barley, but less important in spring barley. Pyraclostrobin (Vivid®) was an important component of a mixture where rhynchosporium eradication was required. Chlorothalonil (Bravo®) was a useful mixing partner, but in two-way mixtures, rhynchosporium eradication was reduced where the dose ratio was 1:1. This effect was not seen in a three-way mixture where the dose ratio of chlorothalonil to other fungicides was 0.5:1 (Oxley *et al*., 2007).

Previous work has shown that *R. commune* DNA quantification late in the season can give a greater understanding of the effect of fungicide applications on yield response (Oxley *et al.*, 2007). The lowest yield responses occurred where both pathogen DNA levels and disease symptoms were low in the upper leaves. Plant breeders may need to re-define a resistant variety as one where visual disease symptoms and fungal DNA are both absent. The results suggested that varieties can respond both to fungicide in the absence of visual disease symptoms and also in cases where the fungus is detectable at 10-40 pg DNA levels inside symptom-free plants.

A number of fungicide groups are known to be active against *R. commune* including the triazoles, QoI's (strobilurins) and succinate dehydrogenase inhibitors (SDHI's). However the SDHI's carry an increased risk of resistance development and are not recommended as a straight fungicide application. At present it is not clear whether varietal susceptibility has any effect on the sensitivity of *R. commune* isolates present on the crop.

The use of molecular tools to quantify fungal DNA load in crops is an area of increasing interest in crop protection. *R. commune* has been shown to be carried on the surface of barley seed and so may be affected by fungicidal seed treatments. However, the use of highly infected home-saved seed could lead to higher early disease levels in crops and associated yield loss. *R. commune* has been shown to grow asymptomatically in barley for several generations until

environmental conditions are conducive for symptom development. (Newton *et al*., 2010). Therefore it was worth examining any potential relationship between fungal DNA, site, disease levels and yield loss.

The project described in this paper had three main objectives: i) Determine the importance of seed borne *R. commune* infection on early disease outbreaks for both susceptible and resistant varieties in different regions of the UK; ii) Establish whether seed infection plays an important role in differential selection for *R. commune* populations with reduced fungicide sensitivity; and iii) Produce advice to growers on the importance and management of seed infection and on the best fungicide mixtures to use on both susceptible and resistant varieties for cost effective disease control.

MATERIALS AND METHODS

Field Trials

A series of field trials was carried out over the three years of the project. Eight winter and eight spring barley varieties with a range of resistance ratings to rhynchosporium were grown at three different geographical sites (Aberdeenshire, Northern Scotland, Fife, Central Scotland and Northern Ireland). Plots were 10m x 2m. Seed was collected from different sources: home-saved (untreated), certified commercial (triadimenol (150g/l) + triflumuron (4g/l) (Baytan®) treated), and home-saved (treated). Home-saved treated seed was treated with triadimenol + triflumuron in year one but in the remaining years was treated by a hot water treatment (60°C for 2 h, then 72 h at 25°C in an oven to dry the seed). Disease levels were assessed at regular intervals and plots were yielded.

R. commune DNA quantification

Seeds, plants at GS31 and F-1 leaves late in the growing season were sampled. DNA was extracted from plant material and DNA quantified using methods described previously (Fountaine *et al.,* 2007).

Sensitivity assays

Leaf samples from the field trials were sent to SRUC in Edinburgh and the fungus isolated from lesions as described previously (Oxley *et al.,* 2007). The sensitivity of the fungal isolates to six fungicides was determined using the 96 multiwell plate assay described previously (Oxley *et al.,* 2007). Fungicides chosen were prothioconazole (pro), cyprodinil (cypr), epoxyconazole (epoxy) (Opus®), pyraclostrobin (pyr), fenpropimorph (fen) (Corbel®) and fluoxystrobin (flu). Isolates were collected from infected leaves as described previously (Oxley *et al.,* 2007). 172 isolates were tested in total.

Relationship between disease and yield loss

Disease data from 283 spring barley crops and 59 winter barley crops was used along with yield data to investigate the relationship between symptoms and crop yield (Figure 1). Regression analysis was carried out by analysis of covariance in a general linear model using the Minitab statistical software (v16).

Statistical analysis

The interactions between crop symptoms, pathogen DNA levels and crop yield, and the influence of other factors on each component were analysed using REML analysis in the Genstat 12 statistical package (VSN International). Resistance levels were categorised into low, medium and high for analysis purposes.

RESULTS

In general disease levels varied from year to year and site to site.

Table 1. Significant interactions between fixed effects (factors) and disease levels and yield and quality in trials.

Factor	Winter Barley				Spring Barley			
	Yield (t/ha)	Specific wt (kg/hl)	Early Rhynch (%dis)	Mid Rhynch (%dis)	Yield (t/ha)	Specifi cc wt (kg/hl)	Early Rhynch (%dis)	Mid Rhynch (%dis)
Year	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.
Site	*	*	n.s.	n.s.	*	**	n.s.	n.s.
Variety	*	**	***	***	n.s.	**	n.s.	n.s.
Seed Treat	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Inter-action	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

* < (*P*=0.05), ** < (*P*=0.01), *** < (*P*=0.001), n.s. = not significant

The factor with the greatest influence on yield and disease levels was Variety (Table 1).

Table 2. Significant interactions between fixed effects (factors) and *R.commune* DNA levels (pgrams) in seed, crop and harvested grain.

Factor	Winter Barley					Spring Barley			
	Seed	Early crop	Mid crop	Leaf F-	Grain	Seed	Early	Mid	Grain
Year	n.s.	n.s.	*	n.s.	*	n/a	n.s.	n.s.	n.s.
Site	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
Variety	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.
Seed Treat	***	**	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
Interaction	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

* < (*P*=0.05), ** < (*P*=0.01), *** < (*P*=0.001), n.s. = not significant (*P*>0.05)

The factor with the greatest influence on *R. commune* DNA levels was the seed treatment applied to the grain. *R. commune* DNA levels in seed and early crops were reduced in winter barley and *R. commune* DNA levels in seed in spring barley (Table 2).

Table 3. Significant interactions between fixed effects (factors) and *R. commune* sensitivity to fungicides.

Factor	Epoxy (ppm)	Pyr (ppm)	Fen (ppm)	Flu (ppm)	Pro (ppm)	Cyp (ppm)
Resistance Rating	*	n.s.	n.s.	n.s.	n.s.	n.s.
Crop	n.s.	**	**	*	**	***
Year	***	***	***	***	***	***
Site	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Treat	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Growth Stage	n.s.	**	n.s.	n.s.	n.s.	n.s.

* < (*P*=0.05), ** < (*P*=0.01), *** <(*P*=0.001), n.s. = not significant (*P*>0.05)

The most significant effect on *R. commune* sensitivity to fungicides was year (significant for all fungicides), with crop also being a significant interaction (Table 3).

Covariance analysis was undertaken to determine the significance of the interactions between disease levels, *R. commune* DNA and fungicide sensitivity.

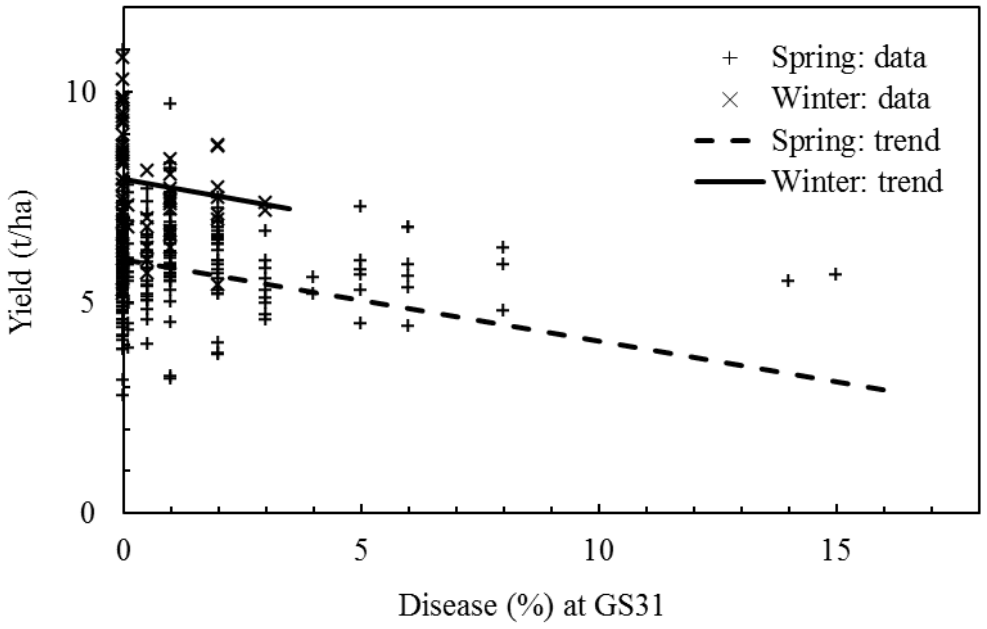

Figure 1. Effect of yield (t/ha) and rhynchosporium disease symptoms at GS31.

A trend was observed between visual disease symptoms and yield loss in trials. Both lines had a common slope but different intercepts (Figure 1). Yield loss is approximately 0.2 t/ha/% visual disease at GS31.

DISCUSSION

Analysis of the field trial results indicated the strong influence of variety on disease levels in the crop (Table 1). Varietal resistance is one of the most effective tools available to growers with spring barley varieties having ratings of 8 and 7 (HGCA, 2014). The greater influence of variety on winter barley rather than spring disease levels in the analysis may reflect lower genetic resistance but perhaps the longer period the fungus has to establish infection in the crop. The strong interaction between seed treatments and fungal DNA (Table 2) highlights the strong influence of seed infection on crop infection in winter barley (Fitt *et al.*, 2012). However, covariance analysis of results suggests seed infection is not critical to early disease epidemics (data not shown). This could be a result of asymptomatic growth. Fountaine *et al.*, (2010) found a four month time lag between sowing infected winter barley seed and symptoms developing. Sensitivity assays indicated that the fungus remains susceptible to the major fungicides used in rhynchosporium control (data not shown). There were significant correlations between DNA levels and symptoms in the mid and late periods of the growing season (data not shown) and also between disease levels and final yield and quality, especially in winter barley. This confirms the importance of disease levels and final yield at GS31 demonstrated in the data from SRUC trials (Figure 1). The importance of disease levels at GS31 will allow growers to tailor their fungicide programmes to minimise rhynchosporium disease at stem extension.

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FUNGICIDE SENSITIVITY IN POPULATIONS OF *RAMULARIA COLLO-CYGNI*

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Summary: *Ramularia collo-cygni* (Rcc) is now one of the major pathogens of barley in the UK and is extending its geographical range across Europe. Control of the pathogen relies on chemical control as no complete varietal resistance is yet available A range of fungicides are now used to control the disease during the growing season. However, Rcc has shown itself to be capable of rapid mutations which lead in turn to the increased risk of fungicide resistance developing. Fungicide sensitivity in Rcc (in the form of EC_{50} values) has been compared between isolates from 2012. The implications for continuing effective disease control are discussed.

INTRODUCTION

Ramularia collo-cygni (Rcc) is the fungal agent responsible for Ramularia leaf spot (RLS). It has been present as a major pathogen of barley in Scotland since the late 1990s (Oxley *et al.*, 2002). Several different classes of fungicides such as the succinate dehydrogenase inhibitors (SDHIs), sterol demethylation inhibitors (DMIs) and chlorophenyls e.g. chlorothalonil can be used to control disease (Havis *et al.*, 2012). During the late 1990's RLS was controlled by QoI (quinone outside inhibitor) fungicides. However, during 2002 there was a marked decline in activity to QoI fungicides in comparison with previous years (Oxley & Hunter 2005). This was due to the development of QoI resistance among populations of Rcc in the UK between 2001 and 2002 (Fountaine & Fraaije, 2009). The rapid appearance of QoI resistance highlights the importance of monitoring the efficacy of the most important fungicides used in RLS control, so that any shift in sensitivity can lead to the rapid introduction of new anti resistance strategies.

MATERIALS AND METHODS

Leaf samples, were collected from SRUC field trial site at Boghall Farm, Midlothian (Eastern Scotland) during the 2012 season.

Single spore Rcc cultures were produced by careful isolation of conidia from infected lesions placed onto Potato Dextrose Agar supplemented with the antibiotic streptomycin. Mycelial suspensions were produced from single spore isolates grown in Alkyl Ester Broth for 10 days at 16°C on an orbital shaker. The suspensions were filtered and diluted to a concentration of 5 x 10^6 pieces of mycelium per ml. (M Piotrowska, unpublished).

The 96 well plate assay was conducted using final fungicide concentrations of 50, 10, 5, 1, 0.5, 0.1 and 0.05 ppm in a total volume of 200 µl (100 µl of mycelial suspension added to 100 µl of fungicide amended media). Plates were incubated at 16^{o}C, with continuous shaking, for one week before being read in plate reader (BMG Omega reader, Switzerland) at a wavelength of 400nm, effective concentration (EC50) values were calculated by the Omega software.

RESULTS

The Bush Rcc isolates were tested against 4 technical grade fungicides – prothioconazole, fluxapyroxad, zoxamide and epoxiconazole (Figure 1). Epoxiconazole, a DMI fungicide showed the highest EC50 value range (0.025 to 1.025). Next was the carboximide, fluxapyroxad which ranged from the lowest EC50 of 0.0002 to 0.266. Both zoxamide (MBC) and prothioconazole had very similar EC50 values 0.010 to 0.039 and 0.007 to 0.057 respectively.

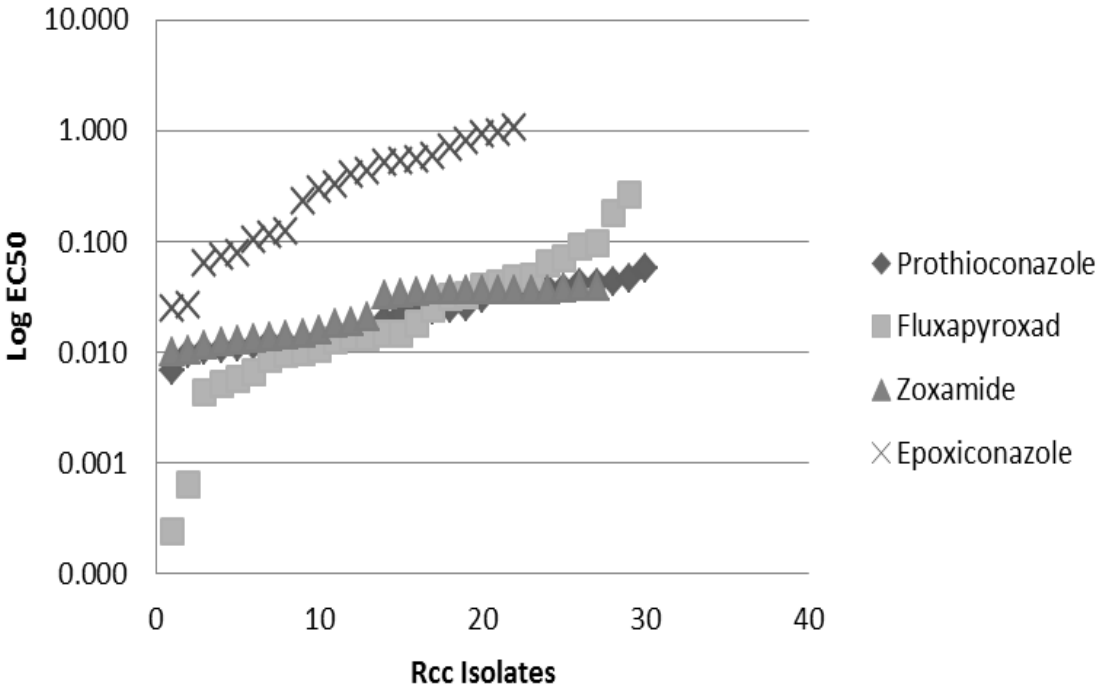

Figure 1. EC50 values for Rcc isolates (Bush 2012). Mean (ppm) Prothioconazole 0.024, Fluxapyroxad 0.041, Zoxamide 0.026, Epoxiconazole 0.403. LSD ($P<0.05$) 0.08.

The QoI fungicide, picoxystrobin and MBC fungicide, carbendazim were also tested. However, very few of the Rcc isolates showed any sensitivity to these fungicides. The majority of isolates showing complete growth in all the fungicide concentrations tested and are therefore not included in the presented data.

DISCUSSION

A range of fungicides, often in a combination, are now used to control the disease during the growing season. However, Rcc has shown itself to be capable of rapid mutations which lead in turn to the possibility of fungicide resistance. Initially RLS was controlled by the use of QoI fungicides. However, from 2002 onwards there was a marked decline in their efficiency which was due to the development of QoI resistance among populations of Rcc. This situation has been contributed to a single amino acid substitution changing glycine to alanine at codon 143 in cytochrome *b* gene (Fountaine & Fraaije, 2009) . The Bush Rcc isolates are all QoI resistant as some growth occurred even in the presence of picoxystrobin fungicide at a concentration of 50 ppm.

Of the two DMI fungicides tested Rcc isolates had higher EC50 values for epoxiconazole than for prothioconazole. Fungicide guidelines indicate that prothioconazole can still be applied to give good protection against *Ramularia* whereas epoxiconazole is applied as a mixture with fluxapyroxad (SDHI). These sensitivity figures reflect the different performance achieved by both fungicides in HGCA sponsored trials and reported previously (Havis *et al*., 2012)**.**

Zoxamide gave very similar range of EC50 values to prothioconazole, despite not being used in barley crop protection programmes. Zoxamide resistant isolates have a mutational change at codon 200, (Fountaine & Fraaije unpublished), however there appears to be none of these highly resistant isolates among the Bush population. The decline in use of MBC fungicides and the selection pressure from other groups may have lead to the increased sensitivity within the Bush population.

To date only the Rcc isolates from Bush have been screened in this fungicide assay. Single spore Rcc from Lanark (Central Scotland) are currently been tested against the fungicides and their EC50 values will be presented at a later date. A comparison will be made between the Rcc isolates EC50 values for the fungicides used, to observe if the trends are similar to the Bush isolates or if there is a geographical difference.

Any isolate showing high resistance, sensitivity or cross resistance can be further investigated by DNA sequencing to establish if any mutation is present.

ACKNOWLEDGMENTS

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FROM *RHYNCHOSPORIUM COMMUNE* GENOME SEQUENCE TO BARLEY RESISTANCE

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Summary: The development of sustainable strategies for the management of *Rhynchosporium commune*, one of the most destructive fungal pathogens of barley, depends on an improved understanding of its biology and interaction with barley. We are exploiting the pathogen genome sequence to identify genes encoding secreted effector proteins, especially the ones essential for pathogenicity. We are also exploring the possibility of using these fungal effectors as molecular triggers of novel, potentially durable, plant disease resistance genes that may be present in the James Hutton Institute collection of barley varieties and landraces exhibiting scald resistance in the field.

Leaf scald caused by the fungal pathogen *Rhynchosporium commune* is one of the most economically significant diseases of barley worldwide. The disease regularly occurs in wetter parts of the UK, particularly southwest and northern England, as well as Scotland and Northern Ireland (HGCA, 2011). It can decrease yield by 30-40% as well as reduce grain quality (Shipton *et al.*, 1974). Despite routine fungicide applications *R. commune* costs the UK economy £7.2 million per year (assuming a current average barley price of £150 per tonne) (HGCA, 2013). *R. commune* development is characterised by a long phase of asymptomatic growth between penetration of the leaf cuticle and occurrence of the disease symptoms, necrotic lesions with dark brown margins (Davis & Fitt, 1990; Lehnackers & Knogge, 1990). Current control strategies rely heavily on fungicide application. Legal restrictions on current chemical classes and decreasing fungicidal efficacy necessitates the introduction and maintenance of effective cultivar resistance in order to achieve sustainable disease management.

All pathogens trigger non-host resistance (NHR) in plants. Successful pathogens can suppress or manipulate NHR by secretion of small proteins called 'effectors'. Once NHR has been suppressed, plants deploy a second layer of defence in the form of resistance (*R*) genes. The products of *R* genes are thought to detect pathogen effectors, termed 'avirulence' (Avr) proteins, and activate resistance responses. To avoid recognition by resistance proteins pathogens can lose either the expression or function of some effectors with no apparent cost to pathogen fitness. Both of these strategies have been deployed by *R. commune* to overcome

Rrs1-mediated resistance within 5 years (Avrova & Knogge, 2012; Houston & Ashworth, 1957). Similarly, while most of the more recently developed spring barley cultivars carry another gene for resistance to *R. commune* called *Rrs2*, their continued use has already led to rapid breakdown of this new resistance. Thus, a deeper understanding of the biology of *R. commune* and its interaction with barley is required for the development of sustainable management strategies for this pathogen.

Sequencing of the *R. commune* genome and transcriptome (protein coding RNA) from germinating conidia and barley leaves infected with *R. commune* provided a unique opportunity to identify the putative fungal effectors mediating interactions with its host plant barley. Genome comparison of 9 *R. commune* strains allowed rapid prediction of candidate effectors that are either conserved or not very variable in *R. commune* populations. These effector types are more likely to be essential for pathogenicity. Plant *R* genes targeting essential pathogen effectors are likely to provide more durable resistance. Transcription of candidate *R. commune* effectors has been profiled during barley infection using quantitative RT-PCR. Targeted gene disruption of candidate effectors found to be highly abundant early during barley infection will help to determine their importance for pathogenicity. At the same time *Barley stripe mosaic virus* (BSMV) mediated transient expression system is being used to aid functional characterisation of predicted effectors. The aim here is to screen genetically diverse barley accessions and identify those 'responding' to application of *R. commune* effectors by mounting a defensive reaction in the form of cell death. The *R* genes mediating fungal effector recognition in these accessions will then be characterised and eventually exploited as novel sources of potentially durable resistance to *R. commune.*

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CEPHALOSPORIUM GRAMINEUM – A POTENTIAL THREAT TO CROPS GROWN IN SHORT ROTATIONS

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Summary: The fungal pathogen, *Cephalosporium gramineum*, causes leaf stripe symptoms in cereal crops. Disease symptoms have been observed in cereal crops grown in short rotations and the disease is described as trash-borne. As part of an HGCA funded study the severity of leaf stripe symptoms and their effect on grain yield was recorded in winter wheat recommended list trials. Leaf stripe was also recorded in winter barley and winter oat crops. Disease severity was found to vary depending on variety and season. In general, wheat was more susceptible than barley or oats. The susceptibility of wheat varieties to the fungus was quantified and resistance ratings calculated for current varieties. The effect of rotation and cultivation techniques on *C. gramineum* levels within soil has also been examined and the implications for disease control are discussed.

INTRODUCTION

Cephalosporium leaf stripe is caused by the fungus *Hymenella cerealis*, (*Cephalosporium gramineum).* Affected tillers appear randomly through the crop and show a distinct yellow stripe which extends to the leaf sheath. The fungus has a wide geographical range and has been reported in Germany, Italy, Netherlands, USA, Poland, Japan and UK (Martynuik, 1995). Infection is more common in crops grown in short rotations. Wheat is the major economic host, but other cereal hosts include oats, barley, rye, triticale and grass hosts include *Bromes* sp., *Dactylis glomerata* and *Agropyron repens* (Howell & Burgess, 1969). The causal fungus is a slow growing fungus in the soil but it is favoured by wet soil conditions and continuous cereal cropping.

The soil-borne fungus enters plants via the roots during winter and early spring. There is evidence that the fungus can be transmitted by seed (Murray 2006), but there is no information on the impact fungicide seed treatments have on the disease at these early stages. Once inside the plant, the fungus moves up the plant causing blockage at the nodes, distinctive leaf symptoms and stunting. At harvest the fungus returns to the soil in the trash. Removing straw, ploughing and, where permitted, burning are the most effective ways to prevent a build up of the problem (Christian *et al.,* 1983). If straw removal is not practical, then deeper ploughing to remove the straw from the root zone may help. In the USA yield losses of up to 80% have been recorded in susceptible varieties (Quincke *et al.,* 2012). In the UK yield losses have been estimated to be in the region of 0.5 t/ha but there was previously no measure of yield loss in replicated field trials. An HGCA-funded project was established to look at the incidence of leaf

stripe and the relationship between disease levels and yield in cereals. The project also had the aims of identifying useful crop protection chemicals and investigating the impact of cultivation and straw management on pathogen survivability. A quantitative PCR for *C. gramineum* was developed as part of the project and its use is reported at this conference (Gorniak & Havis, unpublished)

MATERIALS AND METHODS

Field Trials

A series of field trials were carried out a site in East Lothian, Scotland between 2009 and 2011. Leaf stripe symptoms had been observed in the preceding winter wheat crop prior to the first year of trials. The winter wheat Recommended List was sown in 10m x 2m plots. The plots received fertiliser, herbicide and growth regulator but no fungicide. Plots were assessed regularly for disease and yielded at the end of the trial. In 2010 and 2011 winter barley and winter oat recommended lists were also sown at the site and assessed as above. Ten additional plots of cv. Alchemy were also sown. Five plots were prepared conventionally using a plough and five using minimum tillage. Disease levels were assessed throughout the trial.

Sensitivity assays

C. gramineum was isolated from leaf samples in 2009 using the method of Stiles and Murray (1996). Isolates were grown in Potato Dextrose Broth at 20 ºC for 7 days and a spore suspension produced by sonicating the culture for 1 minute and vortexing for 2 minutes. Sensitivity assays were carried out on a 98 well plate with wells containing 20 µl of spore suspension and 180 µl of fungicide amended media. Each concentration was tested in triplicate and plates were placed on an orbital shaker at 20 ºC for 7 days. Plates were read at 400nm absorbance on a plate reader (Omega, BMG, Germany) and EC_{50} values calculated using the MARS data software.

RESULTS

Results from the 2009 season indicated a relationship between leaf stripe symptoms and yield in the winter wheat Recommended List (Figure 1). Data on disease incidence for the wheat trials over the length of the project were analysed by HGCA to produce provisional resistance ratings for the recommended list.

The calculated resistance ratings show a range from 4 to 8 indicating a spread in susceptibility to the pathogen in UK wheat varieties (Table 1).

The figures on fungicide sensitivity (Table 2) show a wide range in the sensitivity of the pathogen to seed treatment chemicals. The highest mean is 9.99 ppm for fluquiconazole but the range of sensitivities recorded was very wide.

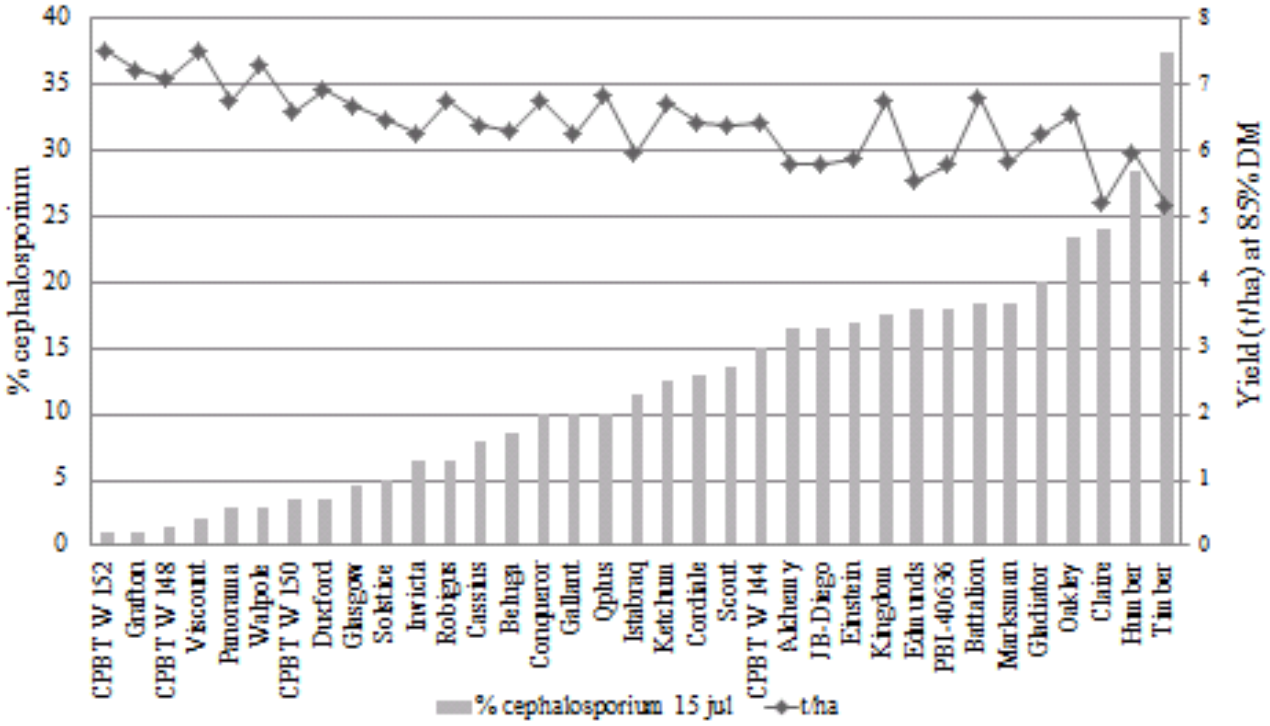

Figure 1. Leaf Stripe symptoms and yield in winter wheat, East Lothian 2009. LSD (P=0.05), Yield=0.72t/ha, % Cephalosporium=13.34.

Table 1. Provisional Resistance Ratings for *C. gramineum.*

Variety	Resistance Rating	Variety	Resistance Rating	Variety	Resistance Rating
Denman	4	Gallant	6	Duxford	7
Timber	4	Invicta	6	Gravitas	7
Torch	4	JB-Diego	6	KWS-Gator	7
Crusoe	5	KWS-Solo	6	KWS-Podium	7
Horatio	5	KWS-Target	6	KWS-Santiago	7
Oakley	5	Monterey	6	KWS-Sterling	7
Alchemy	6	Scout	6	Relay	7
Beluga	6	Solstice	6	SY-Epson	7
Cocoon	6	Stigg	6	Tuxedo	7
Conqueror	6	Warrior	6	Viscount	7
Cordiale	6	Claire	7	Chilton	8
Einstein	6	Delphi	7	Grafton	8
				Panorama	8

Table 2. Sensitivity of *C. gramineum* isolates to the major seed treatment chemicals.

Active Ingredient	Seed Treatment	EC_{50} Range (ppm)	EC_{50} Mean (ppm)
Carboxin	Anchor ®	0.06 – 9.34	2.84
Fludioxonill	Maxim XL ®	0.001 – 4.61	0.83
Fluquiconazole	Jockey ®	0.047 -49.5	9.99
Epoxyconazole	Tracker ®	0.002 – 90.4	6.19
Prochloraz	Kinto ®	0.01 – 21.04	3.38
Prothioconazole	Redigo ®	0.099 -14.3	2.33
Silthiofam	Latitude ®	0.005 – 4.09	1.18
Triticonazole	Kinto ®	0.29 – 20.98	2.49

DISCUSSION

Analysis of the data from the field trials over the length of the project indicated seasonal variation in disease severity. Mean disease levels were greater in 2009 than 2010 and 2011 respectively. This was the only year that the trial plots were a third consecutive wheat crop. In other years the wheat plots followed winter barley or oilseed rape. Rainfall has been associated with spreading fungal inoculum from the soil surface to the plant roots (Mathre & Johnston, 1975). Examination of the weather data for East Lothian for the trialling years showed no significant difference in winter/ spring rainfall between the sites. The fungus is also known to enter via damaged roots (Murray, 2006). This damage can be the result of freezing and thawing cycles in the soil or low pH. Weather data indicated lower temperatures during the winter of 2010 but this did not lead to increased disease levels. Previous crop seemed to be exerting the greatest influence on disease levels at this site.

The effect of insect damage on cereal crop roots has also been highlighted as a factor in Cephalosporium epidemics. (Slope & Bardner, 1965). Wireworm damage in wheat crops following a grass ley contributed to leaf stripe levels of 7% in field experiments. Wheat crops following oats or beans were free from disease symptoms.

Figure 1 shows a general trend in the winter wheat varieties between disease symptoms and yield in 2009. In the following year's trials yield seemed relatively unaffected by Cephalosporium leaf stripe (data not shown). Over the course of the trials disease levels were higher in wheat than barley and oats (data not shown). This has been observed in Canada and the US, where Cephalosporium leaf stripe is only considered to be economically important on wheat (Mundt, 2002).

Provisional resistance ratings indicate a spread in susceptibility to the pathogen within wheat varieties (Table 1). This range has also been observed in US varieties, with only a few resistant varieties produced in breeding programmes (Quincke *et al.,* 2012). Varietal resistance to the fungus relies on either exclusion or suppression of the fungus post entry (Morton & Mathre, 1980).

The fungus has a wide range of susceptibility to seed treatment chemicals (Table 2). In Canada/US control of the pathogen relies solely on cultural methods (Quincke *et al.,* 2012).

However, the results obtained from our trials indicate that growth of many fungal isolates can be controlled by fungicides.

The spores of the pathogen are known to have a limited half-life in soil (Weise & Ravencroft, 1975). Cephalosporium leaf stripe is a disease which has been described as more prevalent in minimum tillage systems (Bokus & Shroyer, 1998). Our experiments showed conflicting results in that foliar symptoms appeared to be higher in ploughed plots in 2010. In contrast, disease symptoms appeared higher in the minimum tillage plots in 2011 and ploughed plots produced significantly higher yield.

The results from this project show that *C. gramineum* is a pathogen which is present in the UK and that seed-borne movement may allow it to become a more serious problem in shorter rotation, minimum tillage systems

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ON THE PATH OF DISCOVERY: *RHYNCHOSPORIUM COMMUNE* EFFECTORS ACTIVATING SCALD RESISTANCE IN BARLEY

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Summary: This research aims to identify durable forms of resistance to *Rhynchosporium commune* - a destructive fungal pathogen of barley. There is scope to improve breeding strategies and disease management through the discovery of novel avirulence (*Avr*) genes that are required for pathogenicity. Resistance genes recognising these essential avirulence genes could prove to be a form of more durable resistance that is not so easily overcome by this pathogen. Little is known about other *Avr* genes apart from the necrosis inducing peptide, *Nip1* (*AvrRrs1*). This project has the potential to reveal some interesting information on the *R. commune* - barley pathosystem. Whilst *Avr* gene discovery is at the core of this research, the knowledge and data gained from this work will lead to a better understanding of this agronomically important pathogen, its infection process and will provide important information for the deployment of an integrated pest management scheme.

Plants and pathogens evolve in response to each other. The production of pathogen effector proteins, which can manipulate host defense mechanisms, has resulted in the evolution of plant resistance genes, which have the ability to detect these molecules (Jones and Dangl, 2006). Avirulence (*Avr*) genes encode effector molecules that are recognised by resistance proteins and are subsequently a liability to the pathogen, negatively affecting the pathogens ability to infect its host. Three necrosis inducing peptides have been identified to date in *Rhynchosporium commune*, one of the most destructive fungal pathogens of barley worldwide. *R. commune* causes barley scald and is responsible for reduced grain quality and yield losses of up to 40% (Shipton *et al*., 1974). Despite numerous barley resistance genes being identified, only one *Avr* gene (*AvrRrs1 – Nip1*) has been discovered in the pathogen so far (Rohe et al, 1995*). AvrRrs1* (*Nip1*) is recognised in barley cultivars carrying the cognate resistance gene *Rrs1*. However this resistance to *R. commune* has not proved durable. (Rohe *et al*, 1995)

Pathogens have evolved diverse strategies to avoid plant recognition through the inactivation of avirulence genes. Alteration or deletion of the *Nip1* gene are two fitness cost-free mechanisms *R. commune* has adopted to evade recognition (Schurch *et al*., 2004). This indicates that *Nip1* gene is redundant or its function is non-essential. *Avr* genes that are present in all isolates and show less variation are more likely to be required by the pathogen and not so easily disregarded. The discovery of novel *Avr* genes that are required for pathogenicity is an important step to identify more sustainable forms of resistance to this disease.

Less variable candidate genes have been selected from a panel of predicted effector sequences, from which further characterisation can begin, and this has set the platform for avirulence gene discovery. To reveal *Avr-R* gene interactions, sequenced *R. commune* strains have been tested on a set of differential cultivars containing major *R* genes to obtain phenotypes of isolates and cultivars using a detached leaf assay (Newton *et al.*, 2001). So far, one *Avr* gene candidate has been identified by correlating single nucleotide polymorphisms (SNPs) within each sequence with the data obtained from virulence testing. To further validate this result, the gene has been amplified from additional diverse strains with known virulence phenotypes.

The expression of selected *R. commune* candidate effector genes have been analysed during barley colonisation using quantitative RT-PCR. The up regulation of these candidates *in planta* supports a role in pathogenesis. Transcription profiling of candidate effector genes revealed that many of them are expressed at the biotrophic stage of infection. Interestingly, the expression profiles of these candidates showed a similarity to the profiles of the previously identified effectors - the three necrosis inducing peptides (NIPs) (Kirsten *et al*., 2012).

Candidates highly expressed during infection have been selected for functional characterisation to determine if any of these genes are essential for pathogenesis. Additionally, the most fundamental part of *Avr* gene discovery is the actual recognition by the corresponding *R* gene in the barley host plant. Further characterisation will include *in planta* expression for cultivar-specific recognition using co-bombardment and/or BSMV-mediated expression.

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DEVELOPMENT OF A REAL TIME PCR ASSAY TO DETECT AND QUANTIFY *CEPHALOSPORIUM GRAMINEUM* IN CEREALS

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Summary: The fungal pathogen *Cephalsoprium gramineum* (*Hymenella cerealis*) causes leaf stripe disease symptoms in wheat, barley, oats, rye and triticale. This vascular wilt disease is common in wheat in the UK. The soil and trash-borne fungus produces symptoms on wheat tillers as a long yellow stripe which continues onto the leaf sheaths. The pathogen is thought be transmitted to new crops via infected crop debris.

A quantitative PCR assay was developed to rapidly and reliably detect and identify this pathogen. ITS (internal transcribed spacer) region was used to design specific primers and hydrolysis probe. The detection level of the *Cephalosporium* real-time assay was found to be 0.13pg. The detection of fungal DNA in wheat seed presents another possible way in which the fungus can move from one location to another.

INTRODUCTION

The fungal pathogen *Cephalsoprium gramineum* (*Hymenella cerealis*) causes leaf stripe and vascular wilt on a range of cereal crops e.g. wheat, barley, oats, rye and triticale. The fungus has been reported in UK, Germany, Italy, Netherlands, USA, Poland and Japan (Martynuik, 1995). Yield losses in wheat in the UK have been estimated at 0.5 t/ha. Symptoms are common in the UK with wheat the most commonly affected and are prominent in crops grown in short rotations. The fungus appears on tillers as a long yellow stripe, which continues onto the leaf sheaths. Plants infected by disease may show shorter culms, small heads, and light-weight shrunken kernels compared with healthy plants (Mathre, 1977). Wet soil conditions and constant cereal cropping are ideal conditions for the growth of a soil-borne fungus. During winter months and early spring, wheat is infected by *C. gramineum* via the plants root system. Infected seeds have been proposed as a source of the disease and infection may take place in asymptomatic and diseased plants (Murray, 2006).

MATERIALS AND METHODS

Primers and Probe design

Forward CephF1 (Table 1) and reverse CephR1 (Table 1) primers were design by Sigma-Aldrich Co. LLS. Primer forward corresponded to the region from base 316 to 339 of the sequence (KF053537) and primers reverse to the region from 504 to 522 of the same sequence (Figure 1). Hydrolysis probe CephP1 (Table 1) designed by Sigma (described previously) was

labelled at the 5' end with the reporter dye FAM (6-carboxy-fluorescein) and with BHQ-1 dye (Black Hole Quencher 1) at the 3' end. The specificity of primers and probe was checked using the National Centre for Biotechnology Information BLAST server. The absence of nonspecific product on gel electrophoresis, respectively, confirmed the specificity of the assay.

Real-time PCR analysis

Amplification reactions were performed in total volume of 25µl and were run on the Agilent MxPro - Mx3000P (Agilent Technologies UK Limited, Stockport, UK) under the following conditions: 1 cycle at 95 °C for 4min followed by 50 cycles 95°C for 20, 56 °C for 40 s; with a final extension at 72 °C for 30 s. The optimum PCR concentrations of primers and probe were experimentally determined, for each of primers forward and reverse (Table 1) at 300 nM and hydrolysis probe (Table 1) at 100 nM. The real-time PCR reactions were carried out using Brilliant III Ultra-Fast QPCR Master Mix (Agilent Technologies UK Limited, Stockport, UK), 5µl DNA template (20ng), ROX reference dye (Agilent Technologies UK Limited, Stockport, UK) 30nM, sterilized distilled water and primers/probe, as descried previously.

All qPCR reactions were carried out in duplicate for each DNA extract. Sterile distilled water was used as no template negative control. The crossing point value (Cq), which refers to the cycle number where the sample's fluorescence significantly increases above the background level, was calculated automatically by the MxPro – Mx3000P software (version 4.10) as the first maximum of the second derivative of the curve.

Table 1. Oligonucleotides used for cloning and real-time PCR.

Oligonucleotide name	Oligonucleotide type	Sequences (5′–3′) and name
CephF1	forward primer	TGATGTCTGAGTACTATATAATAG
CephR1	reverse primer	GTTATAATGACGCTCGAA
CephP1	TaqMan®probe	FAM-ATCTCTTGGTTCTGGCATCGBHQ1 [a]

[a] Probe labelled with 6-carboxy fluorescein (FAM) and Black Hole Quencher 1 (BHQ-1).

Standard curve preparation and spike test

Standard curves were obtained using five-fold dilution series (from 10ng to 0.128pg) by plotting known amounts of target genomic DNA against Cq values. All standard curve samples were run simultaneously with test samples in each real-time PCR experiment. The amplification efficiency, *E*, was calculated from the slope of the standard curve using the following formula (Bustin *et al*., 2009):

$E = 10^{-1/slope}$, % Efficiency = (E – 1) x 100

Varying amount of fungal genomic DNA (0.01, 0.1 and 1ng) were spiked into fixed amount of plant genomic DNA (1, 5 and 20ng) and used as template in the qPCR assay. The correlations between Cq values and the concentration of input template was then determinate. For all experiments conducted in this study, standard curves with R2 values >0.98 were obtained.

PLANT MATERIAL TESTING

Wheat leaves with distinct lesions were collected in 2010 and area infected was assessed. Leaves were then ground in liquid nitrogen until they were a fine powder. Three 0.1g samples were weighed out and DNA extracted using the Illustra™ Phytopure™ DNA extraction kit (GE Healthcare, UK). Seed samples from the wheat RL trial in East Lothian and from crops across the UK were collected for testing for the presence of *C. gramineum*. Samples of 100 seeds were ground in a food processor (Kenwood, UK) for 5 minutes until they reached the consistency of a fine powder prior to DNA extraction (Lee & Tewari, 2001). DNA was extracted using the Phytopure kit described above.

RESULTS

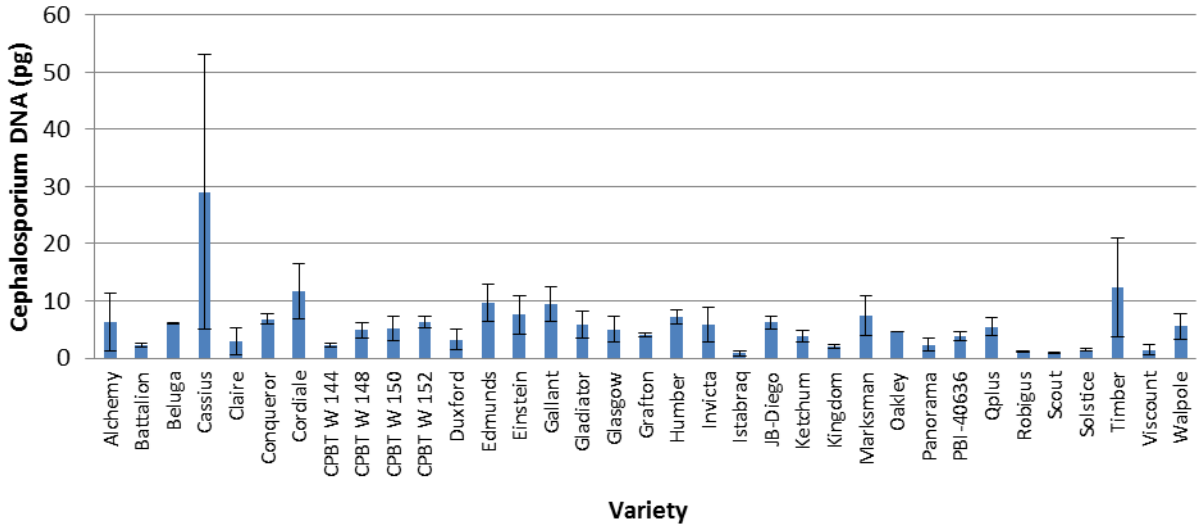

Figure 1. *C. gramineum* DNA in harvested wheat trial in 2009.

Results from Woodhead site from the 2009 season show a wide range of amount of *Cephalosporium* in seeds. The highest mean quantity of the pathogen was recorded in the variety Cassius (29pg).

Table 2. *C. gramineum* DNA levels in seed from 2011 season.

Variety	Region	DNA (pg)	Variety	Region	DNA (pg)
Viscount	East Lothian	3.93	Robigus	Borders	1.02
Viscount	East Lothian	4.05	Gallant	Roxburghshire	0.9
Robigus	Berwickshire	2.32	Claire	Roxburghshire	0.61
Alchemy	Caithness	3.12	Robigus	Perthshire	0.61
Claire	Kincardineshire	0.72	Istabraq	Fife	0.56
Viscount	Aberdeenshire	4.13	Viscount	Berwickshire	0.51
Istabraq	Angus	1.70	Alchemy	Berwickshire	0.73

DISCUSSION

The new diagnostic assay was able to detect *C. gramineum* DNA down to 0.13pg levels. Testing of symptomatic leaves did not produce a clear correlation between visual symptoms and the quantity of fungal DNA in infected leaves (data not shown). This correlation has been shown for other wheat pathogens (Fraaije *et al.*, 2001). The results for *C gramineum* could be due to the limited sample size used in our tests (20 leaves) and the fact that the fungus blocks the plant nodes to induce foliar symptoms. Testing of stems may have revealed a better correlation between foliar symptoms and *C. gramineum* DNA. A relationship was demonstrated between fungal DNA levels and new resistance ratings for winter wheat varieties calculated by HGCA (Havis *et al.*, 2014).

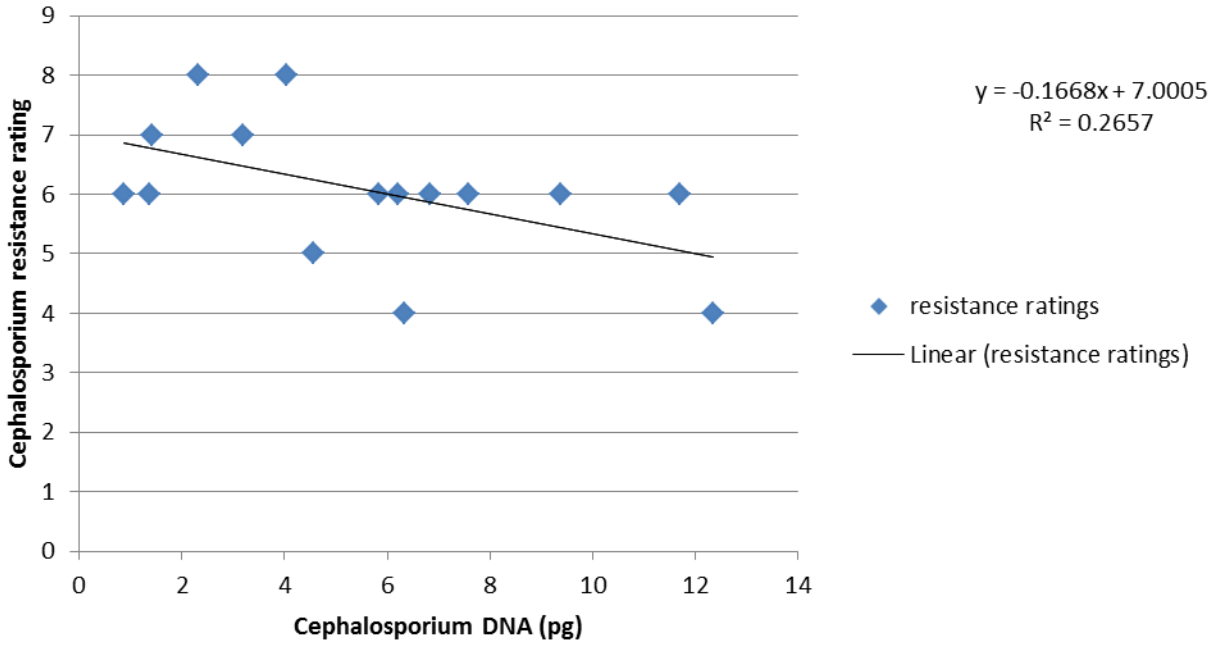

Figure 3. *C. gramineum* DNA vs resistance ratings.

Although the fungus is mainly transmitted via trash and crop debris, the quantification of fungal DNA in seed has shown that the disease can be spread via seed movement and may explain the spread of the disease to new geographical locations. The detailed varietal results presented in this paper were obtained from a trial site in East Lothian, where disease symptoms had been observed in wheat crops. However, testing seed samples from a wider area shows that *C. gramineum* can be detected in wheat from a number of sites across Scotland. Samples obtained from England in 2011 had no detectable *C. gramineum*, although sample size was very low. Cereal growers with short rotations and minimum tillage techniques have to be aware of the potential threat of Cephalosporium leaf stripe. Although no seed treatment chemicals have been approved for control of leaf stripe, recent work has shown that the fungus is sensitive to a number of chemicals used in seed treatments (Havis *et al.*, 2014). The new diagnostic could be used to monitor the efficacy of chemicals against the fungus and also how the fungus can colonise a developing seedling.

The production of new varietal resistance ratings for winter wheat also allows growers to utilise some genetic resistance to reduce the levels of Cephalosporium leaf stripe in their crops. The effect of resistance on infection and spread of the fungus can be tracked now using the qPCR diagnostic.

ACKOWLEDGEMENTS

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THE ROLE OF RHYNCHOSPORIUM COMMUNE CELL WALL COMPONENTS IN CELL WALL INTEGRITY AND PATHOGENICITY

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Summary: This aim of this research is to identify and characterise *Rhynchosporium commune* cell wall components as potential targets for new Rhynchosporium fungicides - a destructive disease of barley. *R. commune* disease of barley is spread worldwide and has considerable effect on crop yield, which is problematic for any economy including the UK. Populations of *R. commune* can change rapidly, defeating new fungicides after just a few seasons of their widespread commercial use, increasing the demand for a new and sustainable mode of management of the pathogen. The cell wall plays a key role in the fitness and virulence of pathogenic fungi. It is a protective surface providing structure to the cell and is the first line of defence against the pathogens host. With such an important role, the cell wall is the perfect target against *R. commune* and the disease caused in barley. This project will reveal some much needed information on the *R. commune* cell wall and in turn help to identify which components are essential for *R. commune* growth and interaction with its host.

R. commune is one of the most destructive diseases in barley and causes up to 40% crop yield losses in the UK (Shipton, 1974) this has a considerable impact on the economy. The pathogen can overcome barley resistance genes and methods of pest control e.g. fungicides very effectively - therefore we need a long-term sustainable mode of management for Rhynchosporium. In pathogenic fungi, the cell wall - composed of carbohydrates and proteins - plays a key role in the establishment of pathogenesis. It forms the outer structure, protecting the fungus from host defence mechanisms and initiating direct contact with its host. Information about cell wall composition, disposition and structure of constituent polysaccharides, glycoproteins and proteins is essential for a complete understanding of host-pathogen interactions. Characterisation of *R. commune* cell wall components will enable us to understand their role in cell wall integrity and pathogenicity. Components that are essential for the core biology of the pathogen during infection may represent potential targets for new environmentally friendly fungicides. From extensive research of other pathogenic fungal cell walls e.g. *Candida albicans* we can glean insight into the potential composition of *R. commune* cell wall. In general, the fungal cell walls consist of a network of polysaccharides – the most common being chitin and beta-glucan – which provides strength and rigidity to the cell. This network then allows for the attachment (via bonds such as GPI anchors) of a range of cell wall proteins with a wide variety of functions i.e. adherence and modulating host immune system.

Recent sequencing of *R. commune* genome has generated an extensive list of cell wall associated genes potentially involved in growth and infection (Ruiz-Herrera, 2006; Sohn, 2006). Subsequent expression analysis of the genes (throughout *R. commune* infection) by quantitative RT-PCR supports their thought function. Amongst this list of genes *R. commune* possesses a putative family of chitin synthases, beta-1, 6-glucan synthases, beta-1, 3-glucan synthases and cellulose synthases. This implies that these compounds could be contained in *R. commune* cell wall. In addition, a putative allantoicase gene has been identified. Allantoicase enzymes function to degrade purines a secondary source of nitrogen in nitrogen limiting conditions i.e. the leaf surface. A study in the corn pathogen, *Colletotrichum graminicola*, showed that deletion of this gene leads to loss of pathogenicity (Münch, 2011). The gene families along with allantoicase enzyme will be subject to functional characterisation. Targeted gene disruption, complementation and biochemical studies will help to uncover the composition of *R. commune* cell wall and identify components involved in fitness and virulence of the pathogen.

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BLACK-GRASS (*ALOPECURUS MYOSUROIDES*) CONTROL ACROSS A WINTER WHEAT / WINTER OILSEED RAPE ROTATION

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Summary: Growers of winter wheat and winter oilseed rape must do all they can to control black-grass (*Alopecurus myosuroides*) across the rotation. Propyzamide is a very useful active ingredient in winter oilseed rape, with no black-grass populations reported to exhibit resistance to this herbicide. Dow AgroSciences Ltd recently launched herbicide products based on the new molecule pyroxsulam for controlling black-grass in winter wheat. With the opportunity to target black-grass control across a winter wheat/winter oilseed rape rotation it is important to maximise the efficacy of applied herbicides, with different modes of action, and their impact on black-grass seed return to the soil seedbank in subsequent crops. This paper summarises research conducted with propyzamide and pyroxsulam at a site near Bicester, Oxfordshire, where effects of herbicide use were measured on black-grass populations across a winter wheat/winter oilseed rape rotation. Used correctly in the rotation, propyzamide and pyroxsulam can reduce black-grass infestations in current and following crops.

INTRODUCTION

Black-grass (*Alopecurus myosuroides*) control achieved in winter wheat crops, from the most frequently used herbicides, has declined over recent years and often results in variable grass control. However, good control is still possible in winter oilseed rape, albeit from a limited range of effective products approved for black-grass control in this crop. A distinct advantage of using active ingredients such as propyzamide (Kerb Flo 500 herbicide, 500 g a.i./litre propyzamide) is that, to date, no resistance to propyzamide has been found in any black-grass populations (Moss, 2010). The correct conditions need to be present for optimal control of black-grass using propyzamide, including seedbed establishment method, treatment dose and treatment timing (Roberts, 2011).

New herbicide products for the control of black-grass in winter wheat containing the new active ingredient pyroxsulam have been launched. Based on pyroxsulam, Unite herbicide (6.94% w/w pyroxsulam + 3.71% w/w flupyrsulfuron-methyl-sodium, soluble granule) and Broadway Sunrise herbicide (5.4 g a.i./litre pyroxsulam + 314 g a.i./litre pendimethalin, oil dispersion) provide black-grass control as effective as current market standards.

To investigate the impact of combining the use of pyroxsulam-based herbicides in winter wheat and propyzamide in winter oilseed rape, a rotational trial was established in autumn 2008, with all work undertaken by Oxford Agricultural Trials. Control of black-grass in each

year was assessed in the winter wheat and winter oilseed rape, followed by assessments of initial black-grass populations in the following crop (winter wheat and winter oilseed rape) and, subsequently, the levels of control achieved in those crops. The trial ran for three years to harvest 2011.

MATERIALS AND METHODS

The trial was established in autumn 2008 at Poundon, near Bicester, Oxfordshire. The soil type at the site is a clay loam, providing a good tilth. Overall dimensions of the trial area were 54 m by 104 m. The whole area was drilled with winter wheat, cv. Gladiator, at the rate of 250 seeds m^{-2} and to a depth of 3 cm, on the 24th September 2008 after a seedbed had been established using a plough and press. The previous crop across the whole area was winter oats.

Broadway Star herbicide (7.1% w/w pyroxsulam + 1.4% w/w florasulam, WDG) was applied at 265g/ha with an adjuvant (Activator 90 at 0.1% v/v) on the 18th March 2009, using a pressurized knapsack sprayer fitted with flat fan 110-02 nozzles and calibrated to deliver an equivalent water volume of 200 litres/ha. Black-grass growth stage was between three and five tillers at the time of application. A 6m wide untreated strip was left through the centre of the trial, vertically and horizontally. No herbicides were applied to this untreated area for the duration of the trial.

In autumn 2009, half the trial area, 27m by 104m, was drilled on the 9th September 2009 with winter oilseed rape, cv. Heros at the rate of 100 seeds/m^2 and to a depth of 2cm. The seedbed for half the oilseed rape area (27m by 52m) was prepared, by conventional ploughing to 15cm depth. The other half was prepared using discs, working to a depth of 5cm. The other part of the trial, 27m by 104m, was drilled on the 8th October 2009 with winter wheat, cv. Solstice, after ploughing, at the rate of 250 seeds/m^2 and to a depth of 3cm. This design resulted in half the trial, 27m by 104m, being winter wheat following winter wheat, and the other half being winter oilseed rape following winter wheat.

Herbicide treatments in the crops drilled in autumn 2009 were based on propyzamide in the winter oilseed rape and pyroxsulam programmes in the winter wheat. The black-grass population that emerged in autumn 2009, in an untreated area, was 363 plants/m^2, based on counts from 5 quadrats.

Propyzamide treatments were applied on the 11th November 2009 to both winter oilseed rape areas, using a pressurized knapsack sprayer fitted with flat fan 110-02 nozzles and calibrated to deliver an equivalent water volume of 200 litres/ha. Black-grass plants were at two to three fully expanded leaves at the time of application. Half the ploughed area, 12m by 49m, received propyzamide at 1.75 litres/ha, and the other half at 2.1 litres/ha. The same pattern was repeated on the minimally tilled area, with half being treated with propyzamide at 1.75 litres/ha and the other half with 2.1 litres/ha. Areas measuring 24m by 3m and 52m by 3m in both cultivation types were left untreated.
To control black-grass in the winter wheat part of the trial, a programme of pre-emergence and post-emergence treatments was used. On the 9th October 2009 a pre-emergence treatment of 60 g a.i./litre flufenacet + 300 g a.i./litre pendimethalin at 4.0 litres/ha was applied. This was followed on the 2nd November, when black-grass was at one to two fully expanded leaves, with a tank mix of 6.94% w/w pyroxsulam + 3.71% w/w flupyrsulfuron-methyl-sodium at 270 g/ha

product with 100 g a.i./litre diflufenican + 400 g a.i./litre flufenacet at 0.6 litres/ha. Areas measuring 24m by 6m and 104m by 3m were left untreated.

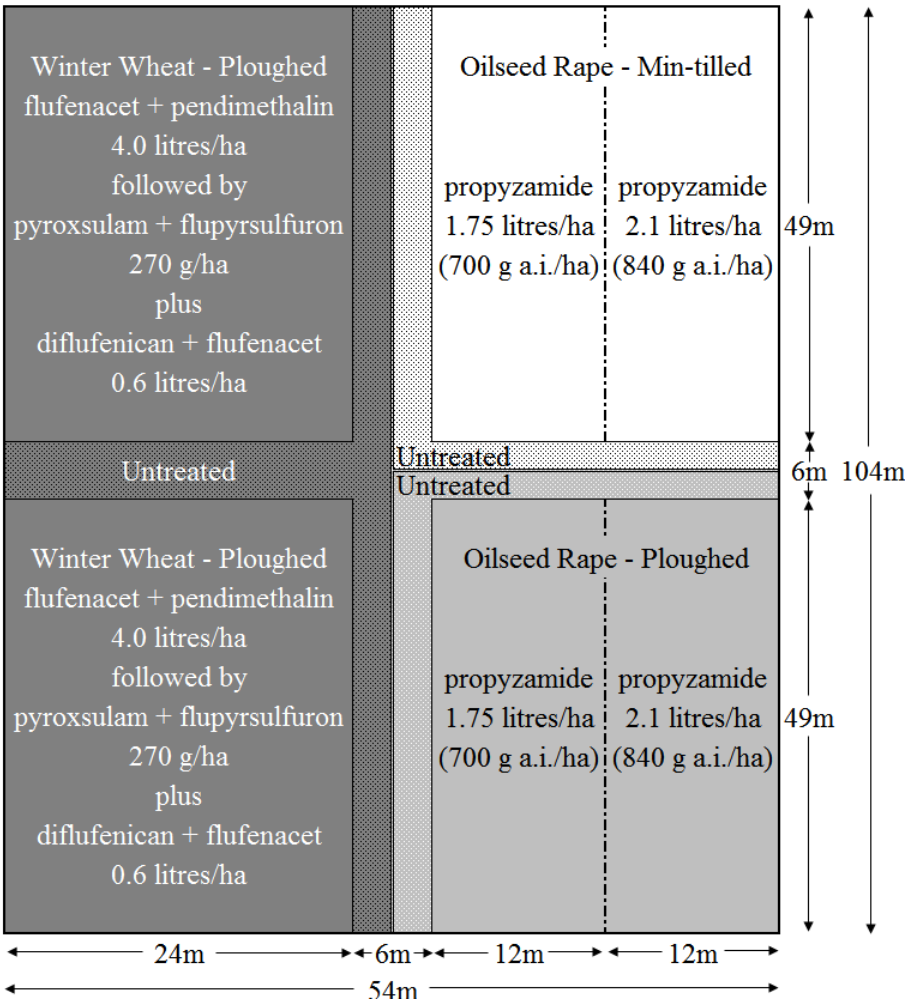

Figure 1. Plan of trial area at Poundon (autumn 2009).

Levels of black-grass control, compared with the untreated areas of each crop, were assessed in January and June 2010. Percentage visual control of black-grass plants was assessed in the winter oilseed rape on the 5th January 2010 (55 days after treatment), and in the winter wheat on the 27th January 2010 (86 days after the post-emergence tank mix was applied). Black-grass head counts were recorded in the winter oilseed rape and the winter wheat on the 15th June 2010 (216 days after the propyzamide treatment in the oilseed rape, and 225 days after post-emergence herbicides were applied in the winter wheat).
In autumn 2010 the whole trial area was ploughed and drilled with winter wheat, cv. Solstice, at the rate of 250 seeds/m^2 to a depth of 3cm. Herbicide treatments were again built upon a programme of a pre-emergence application of 4.0 litres/ha of the 60 g a.i./litre flufenacet + 300 g a.i./litre pendimethalin formulation, with an application of 3.5 litres/ha of the 5.4 g a.i./litre pyroxsulam + 314 g a.i./litre pendimethalin formulation post-emergence when black-grass plants were at one to two fully expanded leaves.

Several assessments were made in this winter wheat crop, after the pyroxsulam + pendimethalin herbicide programme had been applied, to gauge the impact of the previous

cultivations, crop types and herbicide treatments on the population of black-grass. Data collected included: number of black-grass plants m^{-2}; percentage visual control of black-grass from treatments; number of tillers per black-grass plant; number of viable tillers per black-grass plant; and number of seeds per black-grass head.

RESULTS

When the areas sown in autumn 2009 were assessed on the 5th January 2010, the number of black-grass plants in the untreated area of winter oilseed rape drilled after ploughing was 156/m^2. In the area where the seedbed was created by minimal tillage there were 1,660 black-grass plants/m^2. On the 27th January 2010, 190 black-grass plants/m^2 occurred in the untreated area drilled with winter wheat.

Cultivation method had a clear impact on levels of black-grass control achieved from propyzamide in the winter oilseed rape. After a seedbed was established using minimal soil disturbance, black-grass control was 99% and only 50% where propyzamide was used in the ploughed oilseed rape.

A number of assessments were made in the winter wheat crop drilled in autumn 2010. Data were collected from areas of winter wheat following previous winter wheat crops, i.e. a third wheat crop in the rotation; and areas of first wheat, either following winter oilseed rape established after the seedbed was prepared using minimal soil disturbance, or following oilseed rape after ploughing.

Figure 2 depicts the results of these assessments, where '% Control' is the visual percentage control of black-grass plants in the winter wheat crop drilled in autumn 2010 (after herbicide treatment); and 'Tillers/plant', 'Viable tillers' and 'Seeds/head' are data for surviving black-grass plants. Data were split by previous crop and seedbed cultivation method.

Black-grass seed return to the cropping areas was calculated using the above data collected in 2010 for surviving black-grass plants/m^2, the number of viable tillers per plant and the number of seeds counted per head. This estimate was made as a means to determine the combined impact of previous cropping, seedbed establishment technique and herbicide efficacy, on potential black-grass seed return under the conditions of this trial. The results for potential black-grass seed return are shown in Figure 3.

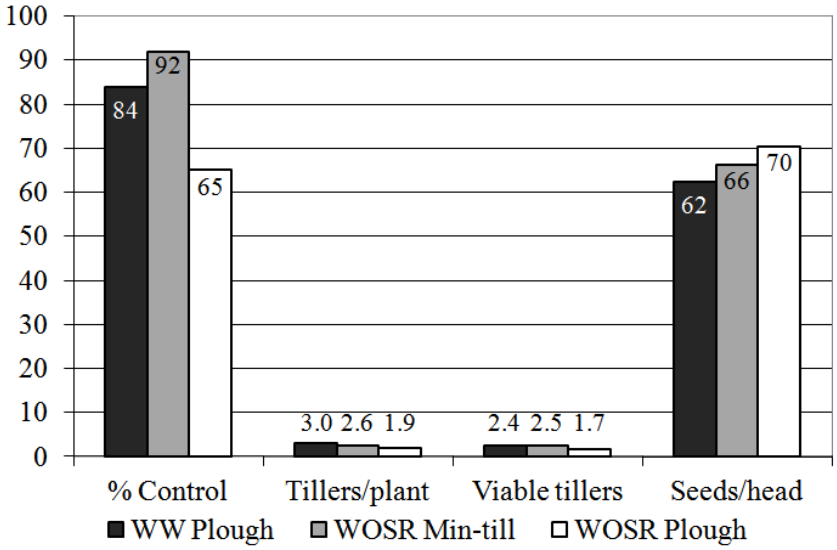

Figure 2. Black-grass assessments in winter wheat drilled autumn 2010.
% Control: control of black-grass plants from herbicides in winter wheat crop.
WW Plough: previous crop winter wheat established after ploughing.
WOSR Min-till: previous crop winter oilseed rape after shallow cultivations.
WOSR Plough: previous crop winter oilseed rape after ploughing.

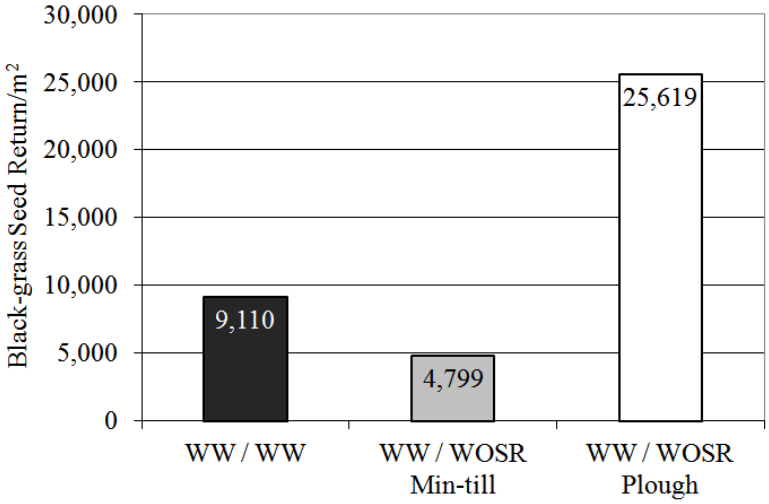

Figure 3. Calculated potential black-grass seed return.
WW/WW: third successive wheat (land ploughed for each crop).
WW/WOSR Min-till: first wheat after oilseed rape (shallow cultivations).
WW/WOSR Plough: first wheat after oilseed rape (ploughing).

The data show the impact of previous cropping, seedbed establishment method and herbicide performance on the potential to manage black-grass populations across a winter wheat/winter oilseed rape rotation. The use of propyzamide on winter oilseed rape in an arable rotation, provided seedbed establishment uses shallow cultivations, can have a marked effect on the population of black-grass in subsequent crops.

Data from this trial indicate black-grass seed return to winter wheat following winter oilseed rape (shallow cultivations) was less than 20% of that where ploughing was used in the oilseed rape crop, and this level was approximately 50% of the burden where winter wheat (ploughing) followed winter wheat (ploughing). Although the calculated black-grass seed return/m^2 is still high (4,799/m^2) in the winter wheat following winter oilseed rape (shallow cultivations), the benefits of this approach to managing black-grass populations across a rotation are clear. Further research work is being conducted to quantify these impacts over a longer period of time.

CONCLUSIONS

Using all available cultural and chemical means of controlling black-grass in typical arable rotations remains crucially important to the ongoing financial security of farmers and growers. Attention to detail, from 'stubble to stubble', in winter wheat/winter oilseed rape rotations is critical if black-grass populations are to be kept in check.

This research into control of black-grass across a typical winter wheat/winter oilseed rape rotation clearly demonstrates how black-grass seed return to subsequent arable crops is affected by the practices adopted and use of herbicides with different modes of action in the preceding crop. A marked reduction in seed return is achievable if relatively simple principles are adhered to.

Propyzamide treatments will control black-grass and confer benefits in the winter oilseed rape crop and in following winter wheat crops. Applications of pyroxsulam-based herbicide products to winter wheat crops are a key part of the cross-rotational approach, particularly when used in a first wheat crop following winter oilseed rape treated with propyzamide, and when treatments are made to small black-grass plants that have already been treated with pre-emergence applied herbicide with activity on black-grass.

Adopting the approaches outlined in this research will help to minimize black-grass seed return and protect the financial viability of farmers whose profitability is heavily reliant upon the winter wheat/winter oilseed rape rotation. The trial at Poundon has been completed but, due to the value of these research findings, a new rotational trial, looking at herbicide use to control black-grass, brome and ryegrass, and the impact of cultivations, was started in autumn 2011 on a much larger site at Dow AgroSciences' research and development facility in Wellesbourne, Warwickshire.

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OVERVIEW OF THE PMTV SITUATION IN SCANDINAVIA

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Potato mop-top virus (PMTV) is transmitted by the soil-borne microbe *Spongospora subterranea* f.sp. *subterranea* that causes powdery scab symptoms on potato. PMTV, in turn, causes spraing which is characterized by necrotic arcs in tuber flesh. The arcs can also be observed on the surface of tubers.

PMTV was first detected in Ireland and Scotland in 1960s. The virus originates most likely from the Andean region of South America as do many other potato viruses. Today PMTV is known to be distributed widely in potato growing areas across South and North America, Asia and North-western Europe. In Scandinavian countries (Norway, Sweden, Denmark and Finland), PMTV was detected and its distribution studied widely for the first time as part of a Nordic programme on soil-borne plant viruses in 1980s. Since then PMTV has been identified as causing significant economic losses to potato production in most potato growing areas in Scandinavia. The most recent survey has been carried out in Sweden and results are currently in preparation for publication. Occurrence of PMTV was studied recently also as part of a large programme involving all countries bordering the Baltic Sea (Santala et al. 2010). Systematic surveys in Poland in six years found no evidence of PMTV, but a single PMTV-infected tuber was detected there in 2008. The source of PMTV was most likely from imported seed potatoes. Surveys in Lithuania, Latvia, Estonia and North-western Russia (Leningrad province) found no evidence of the occurrence of PMTV, but minitubers produced in a screenhouse in Latvia in 2005 were found to be infected. The source of this particular infection remains unknown.

In Finland, PMTV is the only virus causing characteristic spraing symptoms, whereas in Sweden and Denmark the nematode-transmitted *Tobacco rattle virus* is also a common causal agent of somewhat similar arcs in tubers. Long-term trials carried out in heavily PMTV-infested fields in Finland show that most of the infected tubers can remain symptomless in the field and in storage (Santala et al. 2010). For example, up to 95% of the infected tubers of cv. Bintje were found to be symptomless. Furthermore, the portion of infected tubers showing symptoms can vary greatly from year to year. The factors affecting symptom development are still largely unknown, although fluctuating temperature is believed to enhance symptom development. These features make the study of spraing difficult and it is very hard to predict how severe the quality losses caused by PMTV will be when the crop was grown in a PMTV-infested field. Furthermore, control of the disease through certification schemes for seed potatoes is problematic as they are reliant on visual inspection, which is an imprecise tool for monitoring infection. Utilization of the proper detection methodologies for the virus based on serological or molecular methods are needed.

It is clear that PMTV can be disseminated to new fields by the movement of viruliferous resting spores of the vector in soil adhering on seed tubers. Machines moving from field to field without careful cleaning can also disseminate the virus in a similar manner. It was

recently shown at the Swedish University of Agricultural Sciences (SLU) that resting spores of *S. subterranea* remained viable and viruliferous in moist soil maintained in a cold room for 15 years: test plants grown subsequently in the soil became readily infected by PMTV (Santala et al. 2010).

It is proposed that wild potato species growing in Andean mountains may express resistance to PMTV, however, no resistant accessions have been reported as yet. Breeding for resistance to PMTV in potato seems possible based on the studies carried out in the late 1990s by SLU and a Swedish plant breeding company (Sandgren et al. 2002), but the resistance breeding programme was terminated in the process of rearranging the ownership of the company and has not been developed since.

Economic losses caused by PMTV in Scotland and Scandinavia have justified economic investments to research aiming to understand the PMTV-host interactions. Studies were pioneered at Scottish Crop Research Institute (SCRI) in 1990s, including research on the molecular composition of PMTV, and also development of engineered resistance in potato to PMTV (Barker et al. 1998). While some positive results were obtained by genetic engineering, the general anti-GM atmosphere in the European Union has suppressed interest to take this approach further. Development of infectious cDNA clones from the three PMTV RNAs at SLU moved research on PMTV to a new millennium (Savenkov et al. 2003), opening possibilities to identify and critically assess viral and host factors required for infection of plants and transmission of PMTV by the vector. These novel research tools and have been shared with SLU, University of Helsinki and the James Hutton Institute, Dundee. It is envisioned that molecular studies on PMTV-potato interactions will inform new solutions to control PMTV in potato production (Samuilova et al. 2013).

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PVY^N PREVALENCE IN POTATO CROPS: IMPACT OF STRAIN COMPETITION AND DIFFERENTIAL ABILITY TO OVERCOME PLANT RESISTANCE MECHANISMS

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Summary: While virus incidence is low in Scottish seed potato crops, *Potato virus Y* (PVY) has become the most prevalent virus. The monitoring of PVY^N and PVY^O strains has revealed a recent shift towards PVY^N, which now accounts for more than 90% of all PVY cases in seed potato crops. A survey of the molecular and biological diversity of PVY^N isolates indicated that a large majority (~80-90%) belong to the recombinant European (EU)-NTN molecular group with North-American (NA)-NTN and non-recombinant EU-N variants making up the remainder. Timing of transmission and distribution in field trials of a selection of PVY isolates belonging to the three main molecular groups (PVY^O, $PVY^{EU\text{-}NTN}$, $PVY^{NA\text{-}NTN}$) indicate that, in spite of being transmitted at a comparable time by similar aphid species, $PVY^{EU\text{-}NTN}$ was transmitted at the highest rate. Assessment of virus incidence in potato bait plants infected at different times after emergence, revealed a higher capacity of $PVY^{EU\text{-}NTN}$ to infect and spread in older plants in comparison to PVY^O. Altogether our results suggest a differential ability of $PVY^{EU\text{-}NTN}$ in out-competing other PVY strains and overcoming mature plant resistance mechanisms, potentially explaining the prevalence of $PVY^{EU\text{-}NTN}$.

INTRODUCTION

Virus incidence is relatively low in Scottish seed crops with *Potato virus Y* (PVY) predominating in comparison to other potato-infecting viruses. PVY is the most economically important virus to affect cultivated potato both in yield and quality (Valkonen, 2007). PVY is spread by vegetative propagation of potato tubers and by a wide range of aphid species in a non-persistent manner, whereby aphids acquire PVY on their stylets when probing the leaf surface and transmission occurs during further probing of a different plant. Although the virus may only be retained by the aphid for a short time, aphids can quickly transmit virus without colonising the crop.

PVY exists as a complex of strains or variants. The earliest characterization of PVY strains classified them into three major pathogenicity groups: *i.e.*, ordinary or common strain (PVY^O), stipple streak strain (PVY^C leaf drop of potato) and PVY^N (vein necrosis on tobacco) (Singh *et al.*, 2008). Characterisation of PVY by ELISA using monoclonal antibodies can distinguish between PVY^O and PVY^N strains. Molecular typing by genome sequencing identified recombinant PVY^{NTN} (N-Tuber Necrosis) variants that derive from PVY^N and PVY^O strains and fall into molecular subgroups such as $PVY^{EU\text{-}NTN}$ (European), $PVY^{NA\text{-}NTN}$ (North-American) and $PVY^{N\text{-}Wilga}$. $PVY^{N\text{-}Wilga}$ isolates belong to the PVY^O serotype but are biologically related to PVY^{NTN} due to their ability to cause vein necrosis in tobacco and potato tuber necrotic ringspot

disease (PTNRD). We have undertaken the characterization of PVY field isolates by assessing their serology, genome structure and pathogenicity. Within the past six years, surveys of PVY field isolates have revealed a shift from PVY^{O} towards PVY^{N} serotypes in the PVY population in seed potato crops. This change in the population dynamics of PVY prompted us to study the factors that drive their relative prevalence by evaluating field transmission characteristics. We investigated the ability of PVY strains to overcome mature plant resistance mechanisms; a broad spectrum resistance mechanism where potato plants inoculated late in the growing season display increased resistance to PVY infection (Gibson, 1991; Sigvald, 1985).

MATERIALS AND METHODS.

Molecular and Biological Characterization of PVY^{N} and PVY^{O} Field Isolates

PVY ELISA-positive samples (PVY^{N} and $PVY^{O/C}$) intercepted at crop inspection during the 2009-2013 seasons were propagated into tobacco plants *Nicotiana tabacum* cv. White Burley and *Nicotiana benthamiana*. Total RNA extraction, sequencing, phylogenetic analysis and biological typing of PVY isolates were performed as previously described (Davie *et al*., 2012).

Field Transmission Trials

The field transmission trials (Pickup *et al*, 2009; Davie *et al*., 2012) consist of 450 virus-free cv. Maris Piper healthy bait plants with a row of twenty one tuber-borne infected potatoes used as the inoculum source of PVY. Seven plants were infected each with either $PVY^{EU\text{-}NTN}$, $PVY^{NA\text{-}NTN}$ or PVY^{O} isolates (Figure 1), alternately placed along the infector row. Weekly variation in virus transmission is monitored by testing a new set of *N. debneyi* bait plants each week by DAS-ELISA as previously described (Davie *et al*., 2012). Distribution and incidence of PVY was assessed by sampling 4 tubers per plant as previously described (Davie *et al*., 2012). This trial was repeated over a 3-year period (2010-2011-2012).

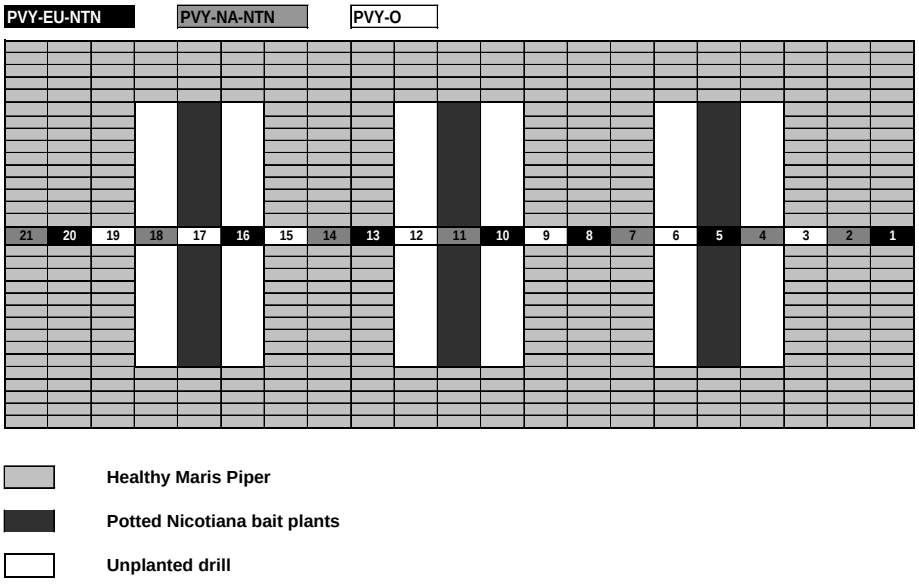

Figure 1. Trial design for monitoring the timing of transmission and distribution of PVY strains.

Mature Plant Resistance Field Trials

Potato cultivars with comparable level of susceptibility to PVY (cvs Estima and Maris Piper) were used. Plants emerged by 4 weeks after planting and were mechanically inoculated at different times after emergence (1, 2, 5, 7 and 10 weeks) with infectious sap of either PVY^{EU-NTN} or PVY^{O} isolates at the same titre (n=12 plants per time-point per PVY isolate). Ten tubers from each plant were tested by growing-on DAS-ELISA to monitor virus incidence as previously described (Pickup *et al*., 2009). Non-inoculated control potato plants (n=24 plants for each of the cultivar tested) were exposed for the whole duration of the trial to assess potential background primary infection from incoming viruliferous aphids. All non-inoculated plants were found to be free of virus.

RESULTS

Dynamics and Population Structure of PVY Strains

The prevalence of PVY^{N} over PVY^{O} has shown a marked increased since 2005. PVY^{N} now represents more than 90% of PVY cases (Figure 2). Data from a previous survey on the molecular nature of PVY^{N} isolates (Davie *et al*., 2012) by sequencing of a recombinant junction of the PVY genome (Schubert *et al*., 2007), indicated that in 2009, the PVY^{N} group was composed of distinct molecular groups with 88% belonging to the European EU-NTN molecular group, 8% to the North American NA-NTN and 4% to the EU-N groups. The molecular nature of PVY^{N} isolates intercepted in 2013 confirmed this trend with PVY^{EU-NTN} and PVY^{NA-NTN} accounting for respectively 92% and 8% of the PVY^{N} population.

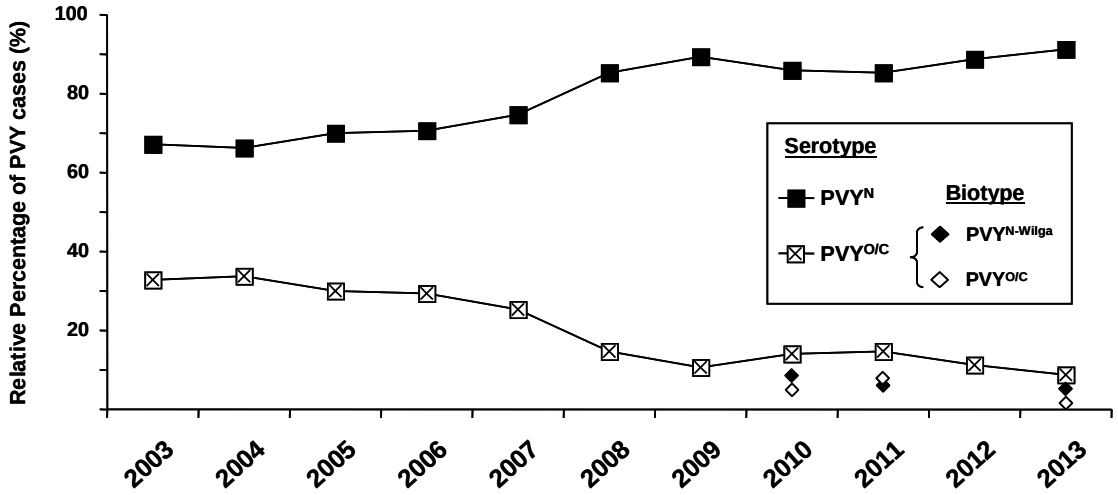

Figure 2. Nature and dynamic of PVY population from 2003–2013. Breakdown of $PVY^{N-Wilga}$ and $PVY^{O/C}$ strains ELISA-positive to $PVY^{O/C}$ antibodies is presented. The relative proportion of $PVY^{O/C}$, PVY^{N} and $PVY^{N-Wilga}$ strains is presented from 2010.

Biological typing of $PVY^{O/C}$ isolates indicates that the majority belong to the $PVY^{N-Wilga}$ biotype (69% of $PVY^{O/C}$ cases in 2013) as opposed to PVY^{O} or PVY^{C} strains (31% of $PVY^{O/C}$ cases).

This suggests that the population of both the PVY N- and O-serotypes are essentially composed of recombinants variants.

Transmission and Distribution of PVY Isolates

Incidence of PVY isolates in field trials was assessed firstly by DAS-ELISA to discriminate between PVY^{O} and PVY^{N} serotypes and by RT-PCR on PVY^{N} positive samples to discriminate between $PVY^{EU\text{-}NTN}$ and $PVY^{NA\text{-}NTN}$ genotypes. Weekly timing of transmission in tobacco bait plants was similar for both PVY^{O} and PVY^{N} serotypes (data not shown) confirming previous results obtained the previous season (Davie *et al.*, 2012) and suggesting that PVY^{N} and PVY^{O} are likely to be transmitted by similar aphid species.

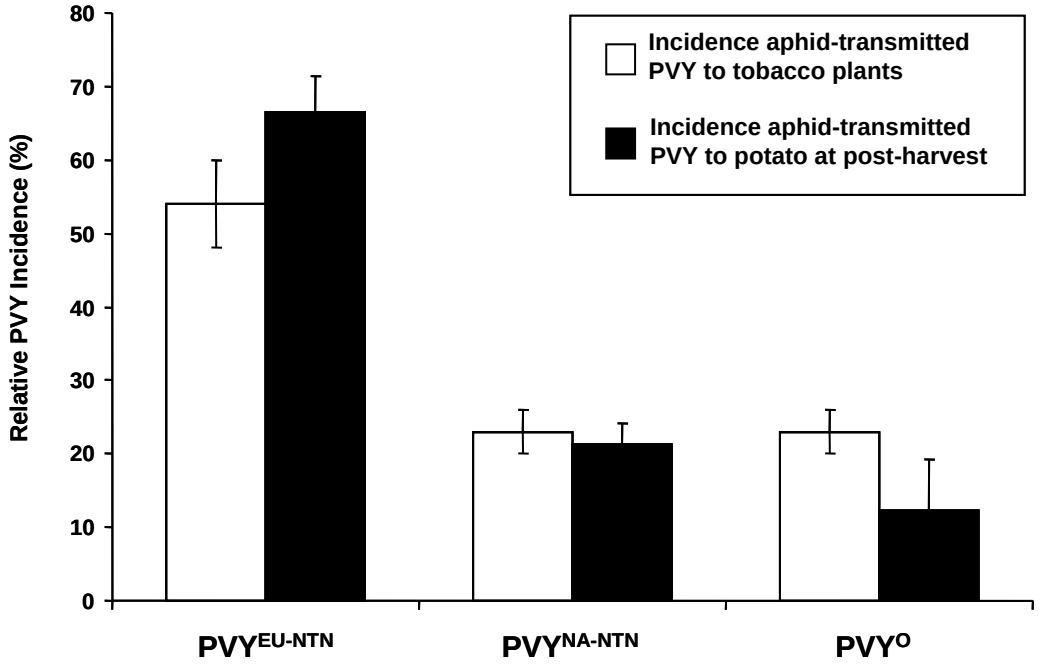

Figure 3. Relative frequency of aphid-transmitted PVY isolates to tobacco bait plants (white) and of PVY incidence at post-harvest in tubers of potato bait plants (black). Relative PVY incidence is expressed as Mean ±SE of infected bait plants from either 2 years (2011 and 2012) or 3 years (2010-2011-2012) of field trials for respectively tobacco bait plants and at post-harvest in potato tubers.

The incidence of aphid-transmitted PVY isolates was significantly higher for $PVY^{EU\text{-}NTN}$ (54% ±6) (P=0.004) than for $PVY^{NA\text{-}NTN}$ (23% ±3) and PVY^{O} (23% ±3) (Figure 3). A skewed distribution towards $PVY^{EU\text{-}NTN}$ was also observed in potato bait plants at post-harvest, where $PVY^{EU\text{-}NTN}$ mean incidence was of 67% (±4.9) (P=0.0013) of infected plants while $PVY^{NA\text{-}NTN}$ and PVY^{O} accounted only for 21% and 12% respectively (Figure 3). The distribution of PVY isolates in single and mixed infections in bait plants was skewed towards $PVY^{EU\text{-}NTN}$ (76% of cases) where $PVY^{EU\text{-}NTN}$ was found in all cases of mix-infected plants tested (data not shown).

Development of Mature Plant Resistance on $PVY^{EU\text{-}NTN}$ and PVY^{O} Infected Plants

We further assessed the effect of mature plant resistance on the ability of $PVY^{EU\text{-}NTN}$ and PVY^{O} isolates to infect potato plants at different developmental stages (Figure 4).

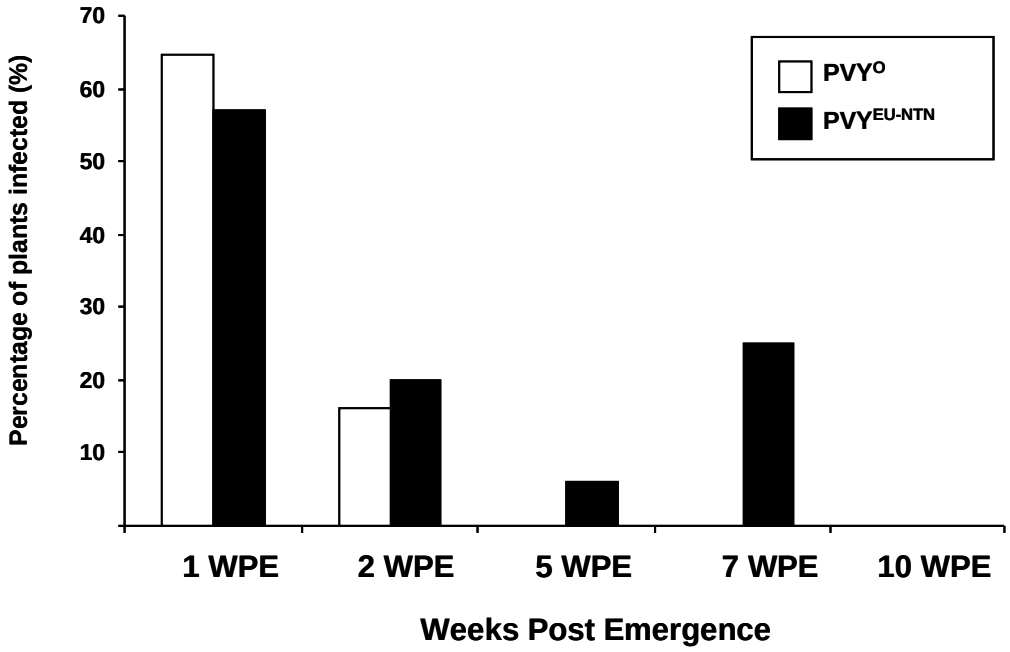

Figure 4. Percentage of $PVY^{EU\text{-}NTN}$ and PVY^{O} infected plants at different inoculation periods after emergence (Week Post Emergence-WPE).

While a comparable incidence in tuber progeny was found between 1 and 2 weeks post-emergence (*i.e.* 6 to 8 weeks post planting) (57% for $PVY^{EU\text{-}NTN}$ and 65% for PVY^{O}), only $PVY^{EU\text{-}NTN}$ was found to successfully colonize their potato host at 5 and 7 weeks post-emergence (*i.e.* 9 and 11 weeks post planting Figure 4). These results indicates that PVY^{O} and $PVY^{EU\text{-}NTN}$ incidence was high and comparable in plants inoculated during the 2 weeks post-emergence (6 weeks post planting) while declining sharply with complete resistance of infection to PVY^{O} after 5 weeks post-emergence. Successful $PVY^{EU\text{-}NTN}$ infection to progeny tubers was observed for longer period after 7 weeks post emergence, suggesting a higher capacity of $PVY^{EU\text{-}NTN}$ to overcome mature plant resistance mechanisms.

DISCUSSION

Recombinant PVY^{NTN} and related PVY variant strains are found to be prevalent worldwide in potato-growing areas and are displacing non-recombinant PVY^{O} and PVY^{N} strains. The reasons for these changes in the PVY population are unknown and likely to be dependant on complex interactions between PVY-plant-aphid vectors and environmental conditions. The aim of our study was to examine the parameters that are driving PVY variant prevalence in Scottish seed crops. $PVY^{EU\text{-}NTN}$ frequency of aphid transmission and virus incidence at post-harvest was found to be higher in comparison to PVY^{O} and $PVY^{NA\text{-}NTN}$. The skewed high incidence and distribution of $PVY^{EU\text{-}NTN}$ cases in single or mixed infections might reflect the outcome of infection dynamics in plants whereby a fitter isolate ($PVY^{EU\text{-}NTN}$) might out-compete PVY^{O} and

PVY^{NA-NTN} for host cellular functions with ultimately resulting in higher incidence in systemic tissues. Further experiments are underway to assess the parameters that condition interactions dynamics between PVY variants during the aphid-transmission and/or co-infection processes in their host plants.

The ability of PVY^{EU-NTN} to successfully infect potato hosts at a relatively late developmental stage (*i.e.* up to 7 weeks post emergence) suggests that PVY^{EU-NTN} might counteract mature plant resistance mechanisms more efficiently than PVY^{O}. This would give another selective advantage for PVY^{EU-NTN} in initiating successful infection events as opposed to other variants (PVY^{O} in this instance). All these factors might explain the prevalence of PVY^{EU-NTN} towards others PVY strains including in our environmental conditions and illustrate the complex nature of PVY dynamics.

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POTATO PHYTOPLASMAS AND *CANDIDATUS* LIBERIBACTER SOLANACEARUM: THE EUPHRESCO PHYLIB PROJECT

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Summary: This paper presents the work being carried out at SASA as part of the EUPHRESCO project on *Candidatus* Liberibacter solanacearum, an emerging threat to potato production, and potato phytoplasmas, an under-studied area of potato disease. This work aims to provide information on hosts, vectors and diagnosis of these pests to inform risk management strategies.

INTRODUCTION

The emergence of *Candidatus* Liberibacter solanacearum (zebra chip) as a pest of economic importance in an increasing range of countries and hosts has raised the profile of both this pest and phytoplasma disease, which can cause similar symptoms. A two year EUPHRESCO project, "Epidemiology and diagnosis of potato phytoplasmas and Candidatus *Liberibacter solanacearum* and their contribution to risk management in potato and other crops" ("PHYLIB" project) started in 2012 and aimed to fill some of the knowledge gaps for both types of pathogen in a collaboration involving institutes from nine countries.

Candidatus Liberibacter solanacearum

Zebra chip (*Candidatus* Liberibacter solanacearum, Lso) is a psyllid transmitted α-proteobacterium that causes disease of solanaceous plants, most notably potato, tomato and pepper. It has also been detected outwith solanaceous hosts in carrot and celery crops. It was first recorded in Mexico in 1994 and in Texas in 2000. Since then it has been reported in several other Western and Middle American states, Central America and New Zealand (Munyanez, 2012a). The common name comes from the streaking symptom in infected tubers that becomes marked when fried, reducing processing quality. Symptoms on aerial parts such as chlorosis, twisted stems with swollen nodes, occasional aerial tubers and vascular discolouration and wilting are also apparent (Secor *et al.*, 2009). These symptoms are similar to those cause by phytoplasma infection and suggest that Lso may be wider spread in terms of host crops, vector and geographical region than is currently recognised (Munyaneza *et al.*, 2010b). It has caused millions of dollars losses to potato growers and is subject to considerable research in multiple countries (http://zebrachipscri.tamu.edu/). In Central and Northern America and New Zealand it is vectored by the potato/tomato psyllid *Bactericera cockerelli*, which was initially thought to be its only vector. The first report of Lso in a non-solanaceous host and outside the Americas and New Zealand was in 14 carrot crops in the south of Finland in 2008 (Munyaneza *et al.*, 2010a). Typical symptoms in carrot crops include reddening and yellowing of the foliage, stunting and adventitious roots, although these symptoms can also be caused by phytoplasmas. (G Teresani, personal communication). Molecular testing of the

psyllid *Trioza apicalis* confirmed that this pest, which has previously been associated with psyllid-yellowing symptoms in carrot, is acting as a vector for Lso (Munyaneza *et al.*, 2010b). In 2011 Lso was reported in both Norway and southern Sweden where 70% of commercial fields were displaying up to 45% symptomatic plants per field (Munyaneza *et al.*, 2012c; Munyaneza *et al.*, 2012b). It is not known if *T. apicalis* is present in the UK. Ossiannilsson, 1992 has been quoted by several authors saying *T. apicalis* "occurs in wider areas of Eurasia from Great Britain to Mongolia" but this statement is unconfirmed. Lso has also been recorded in carrots in mainland Spain and the Canary Islands where it is known to be vectored by a third psyllid species *B. trigonica*. Recent research in this area has also implicated other psyllids such as *B nigricornis* and *B tremblayi* (G Teresani, personal communication). Four Lso haplotypes A, B, C and D have been described (Nelson *et al*., 2013). Haplotype A has been found in central and North America and haplotype B in Mexico, North America and New Zealand. A and B haplotypes are transmitted by *B. cockerelli.* Haplotype C has been found in Finland and haplotype D from mainland Spain and the Canary Islands (Munyaneza, 2013).

EPPO recommends its member countries to regulate Lso haplotypes and its vector *B. cockerelli* as quarantine pests and since 2010 the UK Potato Quarantine Unit (PQU), SASA which operates under The Plant Health Directive 2009/29/EC and Commission Directive 2008/61/EC, has been testing all imported potatoes for Lso.

Potato phytoplasmas

Phytoplasmas are a type of cell wall-less, insect vectored bacteria which are susceptible to some antibiotics (Crosslin *et al.*, 2011). Phytoplasma diseases were first differentiated from viruses in the 1960s but, as they could not be cultured *in vitro*, their study was very difficult until the advent of molecular techniques. Recently, *in vitro* culturing has been reported for a number of phytoplasmas (Contaldo *et al*., 2012). World-wide, nearly 1000 phytoplasma diseases have been identified in a wide range of host plants (Maramorosch, 2011). There are two major systems for classification of phytoplasmas with both systems based on analysis of the highly conserved 16S rRNA gene (Duduk & Bertaccini, 2011). In the most widely used system, phytoplasmas are classified into groups and subgroups, based on 16S ribosomal DNA. Each phytoplasma group is designated by a Roman numeral and each subgroup / strain is designated by a capital letter. There are 12 strains currently associated with disease in potato, although this number is increasing as phytoplasmas characterised from alternative hosts are detected in potato. The most important phytoplasma diseases of potato are potato purple top (PPT), potato stolbur and potato witches broom (PWB) (Ember *et al.*, 2011). PWB and stolbur diseases are both associated with a single 16S group, 16SrVI and 16SrXII respectively, while PPT is associated with phytoplasmas from five ribosomal groups, namely 16SrI, 16SrII, 16SrIII, 16SrVI and 16SrXVIII. In the UK, the only recorded potato phytoplasma is PWB and it is uncommon and rarely causes economic loss. Primary infection in potato crops is spread by leafhoppers from primary hosts such as clovers in the field margins. Tubers grown from infected plants rarely sprout or produce stunted plants and are easily rouged. The disease, therefore, is self-eliminating. Potato stolbur and PPT, on the other hand, cause economic losses in Central and Eastern Europe and North and Central America respectively. Symptoms of potato stolbur in tubers are similar those of zebra chip caused by *Candidatus* Liberibacter solanacearum (see below) and markedly reduce processing quality. In the EU potato stolbur is a quarantine pest on potato and the European and Mediterranean Plant Protection organisation (EPPO) lists PPT and stolbur as quarantine pests.

EUPHRESCO PHYLIB project

The primary aim of the project is to provide information on hosts, vectors and biology of phytoplasmas (potato stolbur) and Lso haplotypes in Europe to enable risk assessment and risk management strategies to be developed.

For phytoplasmas the field work is being conducted in Hungary and Turkey. At SASA the work includes:- 1) examining the most appropriate sampling strategy for potato microplants and glasshouse grown potato plants to enable reliable detection in potato quarantine. 2) evaluating a real time phytoplasma assay developed at SASA 3) surveying for PWB.

For Lso, key objectives are identifying the source of the inoculum for the infections that have occurred in carrots and celery in Europe (e.g. is Lso seed transmitted in these crops or is transmission from as yet unkown alternative hosts such as perennial weeds); establishing whether the Lso haplotypes present in Europe can infect potato; identifying the vectors and whether they can transmit Lso from carrot and celery to potato and evaluating molecular tests for detection and identification of Lso. At SASA the work includes:- 1) surveying carrot crops for *Lso* infection and psyllids; 2) evaluating the most appropriate sampling and testing strategy for use in post-entry potato quarantine.

MATERIALS AND METHODS

Potato witches broom

In 2012 and 2013 an information leaflet was sent to potato growers and Inspectors describing symptoms of potato witches broom (PWB) and asking them to report any suspected cases to SASA. Primary symptoms are an erect staring growth habit downward rolling of the upper leaflets with yellowing between the leaflet veins with sometimes reddening on the leaflet margins. In the second year tubers often fail to sprout or produce weak hair sprouts that die. If the tubers do grow, plants may be dwarfed, bushy and multi stemmed. Leaves may be more rounded than normal. In 2012 and 2013 a total of 5 and 10 plants respectively were collected from Scottish farms and re-planted in pots at SASA. Leaflets from the top, middle and bottom of the potato plant were sampled 3 times during the growing season with DNA extracted from excised leaf midribs using the method of Reid *et al.* (2009). Molecular testing was carried out using nested conventional PCR of P1/tint or R16F2/R16R2 followed by fU5/rU3 and visualised on 1% agarose gels.

Potato stolbur

Progeny tubers from potato plants of 4 cultivars exhibiting stolbur symptoms in the field were supplied by Maria Kölber, Hungary. Eye-plugs excised using a sterilized potato peeler were crushed with a micropestle. DNA was extracted using the InviMag Plant DNA mini kit (Stratec Molecular, Berlin, Germany) and tested using nested conventional PCR as outlined above. Statistical analysis was carried out using Genstat v.14.

Beet leafhopper-transmitted virescence agent (BLTVA)

BLTVA infected microplants cv. Atlantic were supplied by J Crosslin, USA. Infected microplants were allowed to branch, subcultured and then grown on as individual microplants. A total of twelve branched microplants were used to produce 98 microplants with a sketch to show the original relationship between the nodes subcultured. DNA was extracted either from the top of a microplant, or destructively from the whole microplant using the extraction protocol and nested PCR as described for potato stolbur.

Lso and psyllids

In 2012 and 2013 an information leaflet describing symptoms of Lso in carrots and its psyllid vector was sent to 6 and 287 carrot growers in Scotland respectively. Growers were asked to contact SASA if symptoms or the vector were seen. Suspect samples for Lso were extracted using the method of Reid *et al.* (2009) and tested with real-time PCR using the primers LosF/HLBr and probe HLBp. Psyllids were surveyed using yellow water traps (in 2012) or sweep netting (2013). Psyllids were also collected using the Scottish suction trap network.

RESULTS AND DISCUSSION

Potato witches broom

None of the plants collected in 2012 or 2013 tested positive for phytoplasma, despite material from the top, middle and bottom of each potato plant being tested to account for the often patchy distribution of phytoplasma within a host. This may be because the symptoms were not caused by phytoplasma, or that the pathogen titre did not reach a high enough level to allow detection. The plants removed from the field tended to be smaller than the surrounding plants (a symptom of PWB) but when they were re-planted in individual pots at SASA they grew rapidly and PWB symptoms were no longer apparent. This suggests that the onset of PWB symptoms may be competitor mediated and that by removing that pressure, the plants were able to overcome the disease and grow as normal. Progeny tubers from the plants harvested in 2013 will be planted in 2014 to ascertain if there was any disease transmission to the tubers.

Potato stolbur

Using a generalised linear mixed model a significantly greater number of tubers of cultivar 2 were infected with phytoplasma than cultivars 1, 3 and 4 (Table 1). Also, sprouting tubers were as likely to be positive for phytoplasma infection as non-sprouting tubers. Unfortunately cultivar 2 was the least represented in the samples. Tuber infection by potato stolbur was far lower than the 73% reported for the cv. Lady Rosetta (Ember *et al*., 2010). One of the objectives of this work was to produce potato stolbur infected microplants. Infected tubers, however, failed to sprout or if they did grow they failed to produce infected plants *in vivo* or *in vitro*, indicating that tuber transmission may be rare. Crosslin (2011) found that tuber transmission of BLTVA phytoplasma ranged from 0-50% depending on cultivar and year. Stolbur infection may decrease in tubers with increased storage time (M Kölber, personal communication). Due to the time of harvest and the time taken to clear quarantine the tubers had been stored for many months before testing was carried out.

BLTVA

For one of the initial 12 branched microplants, the 4 nodes from the top two branches of the plant were negative for phytoplasma while the bottom 4 nodes were positive for phytoplasma. This pattern, however, was not seen in any of the other microplants with all other nodes testing positive for phytoplasma. It is intended to continue and expand this study to ensure that the PQU sampling protocol of sampling the top of a microplant is appropriate.

Lso and psyllids

In 2012 and 2013 respectively, 1 and 4 samples from carrot crops were sent into SASA to test for Lso. Results were negative. In 2012 no psyllids were caught in the yellow traps perhaps as a consequence of the very wet summer. In 2013 sweep netting of 3 fields in Fife (2 x carrot, 1 x potato, 2 x field boundaries) caught many *Trioza urticae* (a native species found on nettle) on the field boundaries and a few on the carrot and potato crops. Further work is planned to more accurately assess the risk of *T. apicalis* and other potential vectors being present in Scotland.

Table 1. No of progeny tubers infected from plants of four cultivars with stolbur symptoms.

	Plant number										
	1	2	3	4	5	6	7	8	9	10	Total
Cultivar 1											
No. tubers	14	8	14	6	4	8	5				59
No. infected	0	1	0	1	0	2	1				5(8%)
Cultivar 2											
No. tubers	4	8	7								19
No. infected	4	4	6								14 (74%)
Cultivar 3											
No. tubers	21	9	7	7	6	7	9	12	13	19	110
No. infected	6	4	0	0	0	1	0	0	2	2	15 (14%)
Cultivar 4											
No. tubers	10	22	9	19	11	11					82
No. infected	0	0	1	3	1	4					9 (11%)

In Scotland presently we are free from both potato phytoplasmas of any economic significance and Lso. Although historically Scotland has had a strong quarantine system in place for imported potatoes it is important that we have sufficient knowledge in order to assess and counter threats from new and emerging pests such as these. Although the work is at an early stage, being part of EUPHESCO enables collaboration with other countries in order to maximise the scientific returns.

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BREEDING NEW VARIETIES FOR SCOTTISH SEED POTATO EXPORTS

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Summary: Genotypes generated within a potato breeding scheme cannot always be evaluated over the range of environments for which cultivar development is aimed. In a collaborative breeding programme at the James Hutton Institute between Caithness Potatoes and Mylnefield Research Services a cultivar breeding programme aims to develop genotypes which perform well in one or more environments in the UK and/or countries around the eastern Mediterranean basin. Over a four year period up to 30 individual families each comprising between 20 and 40 potato genotypes were selected solely on their phenotypic performance in environments in the UK and Mediterranean sites. While significant genotype x environment interactions for important traits can be identified, the superior families and genotypes tended to perform well over a range of environments. This approach to selection is discussed in relation to an earlier study examining selection of advanced material within a breeding programme for Mediterranean environments.

INTRODUCTION

Potato is the most important root crop used for food and industrial purposes. The International Potato Centre based in Peru estimates that over one billion people eat potatoes and that globally the total crop production exceeds 325 million tonnes, making it a critical crop in terms of food security in the face of population growth and increased hunger rates. The crop is grown over a remarkably wide range of environments from sea level up to 4,700 meters and from southern Chile to Greenland. The World Catalogue (Pieterse and Hils, 2009) now contains more than 4,500 potato varieties that are cultivated in over 126 countries worldwide, with the majority of potato production occurring in Europe and Asia (approximately 80% of world potato production). In Europe, ware potatoes are mainly cultivated in Russia, Ukraine, Poland, Germany, Belarus, France and in the United Kingdom.

Scotland has an acknowledged global reputation for producing high quality seed potato, with seed exports now exceeding 100,000 tonnes per annum. Seventy five percent of exports are to non-EU countries, significantly to Egypt, Morocco and Israel. It is therefore important to breed varieties that are suitable not only for the changing UK climate, but also for different climatic zones of importance to the Scottish and UK seed potato industry. The Scottish seed potato export industry is a key crop for Scotland, with Scottish seed potato production worth around £100 million annually. Around 65,000 tonnes of seed potatoes and 10,000 tonnes of ware potatoes produced each year in Scotland are exported outside the European Union (or to the

Canary Islands). Data from the Science and Advice for Scottish Agriculture (SASA) website for the 2012 to 2013 season on seed potato exports are summarised according to destination in Table 1a below and by variety in Table 1b below.

Table 1a. Seed potato export data from Scotland 2012/2013 by destination. Information from SASA*

Total tonnes	**Country**	**Total tonnes**	**Country**
41320.050	Egypt	67.775	Serbia and Montenegro
10056.500	Morocco	22.500	Macedonia
6542.750	Israel	22.000	Albania
3974.440	Canary Islands	22.000	Croatia
3323.750	Saudi Arabia	21.500	Belarus
1815.425	Thailand	10.325	Ukraine
1500.099	Russia	6.500	St Helena
950.070	Turkey	2.000	Falklands
375.900	Iraq	0.150	Eritrea
209.200	Algeria	0.060	Sudan
197.350	Indonesia	0.010	China
150.000	Brazil	0.000	Iran
135.000	Bosnia	0.000	Tunisia
75.000	Uruguay		
		70800.354	

* http://www.sasa.gov.uk/seed-ware-potatoes/potato-exports.

It is evident from Table 1a that a number of countries with relatively hot climates, notably Egypt, Morocco, Israel, Canary Islands and Saudi Arabia account for approximately 92% of the exports. The data presented in Table 1b below summarises the same data but by variety.

Variety names given in bold print in Table 1b are bred in Britain, accounting for approximately 19% of the seed exported. Of the 70,800 tonnes exported it is worth noting that 60-65%of the export tonnage is based on ‘free’ potato varieties, those varieties without or beyond plant variety rights.

Table 1b. Seed potato export data from Scotland 2012/2013 by variety (export > 10 tonnes.). Information from SASA*

Variety	**Total tonnes**		**Variety**	**Total tonnes**
Hermes	24682.870		**Bounty**	177.500
Desiree	10334.500		Brooke	177.290
Cara	5305.450		**King Edward**	167.500
Valor	5247.050		Kennebec	163.625
Winston	3026.750		**Gemson**	146.250
Burren	2983.825		Habibi	115.125

Table 1b cont. Seed potato export data from Scotland 2012/2013 by variety (export > 10 tonnes.). Information from SASA*

Variety	**Total tonnes**		**Variety**	**Total tonnes**
Banba	2774.425		Shepody	108.750
Atlantic	2417.325		Charlotte	105.000
Lady Balfour	1597.825		Newton	80.004
Slaney	1585.975		Rubesse	75.125
Galactica	1498.200		**Merlin**	75.000
Maris Piper	853.750		Cristina	54.900
Saturna	800.020		Nectar	52.000
Picasso	675.000		Bonnie	50.000
Nieta	602.800		**Sebastian**	50.000
Druid	567.300		**Kerr's Pink**	45.000
Barna	550.800		**Bambino**	40.800
Red Cara	539.525		Electra	30.975
Argos	493.000		**Chicago**	28.000
Russet Burbank	400.000		Spunta	25.020
Paramount	350.000		Ambo	25.000
Horizon	300.000		Ranger Russet	25.000
Maris Peer	289.250		Rudolph	25.000
Nicola	285.020		Umatilla Russet	23.000
Vales Sovereign	247.500		**Casablanca**	20.500
Savanna	222.500		Orla	15.025
Rooster	199.000		Setanta	10.800

* http://www.sasa.gov.uk/seed-ware-potatoes/potato-exports.

The broadly accepted consequences of climate change mean that potato production is vulnerable to a wide and increasing range of pests, pathogens and environmental stresses. The main environmental stresses are drought, heat, cold, mineral deficiency and salinity. Potato is very sensitive to drought and temperature stress, the latter considered to be the most important uncontrollable factor affecting growth and yield. In fact many potato varieties are so temperature sensitive that growth above the optimal average day time temperature of 14-22˚C range sees yield falling off sharply. When these pressures are taken into consideration with an increasing demand for new potato varieties with potential to export seed from Scotland, often to countries with hot climates, it is evident that growers and breeders need to develop both new germplasm and improved varieties and also to examine the methodology whereby new varieties produced in northern Europe can grow and compete in these target environments. In the tropics and subtropics it is evident that heat is a major limiting factor in potato cultivation. However, there are reports of the existence of genetic variability for heat tolerance (Tai *et al*., 1994, Menezes *et al*., 1999).

It is common practice in many crop species to base breeding stations in the region where the resulting new cultivars will be grown in order that selected genotypes will be adapted to that particular region. However, many regions grow potato cultivars that were bred elsewhere. Potato crops are grown in many diverse countries with differing soil types, climates and daylengths, while most breeding programmes are located in the northern latitude countries of Europe and North America. The reason for having many of the breeding programmes in these areas is partly historical, but is also related to the many disease problems which are often associated with repeated vegetative multiplication.

The work summarised in the presentation covers two approaches to selection of heat tolerant germplasm with potential for export. The first summarises earlier approaches at Scottish Crop Research Institute (SCRI, subsequently incorporated in James Hutton Institute) looking at advanced germplasm within breeding programmes and their adaptability to UK growing conditions and subsequently trialled in hot, Mediterranean environments while the second more recent approach examines assessment of relatively unselected material earlier in a targeted breeding programme to assess individual families in target environments.

MATERIALS AND METHODS

Two blocks of material were studied. In the earlier original study, the potato genotypes examined were all derived from the potato breeding research programmes at SCRI, involving a diverse range of parental material. In each of the years, the genotypes under study had previously been selected as a single plant and then in unreplicated four-plant plots at Blythbank Farm in Peeblesshire, Scotland. This farm was used by SCRI's Crop Genetics Department to produce disease-free seed tubers for planting (i.e,. it is a seed site). A further 3 years of assessment followed at a typical ware-growing site (Murrays Farm, East Lothian, Scotland), using replicated yield trials. Following 3 further years of selection, surviving selected material was trialled at four sites in England and subsequently at four sites in the Mediterranean area. Full details of the material, trials and variates recorded are given in Brown *et al*. (1996).

In the second more recent study, thirty seedling progenies were grown in the glasshouse and subsequently for two further years (as single plants and then as 6 plant plots) with basic selection to ensure reasonable yield, tuber number and shapes. After these two years of seed multiplication the thirty progenies were grown at one site in the UK, Egypt, Northern and Southern Greece using replicated trials. All non-UK sites were hot Mediterranean environments. Samples of all the individuals within the seedling progenies (minimum of twelve clones from each of the selected progenies) were represented as four replicate plots within individual sites, allowing the assessment of not only the progenies, but also of the parental material and of the effectiveness of selection for export markets.

RESULTS AND DISCUSSION

Due to the considerable body of data analysed over genotypes, seasons, sites and variates, in both studies and blocks of material it is not practical to present all data on individual genotypes. As a consequence, summary statistics for the variates are presented here. Mean squares from the analysis of variance of data from genotypes grown in the four environments in England and the four environments in the Mediterranean area are shown for saleable yield

recorded on each of the three seasons (Table 2). The effects of environments are partitioned, using orthogonal contrasts, into the difference between the English and Mediterranean environments, between the English environments and between the Mediterranean environments. In the analyses of saleable yield (and other variates considered), the interaction terms were tested against the replicate error pooled from the analyses of individual sites. There were significant differences (P< 0-001) observed between clones in each season. The UK environments produced significantly lower saleable yields (P<0-001) than did the Mediterranean environments (OS) and within both regions there were significant differences between environments (P < 0.001). The interactions between genotypes and the different environments (UK and OS), between genotypes and environments in all seasons and the interaction between genotypes and the environments within the Mediterranean region were also significant (all at $P < 0.001$). In contrast, the interaction between genotypes and environments within the UK region was relatively small and non-significant in all trial seasons (see Table 2).

Table 2. Mean squares from the analyses of variance of saleable yield (kg/plot) of clonal selections grown in 4 environments within England (UK) and 4 environments surrounding the Mediterranean Sea (OS) over 3 separate seasons. The effect of sites is partitioned into (1) English sites compared to Mediterranean sites (UK v. OS); (2) differences between English sites (within UK) and (3) differences between Mediterranean sites (within OS).

	Season 1		Season 2		Season 3	
Source	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Sites						
(1) UK x OS	1	1259.8***	1	349.7***	1	1147.8***
(2) *within* UK	3	734.5***	3	483.5***	3	278.9***
(3) *within* OS	3	546.1***	3	915.7***	3	385.1***
Clones	49	43.0**	39	51.8***	41	45.0***
Clones x (1)	49	16.5***	39	11.8***	41	11.8***
Clones x (2)	147	3.2	117	3.8	123	3.9
Clones x (3)	147	14.3***	117	15.3***	123	10.4***
Replicate error	400	3.9	320	4.6	336	4.7

** = $P < 0.01$, *** = $P < 0.001$

In Table 3, the correlation coefficients between saleable yield at the Scottish trial site and saleable yield at each of the English region environments were all significant (P < 0.001). In contrast, similar correlation coefficients of the Scottish site yield data with that of each environment from the Mediterranean region were generally lower. With regard to the Mediterranean environments, no obvious pattern was observed. When the correlation coefficients between the Scottish site and each of the other environments were averaged over the four environments in each region, the English means were 0.78, 0.76 and 0.63 for season 1, 2 and 3, respectively. In comparison, similar averaged coefficients from the Mediterranean region were correspondingly lower in season 1, 2 and 3 (r = 0.51, 0.56 and 0.44, respectively). In general, the average coefficient from correlation of the Scottish site yield data with the Mediterranean region was of a similar magnitude to the coefficient obtained by correlation of the average saleable yield of the English region with the average saleable yield of the Mediterranean region. The association between yield performance between environments within regions was also examined by correlation (Table 3). On average, the correlation

between any two environments within the English region was 0.59, 0.51 and 0.52 in the three seasons, respectively. A lower association between saleable yields at the Mediterranean locations was observed with coefficients averaging 0.29, 0.34 and 0.41. The range of coefficients within each region is shown in parentheses in Table 3.

Table 3. Correlation coefficients between clonal means of saleable yield of tubers produced at Scottish trial site and corresponding clonal means at four environments in England (Arthur Rickwood, Terrington, Gleadthorpe and Stockbridge House) and four environments around the Mediterranean (Valencia, Burgos, Cyprus, Israel). Mean correlations shown are simple arithmetic averages of correlation coefficients.

	Season 1	Season 2	Season 3
	n = 50	*n* = 40	*n* = 42
UK			
Arthur Rickwood	0.57***	0.55***	0.43***
Terrington	0.70***	0.65***	0.62***
Gleadthorpe	0.64***	0.68***	0.58***
Stockbridge House	0.65***	0.52***	0.51***
Mean UK	0.78	0.76	0.63
OS			
Valencia	0.67***	0.55***	0.33*
Burgos	0.20	0.42**	0.45**
Cyprus	0.21	0.37*	0.49***
Israel	0.52***	0.30	0.00
Mean UK	0.51	0.56	0.44
UK v OS	0.46	0.69	0.61
within UK	0.59 (0.42-0.74)	0.51 (0.24-0.63)	0.52 (0.36-0.62)
within OS	0.29 (0.08-0.46)	0.34 (0.15-0.46)	0.41 (0.19-0.69)
UK v OS	0.29 (0.01-0.55)	0.39 (0.00-0.54)	0.36 (0.11-0.59)

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Within the more recent study involving 30 seedling potato families at the four Mediterranean trial sites, plots were scored on a preference scale (over: 1 poor to 9 excellent) and weighed (yield: kg). Again, as previously, due to the considerable body of data analysed over genotypes and sites and space available, a summary of the superior five seedling families and the five poorest families are presented in Table 4, with fuller data summarised within the conference.

The summary data in Table 4 illustrates that within the first three years of generating clonal material in a targeted breeding programme, it is possible to assess a large range of diverse germplasm in a large number of seedling families. The data presented illustrates that it is possible to identify the poorer families and the superior families, allowing the breeders to focus resources towards the superior families. This allows the breeders to sow much larger samples of these selected families that grow well in the target environments and identify new superior genotypes and varieties. This approach also allows the identification of superior parental material, with the parents Almera, Argos and JHI clone : 97.MT.187 b 146 all contributing to the better seedling families. The data from small numbers of advanced clones in the first study

using advanced lines following some six years of UK selection does not inform the breeder of parental worth.

Table 4. Mean data for 5 poorest families and the five superior families, extracted from data on 30 seedling families. Progeny identity codes, pedigrees and yield (yld) and breeders preference (over) for the 4 environments. Significant lower or higher values in bold type.

Progeny	Pedigree	YldUK	YldGr1	YldGr2	YldEg	OverUK	OverGr 1	OverGr 2	OverEg
06.CP.4	TORRIDON*AGATA	15	19.3	**12.0**	5.8	3.7	**2.8**	**4.0**	**2.2**
06.CP.26	VALOR*SEBASTIAN	15	18.3	**13.0**	5.9	3.6	3.4	4.3	3.1
06.CP.28	TORRIDON*VALOR	**14**	**16.3**	18.0	5.8	**3.4**	3.9	4.2	2.9
06.CP.46	TORRIDON*KINGSTON	16	17.5	17.0	4.5	3.6	**2.5**	**3.8**	**2.4**
06.CP.76	E. BALFOUR*VALOR	15	18.0	16.0	**4.0**	4.0	3.5	5.0	3.3
06.CP.control 1	control var	**20**	**23.8**	**26.0**	**8.7**	**5.0**	**4.1**	**4.9**	**4.6**
06.CP.control 2	control var	**24**	**23.5**	**23.0**	**6.6**	**4.8**	**4.1**	**5.3**	**4.7**
06.CP.5	**ALMERA*97.MT.187 b146**	**21**	**22.8**	**22.0**	6.7	3.8	3.0	4.8	3.1
06.CP.7	**ARGOS*ALMERA**	**19**	**23.5**	**23.0**	**6.2**	4.1	3.7	5.0	**3.7**
06.CP.10	**ARGOS*97.MT.187b146**	**20**	20.0	**23.0**	6.2	**4.2**	3.2	4.5	3.3
06.CP.23	G 7707 2*VALOR	15	17.8	**24.0**	6.7	3.8	3.7	**5.0**	3.6
06.CP.24	G 8776 4*VALOR	17	20.3	**22.0**	6.3	**4.3**	3.4	**5.0**	**3.6**
	Mean	17.58	20.07	19.92	6.12	4.03	3.45	4.65	3.38
30 progenies plus 2 control “progenies”, 4 reps of 12 clones/rep. 4 sites are: UK, Greece 1 – Gr1, Greece 2 – Gr2 & Egypt - Eg									
Yld = Mean plot yield in Kg									
Over = Mean plot overall breeders score, 1 is poor, 9 is excellent									

The data here utilising a progeny approach to selection for hotter environments gives encouraging information to breeders that it is possible to identify superior germplasm and parental material and to ultimately identify new adapted varieties from within breeding programmes in northern Europe. The evidence from the earlier study revisited here supports the view that, given a good association for the important characteristics such as yield, appearance and internal condition, between the performance of genotypes at early generations of a programme in a limited number of environments and in later generations across more diverse environments (e.g., in regions far removed from the original sites), it is possible to select apparently superior potato genotypes with a degree of confidence. The results illustrate the need for plant breeders to assess the efficiency of selection procedures within breeding programmes for individual characteristics and also the reliability of assessing different characteristics across a diverse range of environments. Further studies using data on progenies early in breeding programmes to allow the identification of superior parental material and will lead to the identification of the important genes involved which contribute to the superior genotypes and varieties and ultimately to mapping the important traits and genes responsible..

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POTENTIAL FOR MOLECULAR MARKER DEVELOPMENT AND UTILIZATION IN POTATO BREEDING PROGRAMMES BASED ON A GENETIC STUDY IN A DIPLOID POTATO POPULATION

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Summary: The integration of marker assisted selection (MAS) into conventional potato breeding programmes is highly desirable. It offers a more efficient and accurate approach than conventional breeding alone, with the potential for earlier screening of larger numbers of genotypes and analysis of multiple commercially important potato traits. This reduces selection time, space and the resources required, increasing the chances of achieving the desired outcome. Through extensive phenotyping and genetic mapping of a highly heterozygous diploid potato population (06H1), it was possible to identify quantitative trait loci (QTLs) linked to commercially important traits. This study offers the potential to turn these markers into tools for effective trait selection in commercial potato breeding programmes.

INTRODUCTION

Conventional potato breeding is a method of genetic improvement that involves the hybridisation of parental potato clones with commercially desirable traits and the subsequent selection of new clones with superior qualities. This method of potato improvement involves several years of field trialling, disease testing and assessment of many commercially important traits, during which the number of clones are reduced until the best clones are identified as new varieties or used as superior parental breeding material.

To ensure that modern potato breeding becomes more efficient in meeting changing needs, there is a strong requirement to develop effective links between modern molecular genetics and the improvement of potato. Typical potato breeding programmes rely on multiple rounds of phenotypic selection rather than directly exploiting genotypic information. This can result in clones possessing commercially important traits being discarded in the early stages of a breeding programme. MAS complements conventional breeding methods, improving selection efficiency and enhancing the probability of achieving the desired cultivar.

Genetic analysis in tetraploids is challenging, so diploid populations with half the chromosome complement are often used in genetic studies. The aim of this study was to assess several commercially relevant potato traits in a highly polymorphic diploid potato population (06H1) (Young, 2013). With extensive phenotyping and the generation of genetic maps using a large set of newly-discovered molecular markers, this study provides new insights into the genetic control of potato traits and the potential to identify markers linked to commercially important traits with prospects for use in MAS in commercial breeding programmes.

MATERIALS AND METHODS

06H1 population & field trialling

The 06H1 population was developed prior to the start of this project and originated from crossing two different *S. tuberosum* Group Phureja - *S. tuberosum* Group Tuberosum diploid hybrids and comprises 498 clones. Field trialling took place over three years between 2009 and 2011 at Balruddery farm, Invergowrie, Dundee. All trials were planted in 5 plant plots as two replicates in an alpha design. In years one and two, 346 clones of 06H1 were trialled and in year three, 188 clones of 06H1 (the mapping population) were trialled.

Phenotyping

Many potato traits were assessed between 2009 and 2011 (table 1). Agro-morphological traits such as tuber shape, flesh colour, skin colour and internal defects were scored on an ordinal scale and other traits such as yield and dry matter were physically measured. The remainder of the traits were assessed using a 1-9 scale of increasing desirability, a method of rapidly assessing and scoring different traits. For the purpose of this paper, only yield, eye depth and tuber shape will be discussed with reference to other traits where relevant.

Tuber shape (SHAPE) was evaluated using two different approaches. The first method involved visually assessing the tubers from the total produce of individual plots using a descriptive 1-4 scale where 1 = round, 2 = oval, 3 = long oval and 4 = very long oval. The second approach used digital callipers (Mitutoyo UK Ltd) to determine the length/width (LW) ratio of 6-9 tubers randomly selected from each plot (a similar evaluation method as that described by van Eck *et al*.(1994). The tubers with LW ratio higher than ~1.5 are referred to as 'elongated', whereas a LW ratio close to 1 are classified as 'round'. Visual tuber shape (VSHAPE) or tuber shape regularity was assessed on a 1-9 scale where 1 = a very poor shape and 9 = a very uniform and desirable shape. A desirable shape was based on consumer and processing industry preferences e.g. long tubers for chipping and round tubers for crisping with minimal peeling losses taken into account. Eye depth (EYE) was assessed on a 1-9 scale where 1 = very deep eyes and 9 = very shallow eyes.

Table 1. Full list of potato traits phenotyped between 2009 -2011.

yield	maturity	sprouting after harvest
eye depth	dry matter	sprouting during storage
tuber shape (1-9 visual assessment)	flesh colour	longest sprout
tuber shape (measured)	skin colour	number of sprouts
visual shape	internal condition	emergence
visual size	internal defects	after-cooking darkening
fry colour	growth cracking	tuber size distribution

Statistical analyses

Phenotypic data was analysed using Agrobase Generation II SQL (Agronomix Inc., MB Canada) and GenStat for Windows 14th edition (VSN International 2011). Using Agrobase,

data was analysed using either an alpha analysis or RCB (randomised complete block) analysis to generate statistics for clone means, trait means and coefficient of variation. Clone means were further used to calculate broad sense heritabilities, distributions and used for QTL analysis. Trait means were used to calculate correlation coefficients between all traits. GenStat software was used to analyse clone means using a general analysis of variance (ANOVA) with blocking. Means squared (m.s.) and m.s. residual values from the ANOVA were used to calculate the heritability of each trait. The broad sense heritability (h^2) of clone means was estimated as $h^2= \sigma_g2/ \sigma_g2+ (\sigma_e2/2)$. Also using GenStat software, a two-sided correlation test was used to calculate the correlation coefficient (r). Correlations were calculated using mean trait values from the mapping population (188 individuals) for all years (2009-2011). Significant correlations > ±0.198 ($p<0.05$) and > ±0.244 ($p<0.01$) were identified using Rohlf and Sokal (1995) correlation coefficient tables.

DNA extractions & single nucleotide polymorphism (SNP) screening

Leaf material was sampled from 06H1 glasshouse grown plants and DNA extracted from 100mg of leaf material using a Qiagen DNeasy plant mini kit (Qiagen). DNA was eluted in Qiagen EB buffer (10mM Tris 8.5) and quantified using a Nanodrop 1000 (Thermo Scientific). A DNA concentration of 50ng/µl in a volume of 18µl was sent to Genprobe (Livingstone, UK) and 186 06H1 individuals plus the two parents were genotyped using a core set of SNPs known as the "Infinium 8303 Potato Array" using Illumina's Infiniums technology (Illumina, San Diego, CA).

Analysis of SNPs using Genome Studio software

Analysis of SNPs was carried out using Genome Studio software (Illumina, San Diego, CA) to identify all polymorphic SNPs that were segregating in either a 1:1 or 1:2:1 ratio (figure 1). All monomorphic clusters, NormR results <0.2, unbalanced allelic segregations and SNPs with large numbers of missing values were removed.

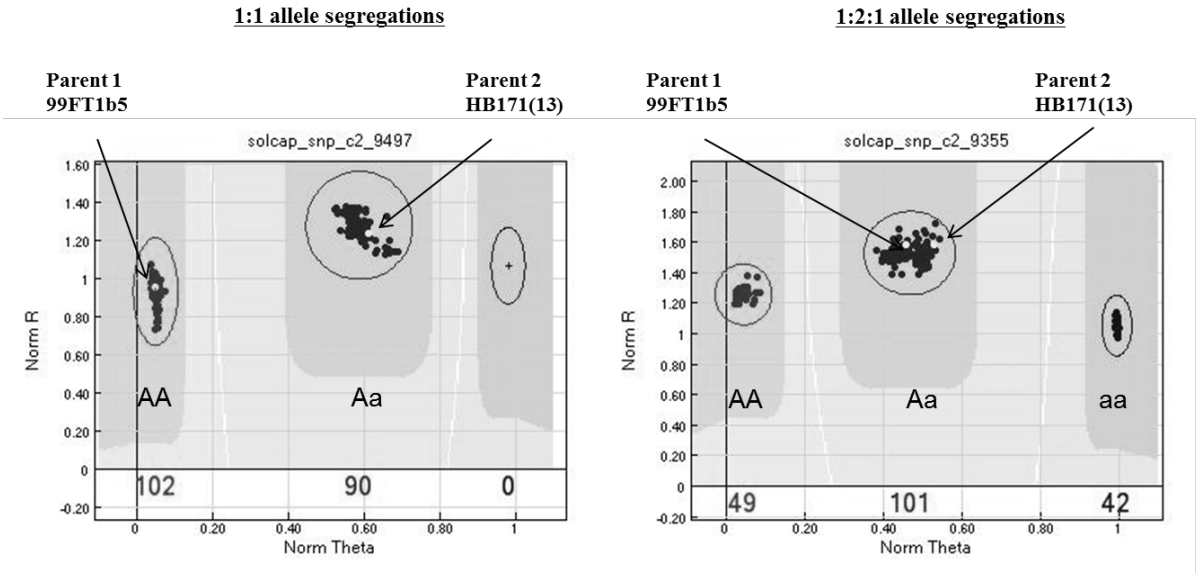

Figure 1. Illustration of identification of polymorphic SNPs using Genome Studio software.

Linkage analysis using JoinMap4 software

JoinMap4 (van Ooijen, 2006) was used to calculate genetic linkage maps of the 06H1 population. All polymorphic markers identified through Genome studio were entered as a cross-pollinated (CP) population type. Data was removed from the analysis if they fitted the following criteria: markers with >20% of missing values, similarity of loci >0.98, markers with severe segregation distortion (P<0.0001) and if any identical individuals were present (one of the individuals was removed). Twelve linkage groups were created using a logarithm of odds (LOD) up to 15. Within each linkage group, suspect linkages were calculated and any with a recombination frequency >0.7 were removed and maximum linkages with recombination frequencies >0.4 with a high LOD were removed. Regression mapping was performed using Haldane's function on combined parental genotypes (hkxhk) and maximum likelihood mapping performed on parent 1 (nnxnp) and parent2 (lmxll) genotypes.

Regression mapping was completed on all data by adding the fixed orders from the parental data on to the hkxhk maps. Twelve linkage maps were generated and linkage groups identified by the known positions of the SNP markers in the potato genome chromosomal pseudomolecules.

QTL analysis

MapQTL5 (van Ooijen *et al.,* 1996) was used to map quantitative trait loci in the 06H1 population. Using this software, QTLs were analysed using the phenotypic data collected on all traits from 2009-2011 combined with the 06H1 mapping data. Interval mapping was carried out for each trait on each chromosome and QTLs identified. To ensure that the QTLs observed were not purely due to chance, permutation tests (based on 1000 permutations) were run to determine above which LOD interval (95% interval) QTLs were classed as significant. To determine which SNP markers were involved in explaining the observed phenotypic variance, a SNP marker or markers with the highest LOD value for the QTL was identified and then the full QTL interval was determined by choosing the highest LOD score and (depending on which number was closest) either subtracting 1 LOD or using the threshold from the permutation test as a cut-off point.

RESULTS AND DISCUSSION

Trait correlations and broad sense heritabilities

Correlation coefficients were highly significant ($p<0.01$) between all traits assessed and ranged from 0.65 to 0.82 and broad sense heritabilities ranged from 0.67 to 0.92 suggesting that the traits assessed are largely controlled by genetic factors. The two methods used for assessing shape were highly correlated (0.91), suggesting that the rapid visual assessment method is a suitable tool for shape analysis in breeding programs but the reproducibility and objectivity of measurement by calliper provides an important advantage for detailed genetical studies.

Strong correlations were also observed between different traits. For example, yield shows high correlations with emergence, visual size, fry colour and maturity ranging from 0.24 to 0.71. These strong correlations observed between different traits are often good indicators of traits

that may be under similar genetic control. These tie in with discussions below where QTLs for these traits co-localise on the same chromosomes.

Linkage maps and QTLS

Through the SolCAP project (http://solcap.msu.edu/index.shtml), 69,011 SNPs have been identified from commercially important processing cultivars Premier Russet, Atlantic and Snowden (Hamilton *et al.*, 2011). From this, SolCAP selected and released a core set of SNPs, known as the "Infinium 8303 Potato Array" that covers 650Mb of the potato genome where 36% of the SNPs are targeted to candidate genes and 64% in other genes spread throughout the genome (Felcher *et al.*, 2012). Analysis of these SNPs through Genome Studio identified 3473 polymorphic markers in the 06H1 population which were used to create genetic maps using JoinMap4 software. Through the filtering process discussed earlier, another 2075 markers were removed and twelve linkage maps were created comprising 1398 markers. Table 2 lists the number of markers mapped to each chromosome with map lengths. The high number of mapped markers (1398) provides dense coverage of the potato genome.

Table 2. Chromosomes identified in mapping, showing numbers of markers in each and mapped length (cM.).

Chromosome	No. of mapped markers	Map length (cM)
1	170	102
2	127	68
3	119	77
4	177	86
5	57	61
6	100	66
7	159	72
8	72	79
9	108	91
10	79	69
11	89	70
12	141	78
Total	1398	919

Yield

YLD QTLs were identified on eight chromosomes (LGI, IV, VI, VII, VIII, IX, X and XI). It is not surprising to identify QTLs spanning several chromosomes as yield is a complex trait and is expected to be under the control of many genes controlling factors affecting growth and yield. On three chromosomes, LG I, VI and IX, YLD and visual tuber size (VSIZE) QTLs co-localised (YLD on LG I, VI and IX accounted for 8 - 15%, 10% and 9% of the observed phenotypic variation respectively and VSIZE on the same linkage groups accounted for 7 - 11%, 10 to 11% and 16% of the variation respectively) with correlations between VSIZE and YLD (2009/2010) being highly significant, $r = 0.43 - 0.69$ ($p<0.01$). Tuber size would be a major contributor to final tuber yields, so the strong association between VSIZE and YLD is

not unexpected and it is reasonable to assume that these two traits may be under similar genetic control.

Schäfer-Pregl *et al.* (1998) reported a strong correlation between yield and tuber starch-content QTLs and Li *et al.* (2010), through an association mapping study, identified candidate genes - *Rca, Sps* and *Pain1* that were highly associated with tuber starch content. Chen *et al.* (2001) developed a potato molecular-function map for carbohydrate metabolism which illustrates the map positions of Schäfer-Pregl *et al.* (1998) tuber starch content QTLs along with candidate genes that may be influencing their control. Interestingly, several of the yield QTLs from the 06H1 population on LG I, IV, VI, VII, VIII and XI align with these tuber starch QTLs and some of the candidate genes implicated in their control are ADP-glucose pyrophosphorylase S (*AGPaseS*), pyruvate kinase (*Pk*), α-glucosidase (*Agl*), plasma membrane H^+-ATPase 2 (*Pha2*) and a sucrose transporter 1 (*Sut1*).

The role of gibberellins in higher plants has been widely studied and is known to play a significant role in plant growth and development. Carrera *et al.* (2000) reported that GA 20-oxidase is a candidate gene involved in the control of potato yield and Ewing *et al.* (2004) identified a QTL for the gene GA 20-oxidase towards the distal end of potato chromosome XI. This may be of significance as a QTL for YIELD 2010 in this study (explaining 10% of the observed phenotypic variation) is also located on LG XI.

Eye depth and tuber shape

During phenotyping of this population, it became evident that there was a link between eye depth and tuber shape as shallow eyes were more prevalent in the longer oval shaped tubers than in round tubers. In this population, correlations between eye depth and shape were highly significant 0.49 – 0.59 ($p<0.01$) and correlations between eye depth and visual tuber shape were 0.48 – 0.61 ($p<0.01$). Eye depth and tuber shape have been reported to be closely linked on LG X (Li *et al.*, 2005). This strong association between EYE, SHAPE and VSHAPE was validated through the co-localisation of major QTLS for these traits on chromosome X. This set of QTLs show the highest percentage explained for phenotypic variation, with EYE, SHAPE and VSHAPE QTLs explaining 43 - 45, 30 - 38 and 16 - 20 percent of the variation respectively. The large QTL for tuber shape and eye depth on LG X almost certainly corresponds to that observed by van Eck *et al.*(1994) for tuber shape and by Li *et al*. (2005) and Śliwka et al. (2008) for eye depth.

Śliwka *et al.* (2008) have reported QTLs for eye depth in LG X, tuber shape in LG II and VSHAPE in LG III. It is highly probable that these same QTLs correlate with some of the minor QTLs identified in this study. However, no previous records of tuber shape or eye depth have been reported on LG VI. QTLs of small effect for SHAPE 2009 and EYE 2010 (explaining 8% of the variation) were identified on LG VI in this study and even though they were not reproducible across years, they may be worth investigating further.

Additional mapping and QTL studies were carried out on eye depth and shape using different methods of analysis (Prashar *et* al., submitted). Prashar *et al*. discuss the mapping and QTL process in more detail, comparing the linkage maps with the potato genome and discussing the early development of tuber shape further.

CONCLUSION

The 06H1 population had not previously been studied, so through extensive phenotyping and statistical analyses, new insights were gained into the population characteristics, trait heritabilities and relationships observed between traits. A comparison between QTLs identified in this study and QTLs reported in the potato literature identified numerous similarities in the locations of the QTLs, along with suggestions as to candidate genes that may be involved in the control of traits. In this study, novel QTLs for several traits, such as eye depth, shape, flesh colour, skin colour and foliage maturity were identified.

This study has identified markers associated with QTLs for economically important potato traits which offer the potential to turn them into tools for effective trait selection in commercial potato breeding programmes. Large populations can be screened rapidly using MAS and desirable traits can be positively identified and selected in the earlier stages of breeding programmes. MAS offers increased accuracy and selection efficiency and in turn will lead to improved and accelerated variety development. Also, the ability to determine marker/gene dosage in parental material will greatly enhance the outcome of breeding for superior potato varieties. For marker assisted breeding to become economically viable, it will be important to develop many valuable markers that can be used simultaneously to carry out multi-trait diagnostics on breeding material.

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USING THE POTATO GENOME TO MAP AND CLONE DURABLE RESISTANCE GENES MORE RAPIDLY

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Potato is the world's most important non-cereal food crop and plays a significant contribution to the UK bio-economy as fresh and processed produce for human consumption, for seed potato tubers, and as a source of starch for food and industry. The continued success of potato, however, relies on a competitively priced product grown in a manner that is safe, eco-efficient and sustainable in both economic and environmental terms. Pests and diseases are a considerable obstacle to the achievement of such goals in all crops. The most significant threat to potato production worldwide is late blight disease, caused by the oomycete pathogen *Phytophthora infestans* that was responsible for the Irish famine in 1845-1846. *P. infestans* is an aggressive, destructive pathogen that causes large yield losses in potato and tomato, and costs associated with chemical control amount to >£3.5 billion globally per year. The consequences of blight infection are serious and current management practices therefore rely on multiple (up to 20) fungicide applications per season for disease control. *P. infestans* is a pathogen with high 'evolutionary potential' and changes in *P. infestans* populations *via* migration and mutation are well documented. Such changes are probably driven by increases in aggressiveness, fitness and virulence against host resistance as well as insensitivity to fungicides. As a consequence, there is a need, when developing a strategy for deploying disease resistance, to respond rapidly to dramatic changes, and therefore novel threats in the pathogen population.

Advances in understanding the molecular processes and mechanisms of disease and inducible resistance provide a molecular framework to search for durable late blight control. All microbes trigger immune responses in plants *via* host receptor-mediated recognition of PAMPs. Successful pathogens suppress or otherwise manipulate PAMP-triggered immunity (PTI). They do this by secretion of virulence factors, called effectors, which re-programme host metabolism to the benefit of the pathogen. Effector-triggered suppression of PTI is the pathogen's front-line of attack in overcoming plant defences. When a successful pathogen has suppressed PTI, plants possess a second layer of inducible defence in the form of resistance (*R*) genes, the products of which typically encode NB-LRR proteins that detect effectors (termed avirulence proteins; AVRs). R proteins subsequently activate resistance responses (effector-triggered immunity; ETI) that overlap with PTI, but are more rapid and include additional responses, such as programmed cell death.

Recently, a number of *P. infestans Avr* genes have been identified. Although each corresponding AVR protein is distinct at the primary sequence level, they all share a signal peptide for secretion, followed by the motif RXLR and an acidic region, often ending in the

sequence EER. The translocation of RXLR-containing proteins inside the plant cell is consistent with their recognition by host NB-LRR resistance proteins. We seek universally expressed, essential (i.e., functionally non-redundant) and sequence-conserved effectors that are targeted by resistances from wild potato and Solanaceous non-host species. Such resistances may be predicted to be more durable than those previously deployed and, by their nature, are intended to meet the challenge of future pathogen population changes.

We have conducted parallel screening of plant germplasm for responses to diverse, contemporary *P. infestans* isolates and also to specific key effectors, and identified new sources of resistance in the Commonwealth Potato Collection (CPC). The CPC, at the James Hutton Institute, comprises >1800 potato accessions held in true seed form and represents an invaluable source of novel late blight resistances. Recent advances in genome sequencing technologies have led to a dramatic reduction in the associated costs, facilitating rapid analysis of entire crop genomes. Eleven years since the sequencing of the model plant *Arabidopsis thaliana*, the genomes of two important Solanaceae crop plants, potato and tomato, were reported. These genomes provide a blueprint to find new genes for important traits, including disease resistance.

In a recent study (Jupe *et al.*, 2012) we identified 438 NB-LRR genes in the sequenced S*olanum tuberosum* group Phureja clone DM1-3 516 R44 (DM), describing their phylogenetic relationship and their physical locations in the 12 potato chromosomes. This study formed the basis of a novel *R* gene enrichment and sequencing platform (RenSeq). By combining the sequences of the newly predicted potato NB-LRRs and the tomato *R* genes alongside functionally validated pepper resistance genes, over 48,500 NB-LRR gene-specific, RNA-based, biotinylated probes were designed using Agilent SureSelect technology. The enrichment for NB-LRR genes has been used to significantly reduce the genome complexity of wild potato species prior to current generation sequencing. When applied to the sequenced clone DM, over 330 additional NB-LRR containing regions were identified (Jupe *et al.*, 2013). This approach can thus be used to re-annotate the NB-LRR gene complements from at least partially available genomes without reference gene models. A comparison between the newly identified NB-LRRs and the corresponding baits with the highest sequence similarity demonstrated that approximately 80% homology is sufficient for enrichment. RenSeq has also successfully been utilised on bulked resistant (BR) and bulked susceptible (BS) samples in segregating populations involving wild diploid outcrossing *Solanum* species. Computational algorithms were developed to enable the rapid identification of sequence polymorphisms that co-segregate closely with resistances. NB-LRR-derived markers that co-segregate with resistance were mapped in segregating back-cross populations. New resistances mapped in all cases so far near previously described NB-LRR gene clusters containing recently duplicated paralogs.

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SOIL PEST MANAGEMENT INITIATIVE: A POTATO CYST NEMATODE FOCUS

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Summary: Potato Cyst Nematodes, *Globodera pallida* and *Globodera rostochiensis* are a serious concern for the potato industry in Great Britain. New infestations exclude production for seed, and for ware production an integrated control strategy exploits field selection, variety resistance and use of nematicides. The industry faces challenges arising from the detection and quantification of soil PCN infestations, particularly for *G. pallida* as it increased significantly following introduction of varieties with full resistance to *G. rostochiensis.* Successful PCN control is dependent upon reliable estimates of field populations and the Soil Pest Management Initiative has been established by industry to improve soil sampling methodology and the consistency and reliability of laboratory PCN extraction and identification. SPMI will underpin the crucial stewardship activities of the product Authorisation Holders and it will develop training programmes to help industry better understand and manage PCN in the future.

INTRODUCTION

Potato Cyst Nematodes, *Globodera pallida* (white PCN: wPCN) and *Globodera rostochiensis* (golden or yellow PCN; yPCN) are a serious problem for potato producers and the industry in Great Britain. Current losses are estimated at £26 million p.a. and withdrawal of nematicides would have a significant impact, with losses increasing to an estimated at £55m p.a. (Twining *et al*, 2009). *G. pallida* is a particular concern as its distribution in GB has increased significantly following the introduction in the 1970's of potato varieties (e.g. Maris Piper) with resistance to *G. rostochiensis*. Control of PCN has been based on strategies that adopt a range of measures including a) rotation to allow PCN populations to decline; b) use of granular or fumigant nematicides to reduce the infective population level, and c) by exploiting variety resistance.

SOIL PEST MANAGEMENT INITIATIVE

The Soil Pest Management Initiative (SPMI) is a recently established cross-industry group which has best practice management of nematodes in potato crops as its initial focus. Key areas of activity include: a) *soil sampling strategies*; with an industry consultation being carried out, b) *development of a proficiency testing system for laboratories* involved in PCN extraction and identification and c) *industry training* to improve knowledge of PCN and management strategies.

The production of best practice guidelines for soil sampling and the proficiency testing for laboratory sampling will be crucial. They both underpin robust and reliable management decisions on any PCN control strategy regarding land selection, use of nematicides and variety choice. Commercial decisions about the choice of specific nematicides or varieties to suit particular end-markets are then a matter for agronomists and advisors. The SPMI work supports the company product stewardship, but is not a replacement for this crucial activity undertaken by approval holders.

The training programmes under development will increase grower and advisor knowledge of PCN populations and the management options available within the legislative and environmental framework that the industry has to comply with. This will include use of a PCN model to demonstrate the effects of wPCN infestation on the yield potential of the potato crop and the consequent population dynamics of the wPCN. The model was developed with Potato Council research funding at SCRI (now James Hutton Institute) and a new online version (Potato Council, 2013) is available to the industry.

SPMI will engage with the industry and research groups exploring alternative management option to ensure up-to-date information and advice is available. The development of complementary management options has included use of trap crops e.g. *Solanum sisymbriifolium* (sticky nightshade) and cover crops, grown as biofumigants which release isothiocyanates when macerated and incorporated into soils. Recent research has highlighted the potential such biofumigant crops may play in an integrated approach to control PCN across the rotation. Longer term, the *G. pallida* genome is being mapped and targets for novel resistance mechanisms are being identified that could be exploited by conventional breeding or GM technologies.

Currently there is a window of opportunity to improve soil sampling, laboratory assessments and PCN identification to better understand and quantify the populations present. This will allow industry to more effectively exploit currently available nematicides, potentially use biofumigants in the rotation, and deploy varieties with improved partial resistance to reduce wPCN populations and contribute to the improved sustainability of the potato industry in GB.

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BIOLOGY AND CONTROL OF *DICKEYA* SPP. AFFECTING POTATO IN THE UK

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Summary: *Dickeya dianthicola* and *D. solani* were first found causing blackleg and soft rot of ware potatoes in England and Wales in 1996 and 2007 respectively. Genome sequence comparisons confirmed phylogenetic relatedness between *Dickeya* species but a lack of diversity within *D. solani*, allowing design of sensitive detection and specific identification methods. Study of the causes of blackleg in UK classified seed crops from 2010-2013 confirmed absence of *Dickeya* from Scottish potatoes, whereas 0.5–2.3% of stocks found with *D. solani* in England and Wales were all of non-UK origin. The pathogen did not survive over winter in soil or common weeds following potato crops with high incidence of *D. solani* blackleg. Three watercourses were found contaminated with *D. solani*, in only one case did the infestation persist in the following seasons. Control recommendations are based on sourcing *Dickeya*-free seed and avoiding irrigation from contaminated water. Scottish legislation introduced in 2010 prohibits planting of potatoes infected with *Dickeya*.

INTRODUCTION

A species of *Dickeya* (syn. *Erwinia chrysanthemi*) has been spreading in the European seed potato trade since the early 1970's and has been most damaging in warm climates (Lumb *et al*., 1986; Toth *et al*., 2010). Recent phylogenetic analysis comparing *recA* and *dnaX* gene sequence homologies (Parkinson *et al*., 2009; Sławiak *et al*., 2009) confirmed the species involved as *D. dianthicola,* originally introduced into Europe around the 1950's and spread as a pathogen of ornamentals such as *Dianthus*. The same phylogenetic analyses also identified another blackleg-causing *Dickeya* strain which had been observed to be spreading on European seed potatoes since at least 2004-5 (Tsror *et al*., 2009; Toth *et al*. 2010). The same strain has also been isolated from ornamental hosts including hyacinth and *Scilla* sp., again suggesting a pathway of spread between ornamental and potato production. This strain has recently been formerly classified as *Dickeya solani* (van der Wolf *et al*., in press). *D. solani* causes a particularly aggressive form of blackleg and soft rot disease, especially when air temperatures exceed 25 °C and in some European countries it has become the dominant cause of potato blackleg, possibly due to its ability to outcompete *Pectobacterium spp.* and *Dickeya dianthicola,* the other common causes of blackleg (Czajkowski *et al*., 2013).

This paper reviews the findings of UK collaborative research focussed on the use of genome sequence data to confirm phylogenetic relationships between *D. solani* and the other *Dickeya*

species, the degree of genetic diversity amongst isolates of *D. solani* from different sources and to develop practical methods for its detection and identification. Application of the diagnostic methods to determine the current incidence of findings of *D. solani* in the UK and to improve understanding of how it spreads and survives under UK conditions is also described.

DESIGN OF REAL-TIME PCR ASSAYS USING COMPARATIVE GENOMICS

Genome sequencing was completed for pairs of *D. solani* and *D. dianthicola* isolates (Pritchard *et al*., 2013a) as well as other genotypes representing all known *Dickeya* species and other, as yet unclassified, *Dickeya* phylogroups (Pritchard *et al*., 2013b). Following assembly of the sequence data, a bioinformatics pipeline was designed using default parameters for selection of primers and probes suitable for real-time PCR analysis (Prichard *et al*., 2013c). The specificity of selected primers and probes were then computationally tested against the other *Dickeya* genomes as well as other sequenced *Enterobacteriaceae* in the GenBank database. The specificity of primers and probes that matched sequences within the *D. solani* or *D. dianthicola* genomes but were absent from all other genomes was then confirmed by testing a panel of 77 reference isolates of *Dickeya* and related genera from the National Collection of Plant Pathogenic Bacteria (NCPPB). The confirmed specificity of primers and probes finally selected for use in real time PCR assays for detection and identification of *D. solani* and *D. dianthicola* is shown in Table 1.

Table 1. Specificity of selected real time PCR assays (DIA and SOL) designed for detection of *D. dianthicola* and *D. solani*.

Identification	# isolates tested	# isolates detected	
		DIA	SOL
Dickeya dianthicola	7	7	0
Dickeya solani	16	0	16
Dickeya dadantii	16	0	0
Dickeya chrysanthemi	10	0	0
Dickeya paradisiaca	1	0	0
Dickeya zeae	11	0	0
Dickeya unidentified clade DUC-2	5	0	0
Dickeya unidentified clade DUC-3	1	0	0
Dickeya SLCI (‘*D. aquatica*’)	1	0	0
Dickeya SLCII	1	0	0
Pectobacterium atrosepticum	1	0	0
P. carotovorum subsp. *carotovorum*	1	0	0
P. betavasculorum	1	0	0
P. carotovorum subsp. *odoriferum*	1	0	0
P. wasabiei	1	0	0
Pantoea agglomerans	1	0	0
Brenneria quercina	1	0	0
Erwinia amylovora	1	0	0

A second approach to develop a further real-time PCR assay with specificity to *D. solani* involved selection of primers and probes based on observed *fusA* gene sequence diversity between *Dickeya* species and related taxa (Kelly *et al*., 2012). Similar levels of specificity were

observed with both *fusA* and SOL assays when tested against the panel of reference strains and in provisional intercomparisons across 6 UK diagnostic laboratories. Further validation is planned across European laboratories within the framework of the EU EUPHRESCO II project.

DIVERSITY WITHIN *DICKEYA SOLANI*

A web-based multilocus sequence typing (MLST) scheme (http://pubmlst.org/dickeya/) based on sequence homology between eight housekeeping genes (Kowalewska *et al*., 2010) confirmed that *D. solani* is phylogenetically distinct from all other *Dickeya* species, as indicated by earlier analyses of individual genes (Parkinson *et al*., 2009; Sławiak *et al*., 2009). Sequence homogeneity observed between all isolates of *D. solani* tested indicated that a single clone has infected and spread between ornamental and potato hosts. Two higher resolution approaches, comparing Single Nucleotide Polymorphisms (SNP) or Variable Number Tandem Repeat (VNTR) sequences, also concluded a lack of micro-evolutionary diversity within *D. solani* isolated from potato or environmental sources either in the UK or elsewhere in Europe and Israel. Six SNP profiles and 3 VNTR profiles were identified (Tables 2 and 3), and profiles were shared between isolates from different countries, from potato and hyacinth and from potato and contaminated surface water, indicating potential application of these analyses for source tracing of individual profiles.

Table 2. SNP profiles amongst *Dickeya solani* isolates.

Source	Number of Isolates	SNP profile
Potato, Israel	1	1
Potato, Poland	3	
River water, Scotland	3	
Potato, Belgium	1	2
Potato, Netherlands	2	
Potato, Israel	1	3
Potato, Spain	1	4
Potato, Netherlands	1	5
Potato, Netherlands	1[T]	6

DISTRIBUTION OF *DICKEYA* SPP. IN UK POTAO CROPS.

Annual surveys were conducted between 2010 and 2013 to determine the occurrence and distribution of *Dickeya* spp. causing blackleg in all (approx. 800 per year) seed stocks entered for classification in England and Wales and in seed and ware stocks considered to be at highest risk from the pathogen in Scotland. The latter included crops produced from non-Scottish origin seed, their nearby crops and crops grown close to previously contaminated fields or watercourses. Around 10% of seed crops produced from Scottish-origin seed were also surveyed each year. A total of 545, 752, 821 and 671 Scottish crops were surveyed in 2010-2013 respectively. All crops were sampled for blackleg plants on 2 official inspection dates. An additional post-harvest test of the Scottish crops was also performed on 200-600 tubers per stock. In all cases, the bacteria were isolated on modified crystal violet pectate medium

(CVPM) and then identified using the new real-time PCR and MLST sequencing methods described above. Survey results are summarised in Table 4.

Table 3. VNTR profiles amongst *Dickeya solani* isolates.

Source	Number of isolates	VNTR profile
Potato, England/Wales	27	1
Potato, France	1	
Potato, Israel	2	
Potato, Poland	1	
Hyacinth, Netherlands	1	
Potato, England/Wales	15	2
Potato, Finland	2	
Potato, Israel	1	
River water, England/Wales	2	
Potato, England/Wales	1	3
Potato, Netherlands	1[T]	

[T]*Dickeya solani* type strain (IPO 2222)

All *Dickeya* findings in the UK were associated with seed originating from outside of the UK and no evidence of spread to crops grown from seed of UK origin was identified within the 4-year period. In England and Wales, blackleg was caused by *D. solani* in only 0.5–2.3% of the crops. *D. dianthicola* was found in only 0.1-1.1% of the crops, whereas *Pectobacterium atrosepticum* was found to be the dominant cause of blackleg in 24.1-28.4% of the crops. In Scotland, *D. solani* was found in only 9 ware potato crops (1.6% of crops surveyed) in 2010. The only other findings of *D. solani* in Scotland were in 4 crops the previous year (2009). Since 2010 there have been no further findings of *Dickeya* spp. in potato grown in Scotland, and they have never been found in seed or ware potatoes grown from seed of Scottish origin.

Table 4. Blackleg findings in seed potato stocks entered for classification in England and Wales and potato crops surveyed in Scotland.

	2010	**2011**	**2012**	**2013**
England and Wales				
% seed stocks surveyed with blackleg	32.1	21.5	33.8	31.0
% with blackleg caused by *D. solani*	2.3	0.5	0.6	0.5
% with blackleg caused by *D. dianthicola*	0.1	0.1	0.6	0
Scotland				
% seed crops surveyed with blackleg	34.2	64.2	67.8	35.6
% total crops sampled with blackleg caused by *D. solani*	1.6	0	0	0
% total crops sampled with blackleg caused by *D. dianthicola*	0	0	0	0

SURVIVAL AND SPREAD OF *DICKEYA* SPP. IN THE UK

Local spread of *D. solani* was studied in field observation plots planted with artificially inoculated seed tubers. Inoculation was performed by vacuum infiltration of the seed tubers in aqueous suspensions containing different concentrations of bacteria. Blackleg incidence after planting was positively correlated with the tuber inoculum loading in each of 3 seasons, but was restricted to plants grown from inoculated seed only. Spread of latent infections to progeny tubers of inoculated and uninoculated neighbouring rows of plants was observed but was related more to the degree of localised soil waterlogging than to the original bacterial loading on the seed. Monitoring of harvested tubers with *D. solani* latent infections showed that he bacterium survived and even multiplied during commercial storage, especially at temperatures of 8-12°C used for processing potatoes.

Following artificial infections under greenhouse conditions, *D. solani* was found to bind to roots and systemically colonise some common weed species (including nettle (*Urtica urens*) and filed pansy (*Viola arvensis*)). However, no evidence of survival overwinter in soil or common weed species was observed under field conditions in the seasons following potato crops with high incidences (over 20%) of blackleg caused by *D. solani*.

Annual surveys of watercourses, undertaken as part of the UK *Ralstonia solanacearum* monitoring programme, identified 3 catchments contaminated with *D. solani* and 2 with *D. dianthicola*. However, persistence in following seasons was recorded in only one of these cases where *D. solani* was repeatedly isolated from a river in South East Scotland over several seasons. *D. zeae* and some as yet unclassified *Dickeya* taxa, including a potentially new species proposed as '*D. aquatica*' (Parkinson *et al*., in press), were occasionally found in surface water but have not been found on UK potato crops, whereas *Pectobacterium carotovorum* was almost always present in surface water.

Potential for spread and transmission of *D. solani* during handling of potato tubers was demonstrated when a single tuber with soft rot caused by *D. solani* was shaken in a chitting tray with 100 healthy seed tubers from a stock which had previously tested free from *Dickeya*. After removal of the rotted tuber, the remaining tubers were immediately planted under disease-conducive conditions in the glasshouse at 25 °C. Disease development due to *D. solani* infection was then recorded in the developing plants as 60% non-emergence and 14% blackleg with only the remaining 36% giving rise to healthy plants.

RECOMMENDATIONS TO CONTROL *DICKEYA* SPP. IN THE UK

Since the source of *Dickeya* spp. entering the UK was clearly infected seed potatoes, the main control recommendation is based on avoidance of further introduction by sourcing seed free from infection or contamination with the bacteria. Protection of the seed producing areas in Scotland was increased in 2010 by plant health legislation requiring a zero tolerance for all *Dickeya* spp. in the seed potato classification system and prohibiting planting of seed potatoes infected with *Dickeya* spp. for either seed or ware production. Monitoring of seed and growing crops is conducted through the annual surveys as described above. Since 2010, there have been no new findings of *Dickeya* spp. on potato in Scotland. A similar approach has been taken in Northern Ireland. In England and Wales, new varieties are more commonly multiplied

from seed of non-UK origin to supply market demand. In this case, *Dickeya* control methods are based on the same seed classification tolerances as for blackleg caused by *Pectobacterium atrosepticum*. The survey results showing a lack of spread of *D. dianthicola* and *D. solani* to seed stocks of UK-origin during their multiplication in England and Wales indicate that this approach has so far been effective.

Since *D. solani* and *D. dianthicola* have been identified in UK watercourses, an additional control recommendation involves the avoidance of irrigation and land prone to flooding from contaminated sources. The lack of findings of *Dickeya* spp. on potatoes grown in the vicinity of contaminated water courses, as well as the very low populations detected (<5 cfu per ml of water) and the lack of persistence of the bacteria in most cases suggest that contaminated surface water is a low risk factor in pathogen dispersal. It is possible, however, that the bacteria could be initially introduced to potato crops *via* this pathway.

Studies have shown that *D. solani* survived for less than a few days on different surfaces (including wood, steel, hessian and rubber) even when mixed in homogenised potato tuber tissue. Nevertheless, it was demonstrated that extensive spread occurs from rotting to healthy tubers during mechanical handling and can lead to high disease incidence when transmission occurs immediately before planting under disease-conducive conditions. Ten commonly used agricultural disinfectants were shown to effectively control *D. solani*, *D. dianthicola* and *Pectobacterium atrosepticum* in aqueous suspensions containing 10^8 cells ml^{-1} within 5 minutes at the manufacturer's recommended doses. These were based on the following active ingredients: Sodium hypochlorite (Domestos™), tosylchloramide sodium (Halamid™), iodophor (V18™), quaternary ammonium (Vanoquat™), acidic-based iodine (FAM-30™), glutaraldehyde and quaternary ammonium (GPC8™), 4-chloro-m-cresol and tar acids (Jeyes Fluid™), peracetic acid (Jet-5™), propanol and ethanol (Mikrozid AF™) and dipotassium peroxodisulphate, dodecylbenzenesulfonate and organic acids (Virkon S™). Whilst it would not prevent tuber to tuber transmission during handling, disinfection of equipment and storage containers may be recommended to prevent transmission of the bacteria between seed stocks.

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R&D INTO BLACKLEG CAUSED BY *PECTOBACTERIUM ATROSEPTICUM*: AN UPDATE

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Dickeya solani has been a significant threat to potato production throughout Europe. As a result, there has been a period of intense research to better understand the pathogen and possible methods for its control. Although *Dickeya* species remains a threat, the closely related pathogen *Pectobacterium atrosepticum* continues to be the major issue for potato production in Northern Britain. Since 2007 there has been a steady increase in seed area downgraded or rejected within the Scottish Seed Classification Scheme as a result of blackleg, caused by *P. atrosepticum*. This increase has been attributed to either changes in the pathogen, management practices or the environment. Certainly, the increase in blackleg has coincided with a series of very wet growing seasons, the removal of sulphuric acid as a means of haulm destruction and increasing consolidation within the industry, resulting in fewer but bigger businesses growing a wider range of cultivars. Further it is clear from growing crop inspections returns that blackleg is strongly influenced by disease incidence in the preceding seed crop. It is therefore concerning that blackleg can be found in Pre-Basic crops as early as the second field-grown generation. More so when considering that disease incidence, in general, will rise steeply to a plateau in subsequent generations once initial infection has occurred.

A three year project, funded by Potato Council and Scottish Government, has recently been commissioned to identify how and when early field generations become infected by *P. atrosepticum*. The rationale being that any delay in the initial infection will have a positive effect in later generations. Experiments will also investigate the effect of removal as a means of haulm destruction. The project has 5 main components:-

- Monitor commercial Pre-Basic 1 crops through a 3 year multiplication cycle.
- Investigate the movement of *P. atrosepticum* from infected to healthy plants.
- Investigate the routes by which daughter tubers become infected once *P. atrosepticum* is present in or on a plant.
- Identify whether a change in the population of *P. atrosepticum* strains has occurred in recent years.
- Compare the effectiveness of sulphuric acid with currently used haulm destruction programmes to determine their relative impact on spread of *P. atrosepticum* to daughter tubers.

Analysis of available data on the distribution of seed crops will also be carried out to determine if proximity to crops exhibiting blackleg symptoms increases the risk of blackleg in Pre-Basic crops and in their subsequent generations.

Monitoring of Pre-Basic crops

In order to understand the entry route of *P. atrosepticum* into high grade seed stocks commercially grown Pre Basic 1 crops will be monitored throughout planting, cultivation, harvest and storage. Mini-tubers will be tested prior to planting for the presence of *P. atrosepticum*. Growing plants will then be extensively sampled (foliage, stem, stolon, roots and tubers) at regular intervals across the growing cycle to determine when the initial infection occurs. The potential for contamination of crops by *P. atrosepticum* through normal agricultural activity will also be assessed by sampling from machinery, boxes etc. that routinely come into contact with seed, growing plants and harvested tubers.

Infield infection by *P. atrosepticum*

Field plots will be established over 2 sites to track the movement of *P. atrosepticum* from infected plants to neighbouring healthy plants during the growing season at harvest. As with the study of commercial crops, plants (foliage, stem, stolon, roots and tubers) will be tested at regular intervals throughout the growing cycle to track disease spread.

Seed to daughter tuber infection

Glasshouse-grown plants will be studied using fluorescent microscopy techniques to study the movement of *P. atrosepticum* through possible entry points, including wounds and roots. A greater understanding of the relative importance of these different routes will ultimately lead to better control.

***P. atrosepticum* population studies**

Molecular tools to characterise the genetic make-up of *P. atrosepticum* are being developed to determine whether the population of *P. atrosepticum* has changed over time. This is to determine whether it is possible to differentiate between recent isolates and those collected in previous studies, as the first steps toward identifying whether a population shift may explain the recent increase in disease incidence. The study will also provide information on whether other blackleg causing bacteria are increasingly present in potato crops grown in Northern Britain, e.g. *P. wasabiae* and *P. carotovorum* subsp. *brasiliensis.*

Effectiveness of sulphuric acid as a haulm desiccant

Plot-scale trials will be used to quantify and compare the impact of sulphuric acid with regards currently available desiccants on the spread of *P. atrosepticum* to progeny tubers. Laboratory studies linked to this work will also evaluate the bactericidal effects of sulphuric acid (or lack of) and those of currently available desiccants. The results of the comparisons will be used to evaluate whether additional work, to determine the impact of the inclusion of bactericides at haulm destruction, on the spread of *P. atrosepticum* may be warranted.

PRACTICAL LESSONS FOR NORTHERN BRITAIN FROM A THREE-YEAR FEASIBILITY STUDY OF CULTIVAR RESISTANCE-BASED INTEGRATED CONTROL OF POTATO BLIGHT (*P. INFESTANS*)

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Summary: The feasibility of matching blight fungicide inputs more closely to cultivar resistance to foliar blight was examined in three years (2009 to 2011) of field trials in Scotland. Separate evaluations of integrated control were made for rapid canopy development and the stable canopy phase of crop growth because these two growth phases normally present substantially different challenges from blight. It was clearly demonstrated that fungicide inputs to plots of more resistant varieties could be substantially reduced without compromising the control of leaf blight, even in the presence of the more aggressive genotype 13_A2 and under conditions more conducive to disease development than those prevailing in growers' crops.

INTRODUCTION

The principle of reducing fungicide inputs on potato cultivars with good resistance to foliar blight without compromising control has been established for many years (Fry, 1978; Kirk *et al*., 2005). Most of the research has been in countries where there is considerable pressure to reduce the use, or environmental impact, of blight fungicides (Kessel *et al*., 2006; Neilsen, 2004; Naerstad *et al*., 2007). The work carried out in these other countries shows that there is potential to adjust fungicide input depending on cultivar resistance. However, results using UK cultivars and fungicides under UK conditions are necessary to give agronomists and growers confidence in integrated control. The aim of the experiments described in this paper was to assess the feasibility of such an approach.

MATERIALS AND METHODS

Experiments were established at SRUC, Auchincruive in 2009, 2010 and 2011. In this work integrated control consisted of a more resistant cultivar compared with the susceptible King Edward, combined with reduced fungicide input (combination of fungicide product, spray interval and dose). The fungicides used and their full rate details are given in Table 1.

Table 1. Fungicides, active ingredients and rates.

	Active ingredient (a.i.)		Rate		EuroBlight efficacy rating	
Fungicide	Common name	g/kg or L product	active ingredient	Product (kg or L/ha)	Foliar blight	Curative activity
Merlin SC	chlorothalonil + propamocarb HCl	375 + 375/L	0.938 + 0.938	2.5 (L)	3.4	++
Infinito SC	fluopicolide + propamocarb HCl	62.5 + 625/L	0.10 + 1.0	1.6 (L)	3.8	++
Shirlan SC	fluazinam	500/L	0.20	0.4 (L)	2.9	0
Revus SC	mandipropamid	250/L	0.15	0.6 (L)	4.0	+
Laminator Flo SC	mancozeb	455/L	1.274	2.8 (L)	2.0	0
Dithane NT WG	mancozeb	750	1.275	1.7	2.0	0

Three cultivars with contrasting foliar blight resistance ratings were planted each year. In 2009 cvs King Edward (foliar blight resistance rating 3), Markies (5) and Cara (rated 7 in 2010 but 5 in 2012) were used. Cultivars King Edward (3), Cara (5) and Sarpo Mira (7) were used in 2010 and 2011.

Effectiveness of integrated control during rapid haulm growth

The experimental design was a split-plot layout with four replicates. Treatments consisted of a factorial combination of three cultivars, four fungicide doses and two application intervals, with an untreated for each cultivar. Fungicide treatments were the whole-plot level and cultivars the subplot level. The test fungicides (Table 2) were applied during rapid canopy only. All plots, including the untreated, were then sprayed with mancozeb (Laminator Flo in 2009 but Dithane NT in subsequent years) until desiccation.

Table 2. Fungicides applied during rapid haulm growth trials.

	Fungicide applied, rate and interval
1	Untreated
2	mandipropamid 0.6 L/ha (4 sprays)[1] at 7-day intervals
3	mandipropamid 0.6 L/ha (3 sprays)[2] at 10-day intervals
4	mandipropamid 0.45 L/ha (4 sprays)[1] at 7-day intervals
5	mandipropamid 0.45 L/ha (3 sprays)[2] at 10-day intervals
6	mandipropamid 0.3 L/ha (4 sprays)[1] at 7-day intervals
7	mandipropamid 0.3 L/ha (3 sprays)[2] at 10-day intervals
8	mandipropamid 0.15 L/ha (4 sprays)[1] at 7-day intervals
9	mandipropamid 0.15 L/ha (3 sprays)[2] at 10-day intervals

[1] 5 sprays in 2011
[2] 4 sprays in 2011

In 2009 and 2010 planting was by hand and fungicide treatment plots were four rows wide by 9.75 m long and separated longitudinally by 1.5 m of bare earth. Each cultivar sub-plot was four rows by 2.75 m long separated by 0.75 m unplanted row length. Seed spacing within rows was 25 cm. In 2011 a planter was used. The fungicide treatment plots were 8.97 m long and the cultivar plots 2.3 m long. The unplanted row length was standardised at 1.61 m. Seed spacing within rows was 23 cm. A single row of cv. King Edward was planted longitudinally between each of the blocks as an infector row.

Treatments were applied in 200 litres of water per hectare, using a tractor-mounted modified AZO compressed air sprayer. The nozzles were Lurmark F03-110 flat fans and the spray pressure 3.5 bars. For all experiments, spray programmes started at the first blight warning or when haulm met along the rows, whichever was sooner. Some treatments were early or late if weather conditions were unsuitable on the due date and there was a risk of inaccurate spraying. Infector rows were not sprayed with fungicide but were inoculated with specific isolates of 13_A2 (Table 3). Foliar blight was assessed regularly for each subplot as a percentage of leaf area destroyed by blight using a modified MAFF key 2.1.1 – Potato Blight on the Haulm (Anon., 1976; Large, 1952).

Table 3. Isolates of *P. infestans* (genotype 13_A2) used for inoculation.

Year	Isolate
2009	07/39
2010	2009_7654A
2011	2009_7654A, 07/39, 2006_3928A and 2008_6082F

Effectiveness of integrated control during stable canopy

The materials and methods were very similar to those described for the rapid canopy experiments. However, to allow the test fungicides to be targeted during stable canopy, over sprays were applied during rapid canopy (Merlin @ 2.5 L/ha) and also after the blocks of test fungicides (mancozeb at 1274 or 1275 g/ha). Three test fungicide products (Infinito, Revus and Shirlan) were evaluated. The fungicide treatments were applied at four rates (full, three-quarter, half and quarter) to all three cultivars. In the stable canopy trial in 2010 and 2011 there were six and four applications of the test fungicides at 7 and 10 days respectively. There were untreated control plots for each variety.

The foliar blight results from the rapid canopy and stable canopy trials were analysed as factorial experiments plus the untreated control. The rapid canopy trial was analysed as (four rates of fungicide x two timing intervals) + control and the stable canopy trial as (three fungicides x four rates x two timing intervals) + control. AUDPC (Area under Disease Progress Curve) values were calculated for each subplot and analyses of variance carried out. All analyses used GenStat for Windows 12th edition.

RESULTS

Figure 1 shows foliar blight severity for the integrated control treatments (ICTs), comprising reduced rates of fluazinam and/or extended spray intervals on the more resistant cultivars Cara and Sarpo Mira. Similar results were obtained in all of the stable canopy trials and also the three rapid canopy trials. To facilitate summarising the results from all trials the foliar blight severity for each of the ICTs was placed in one of four categories depending on its efficacy in relation to the reference treatment (Stable canopy: cv. King Edward treated with propamocarb + fluopicolide at full rate and 7-day intervals; Rapid Canopy: cv. King Edward treated with mandipropamid at full rate and 7-day intervals) (Table 4). In the vast majority of cases the ICTs outperformed the reference treatment. The absence of ICTs from the "significantly more effective" category in 2010 reflects the relatively low blight pressure in 2010. This resulted in uneven blight development between replicate blocks of these large trials, substantially

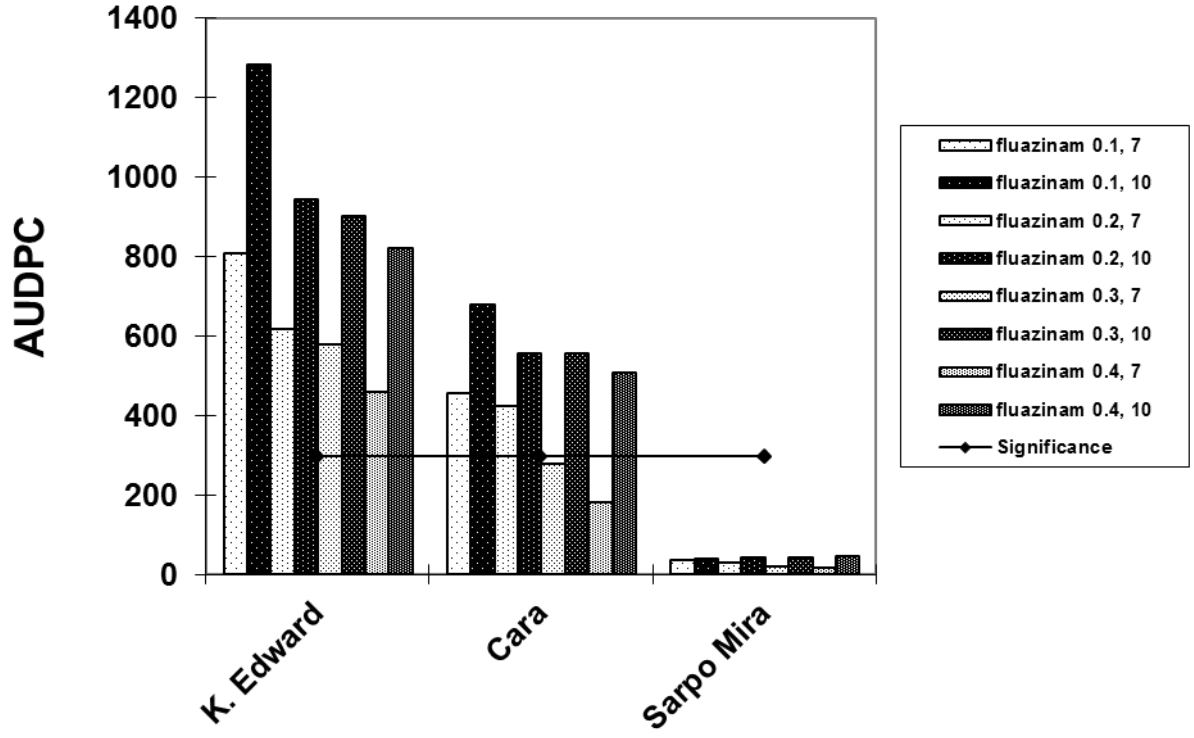

Figure 1. Foliar blight severities (AUDPC) for different combinations of cultivar resistance and fungicide input (fluazinam) in relation to the reference treatment of propamocarb + fluopicolide @ 1.6 L/ha applied every 7 days during stable canopy to the cultivar King Edward in 2011. AUDPC values below the significance value indicate significantly better control (at P=0.05) than the reference treatment.

reducing the statistical significance of treatment differences. Integrated control was superior where the highly resistant cv. Sarpo Mira (7) was used compared with cv. Cara (5) or Markies (5). However, 33.7% of ICTs including cv. Cara or Markies resulted in significantly better control, and 54.3% were more effective, than the reference treatment (Table 4). There were only five significantly less effective ICTs and these were all in the 2011 stable canopy trial.

Fungicide inputs were low for these five treatments: all five were applied at 10-day intervals and the treatments were quarter–rate propamocarb + fluopicolide, quarter–rate mandipropamid and three-quarter-rate, half-rate and quarter-rate of the least effective fungicide, fluazinam.

Table 4. Effectiveness of cultivar resistance-based integrated control versus a standard 7-day spray fungicide treatment of a susceptible variety.

	Significantly more effective	More effective	Less effective	Significantly less effective
a) Rapid canopy trials				
2009	12[1] (12)[2]	1 (1)	1 (1)	0 (0)
2010	0 (0)	14 (7)	0 (0)	0 (0)
2011	13 (6)	1 (1)	0 (0)	0 (0)
Overall %	59.5 (64.3)	38.1 (32.1)	2.4 (3.6)	0.0 (0.0)
b) Stable canopy trials				
2009	21 (21)	23 (23)	2 (2)	0 (0)
2010	0 (0)	46 (23)	0 (0)	0 (0)
2011	33 (10)	4 (4)	4 (4)	5 (5)
Overall %	39.1 (33.7)	52.9 (54.3)	4.3 (6.5)	3.6 (5.4)

[1] Values for ICTs including cvs Cara, Markies and Sarpo Mira
[2] Values for ICTs including cvs Cara and Markies only

DISCUSSION

Cultivar resistance-based integrated control proved to be highly effective in controlling the new population of *P. infestans* under northern Britain conditions, even when disease pressure was artificially high. Similar results were obtained in identical trials carried out concurrently in west Wales. It's anticipated that a system of integrated control can be developed based on 1 to 9 foliar resistance ratings for cultivars and EuroBlight fungicide ratings. Although reduced resistance in some cultivars, associated with changes in the *P. infestans* population (Lees *et al*., 2012), is a setback to implementing integrated control, there remain substantial differences between cultivars and these can be exploited. This is illustrated clearly by the results for the ICTs including varieties with current and representative levels of foliar resistance, i.e., cvs Cara and Markies. The current area of crop with cv. Sarpo Mira's foliar resistance is extremely small. For the time being therefore the results presented for Sarpo Mira indicate the further potential of cultivar resistance-based integrated control. This potential can be realised when future potato cultivars combine the agronomic characteristics required by the industry and high levels of resistance to foliar blight. In the experiments reported here a wide range of fungicide

doses were used. It is important to bear in mind when using the results to estimate the value of integrated control in commercial practice that some treatments used very low fungicide inputs. For example where there were three or four sprays of a quarter dose then total fungicide input was 19 or 25 percent of a full label dose applied every 7 days respectively.

ACKNOWLEDGEMENTS

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INTRODUCTION AND PERSISTENCE OF SEED AND SOIL-BORNE POTATO PATHOGENS WITHIN A ROTATION

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Summary: The Centre for Sustainable Cropping (CSC) is a long term experimental platform. It was established in 2009 to integrate all aspects of sustainability research on arable ecosystems at The James Hutton Institute and to study whole-system responses to sustainable crop management relative to current conventional crop husbandry practice. This study, utilizing the CSC platform, focuses on four seed and soil-borne potato pathogens, *Spongospora subterranea* (powdery scab), *Rhizoctonia solani* (black scurf), *Helminthosporium solani* (silver scurf) and *Colletotrichum coccodes* (black dot). Through quantifying pathogen levels in soil and on seed tubers we are investigating the impact of introducing inoculum into soil on subsequent disease levels on progeny tubers. The experimental platform also enables the effect of host resistance and treatments associated with the conventional and sustainable husbandry on both disease and soil contamination to be investigated. The persistence of soil-borne inoculum (once introduced through contaminated seed) is being monitored through the rotation.

INTRODUCTION

The Centre for Sustainable Cropping (CSC) is a long term experimental platform that was established in 2009 to integrate all aspects of sustainability research on arable ecosystems at The James Hutton Institute. The CSC provides a research facility to test and demonstrate the economic and environmental trade-offs, costs and benefits of sustainable arable land management to the whole arable ecosystem over many decades. This long-term, whole-systems approach is key if multiple benefits are to be identified and the potential conflicts between management for crop productivity and system resilience are to be resolved.

Sustainable management of arable systems for both agricultural production whilst minimising environmental impact must therefore achieve a balance between maximising crop production, conserving arable biodiversity and maintaining ecosystem functions. Thus, the general aims of the platform are:

- To design a sustainable cropping system that, over the course of a series of 6 year rotations, tests and demonstrates the optimisation of inputs (nutrients, water and agrochemicals), yield (quality and quantity), environmental services (water capture, greenhouse gas emissions, soil erosion, nutrient flows), biodiversity (soil microbes, plants and arthropods), and ecosystem processes (photosynthesis, carbon and nutrient transformations and fluxes, decomposition, community dynamics).

- To assess the effect of the sustainable system on long-term trends in yield and system health relative to standard conventional practice.
- To provide a broad framework for whole-systems research and a field-scale test-bed for new 'sustainable' crop varieties for enhanced nutrient and water use efficiency, weed suppression, and pest and disease resistance.
- To provide a demonstration site for knowledge transfer.

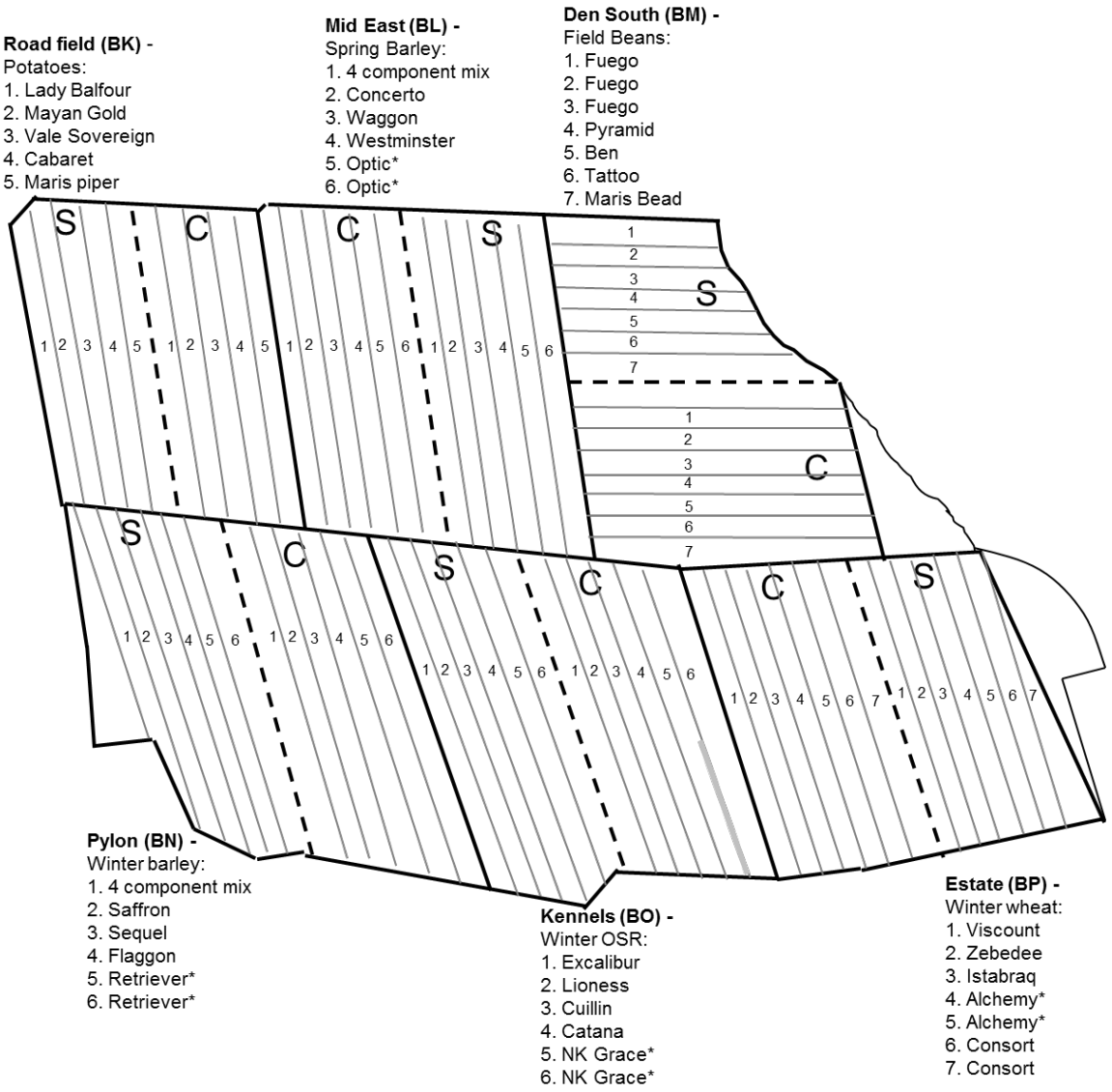

Figure 1. Field layout for 2011/2012 growing season showing the crop rotation, field splits (where C= conventional management and S = sustainable management) and cultivar strips for each of the six fields (BK to BP) of the CSC.

The platform is based on a six course rotation with six 5-6 Ha fields planted with potato, winter wheat, beans, spring barley, winter oilseed rape and winter barley each year (Fig. 1). One half of each field is managed according to current conventional crop husbandry practice and a

sustainable management package is applied to the other half. Further details of the design, sampling and sustainable treatment can be found at www.hutton.ac.uk/csc.

By monitoring the levels of four potato pathogens in soil and on seed tubers, we are investigating the impact of inoculum sources and soil contamination on disease levels on progeny tubers. The CSC platform also enables the effect of host resistance and treatments associated with the conventional and sustainable husbandry to be investigated, although the latter will not be discussed here. The persistence of soil-borne inoculum (once introduced) is being monitored through the rotation. This study focuses on four soil-borne potato pathogens, *Spongospora subterranea* (powdery scab), *Rhizoctonia solani* (black scurf), *Helminthosporium solani* (silver scurf) and *Colletotrichum coccodes* (black dot).

This is a long-term project, and the results from the first three years (2011-2013) of monitoring are reported here. The data presented is from the conventional field plots only.

MATERIALS AND METHODS

Five potato cultivars were planted in each year, both in the conventional and the sustainable field sections: cv. Maris Piper, the top ranked cultivar based on planted area in GB, cv. Mayan Gold which is resistant to a number of pathogens including powdery scab, cv. Vales Sovereign which is known to be drought resistant and yields well in lower nitrogen conditions, cv. Lady Balfour, which is popular in the organic market, and cv. Cabaret which is suitable for both ware and processing.

Soil samples

Soil samples were taken in March (prior to planting of the potato crop) and in October/November (post-harvest of the potato crop) from all the fields within the rotation, except in 2011 when the post-harvest soil samples were not taken until the following March. In the non-potato fields within the rotation, soil samples are taken from each half field. In the fields which have been planted with potato, soil samples are taken from each cultivar strip in the post-harvest sampling. Soil samples taken from the half field consisted of a bulk of 100 x 10 g samples taken from across the field area in a W-shape. When the areas under different cultivars were sampled, 20 x 10 g cores, taken across the area in a W-shape, were bulked.

Soil DNA extractions were carried out according to the method of Brierley *et al.* (2009). Target pathogen DNA was quantified using real-time PCR; *S. subterranea* (van der Graaf *et al.* 2003), *C. coccodes* (Cullen et al, 2002), *H. solani* (Cullen et al., 2001) and *R. solani* (Lees et al., 2002). Soil inoculum is expressed as pg DNA / g soil except for *S. subterranea* where inoculum is expressed as sporeballs / g soil. Amounts of *C. coccodes* DNA*:* <100, 100-1000, >1000 pg DNA/g soil and *S. subterranea* DNA: 0, <10 and >10 spore balls/ g soil are referred to as low, medium and high as determined in Lees et al (2010) and Brierley et al (2013) for *C. coccodes* and *S. subterrranea* respectively.

Potato tuber samples

A visual disease assessment was made on 100 tubers of each seed stock prior to planting in each year. Per cultivar 24 tubers were assessed individually using real-time PCR. The peel

from each tuber was weighed and placed into a grinding bag with 15 mL CTAB-PO_4 Buffer and ground using a Homex 6 grinder (BIOREBA AG.). A 1.5 ml aliquot of the supernatant was removed and processed according to the method of Cullen *et al*. (2001). Target pathogen DNA was quantified using real-time PCR assays described above. The amount of pathogen DNA detected was expressed as ng DNA / g tuber peel. Within a week of the potato harvest, a visual disease assessment was made on 100 tubers of each cultivar from each field treatment and is reported as disease incidence (% of tubers with symptoms) and mean severity (percentage area of tuber covered in symptoms).

RESULTS

Road Field

At the onset of monitoring soil-borne potato pathogens in March 2011, the field in which potatoes were to be planted (Road Field), had medium levels of *S. subterranea* and *C. coccodes* but no detectable *R. solani* or *H. solani* inoculum (Table 1).

Visual assessment of the potato seed stocks planted in 2011 revealed that all five had some silver scurf (44-99 % incidence). Maris Piper and Cabaret had high incidences of black dot (>81%). All cultivars except Mayan Gold (which was free from powdery scab symptoms) had a relatively low incidence of powdery scab (<17%). No powdery scab, black dot or silver scurf were recorded on the progeny tubers of any cultivar, and the amount of detectable soil inoculum of *S. subterranea* and *C. coccodes* did not increase and remained undetectable for *H. solani* post-harvest (Table 1). A more detailed account of *R. solani* inoculum and black scurf on potato grown in Road Field is presented in Figure 2.

Cultivars Cabaret, Vales Sovereign and Lady Balfour seed stocks had some black scurf (28, 7 and 1 % incidence respectively), but stocks of all cultivars including Maris Piper and Mayan Gold had some measurable amount of *R. solani* contamination as determined by real-time PCR (Figure 2). Black scurf was found on cvs Cabaret, Lady Balfour, Vales Sovereign and Maris Piper progeny tubers. *R. solani*, which was undetectable in the field soil pre-planting, was now found in the cultivar strips where disease had developed when sampled the following year, March 2012 (Figure 2). Only cv. Mayan Gold had no black scurf on progeny tubers and no detectable soil inoculum post- harvest. Cv. Mayan Gold is highly resistant to black scurf, although no official resistance rating is available. We are not yet able to determine whether the black scurf on progeny tubers developed from *R. solani* in the soil that was present but undetected, or from seed infections, which in the case of cv. Maris Piper were symptomless. When the soil from this field was sampled again 8 months year later (November 2012) no *R. solani* inoculum was detected (Figure 2).

Table 1. Inoculum levels of four soil-borne pathogens of potato quantified after sampling soil on a number of occasions between March 2011 and November 2013 in three of the fields within the rotation.

Field	Crop	Sampling time	Inoculum level ([a] spore balls/ g soil and [b] pg DNA / g soil)			
			S. subterranea [a]	*C. coccodes* [b]	*H. solani* [b]	*R.solani* AG3 [b]
Road Field	2011 Potatoes	March 2011	4.3	138.6	0.0	0.0
	2012 Winter wheat	March 2012	0.1	123.3	0.0	226.3
		November 2012	0.1	204.8	0.0	0.0
	2013 Winter OSR	March 2013	0.0	0.0	0.0	0.0
		November 2013	0.7	8.8	0.0	0.0
Mid East	2011 Spring barley	March 2011	0.8	0.0	0.0	0.0
	2012 Potaotes	March 2012	0.1	40.5	0.0	0.0
		November 2012	1.8	0.0	0.0	0.0
	2013 Winter wheat	March 2013	1.2	0.0	0.0	0.0
		November 2013	1.0	0.0	0.0	0.0
Den South	2011 Field beans	March 2011	0.0	151.3	0.0	0.0
	2012 Spring Barley	March 2012	0.0	0.0	0.0	0.0
		November 2012	0.0	0.0	0.0	0.0
	2013 Potatoes	March 2013	1.9	10.4	0.0	131.4
		November 2013	0.3	1.1	0.0	0.0

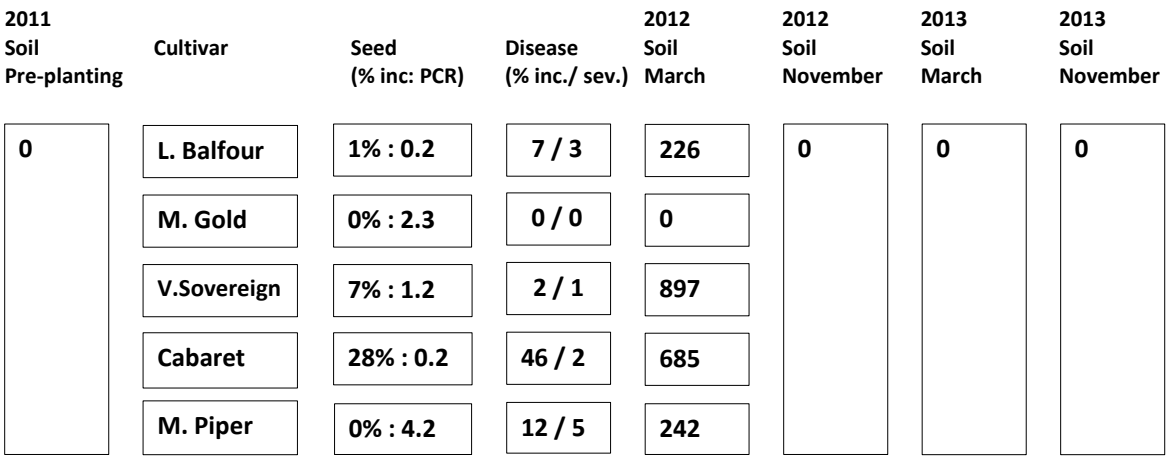

Figure 2. Monitoring of *R. solani* inoculum in soil (pg DNA/ g soil) pre-planting and seed inoculum levels (black scurf incidence (%) and *R. solani* contamination (ng DNA / g peel)) for seed stocks of five cultivars planted in Road Field in 2011. Disease (% incidence and severity of black scurf) on progeny stocks, and soil inoculum in the cultivar strips following harvest of the potato crop are shown (March 2012), following which detectable *R. solani* soil inoculum from the whole field at subsequent sampling occasions is shown.

Mid East

In 2012 Mid East field was planted with potato. Prior to planting, low levels of *S. subterranea* and *C.coccodes* but no *R. solani* or *H. solani* inoculum were detected (Table 1). Seed stocks of cvs Maris Piper and Mayan gold had a high incidence of black dot (92 and 69 % respectively), and cvs Lady Balfour, Vales Sovereign, Cabaret and Maris Piper had relatively low incidences of powdery scab (< 12%). Cultivar Lady Balfour was the only one with any black scurf (4% incidence). The progeny tubers of all five cultivars were free of black dot, black scurf and powdery scab. More details of *H. solani* inoculum and silver scurf on potato grown in Mid East field are presented in Figure 3.

All seed stocks planted in 2012 had silver scurf symptoms and the DNA on the seed stocks ranged from 3 to 498 ng DNA / g peel, all cultivars had silver scurf on the progeny (Figure 3). Whilst cv. Cabaret had the lowest seed contamination level and the least disease on progeny tubers and cv. Lady Balfour the highest amount of seed contamination and the most disease on progeny tubers, there was little difference between the other three cultivars (Figure 3). Despite the relatively high amounts of contamination on seed stocks and resulting progeny, there was no inoculum detected in the soil post-harvest.

Den South

In 2013, Den South (the third field in the rotation) was planted with potatoes. In soil sampled immediately prior to the planting, *S. subterranea*, *C. coccodes* and *R. solani* were detected but not *H. solani* (Table 1). Silver scurf was common (>55% incidence) on all seed stocks planted in 2013, and cvs Lady Balfour and Vales Sovereign had relatively low incidences of black scurf (<13%). Cultivar Cabaret was the only seed stock to have any black dot, and the incidence was low (3%). Details of detectable *S. subterranea* and powdery scab on seed and progeny are given in Figure 4. All five seed stocks planted in 2013 had detectable *S. subterranea* inoculum (real-time PCR), and all but cv. Maris Piper had powdery scab symptoms, with cv. Cabaret having the greatest incidence (95%). Despite the levels of disease on the seed, none of the progeny stocks had powdery scab, most however had severe common scab (data not shown).

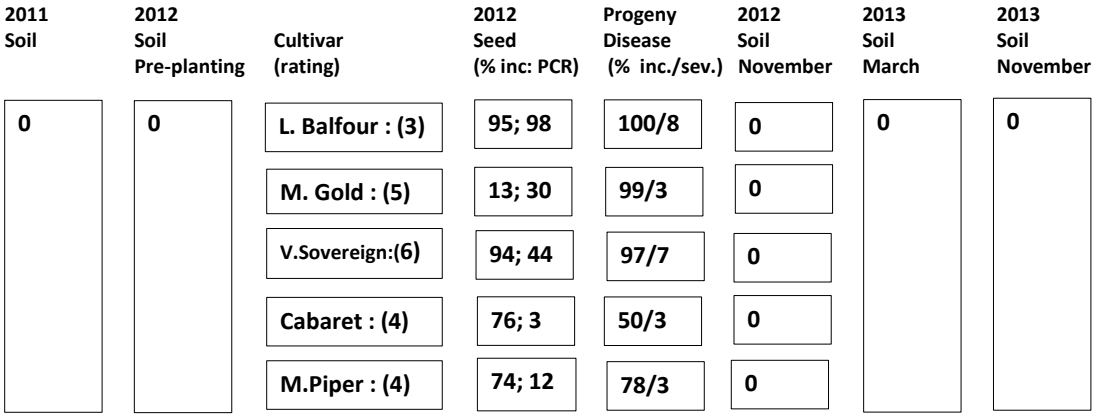

Figure 3. Monitoring of *H. solani* inoculum in soil (pg DNA/ g soil) pre-planting and seed inoculum levels (silver scurf incidence (%) and *H. solani* contamination (ng DNA / g peel)) for seed stocks of five cultivars planted in Mid-East Field in 2012. Disease (% incidence and severity of silver scurf) on progeny tubers, and soil inoculum in the cultivar strips following harvest of the potato crop are shown (November 2012), following which detectable *H. solani* soil inoculum from the whole field is shown for subsequent sampling occasions.

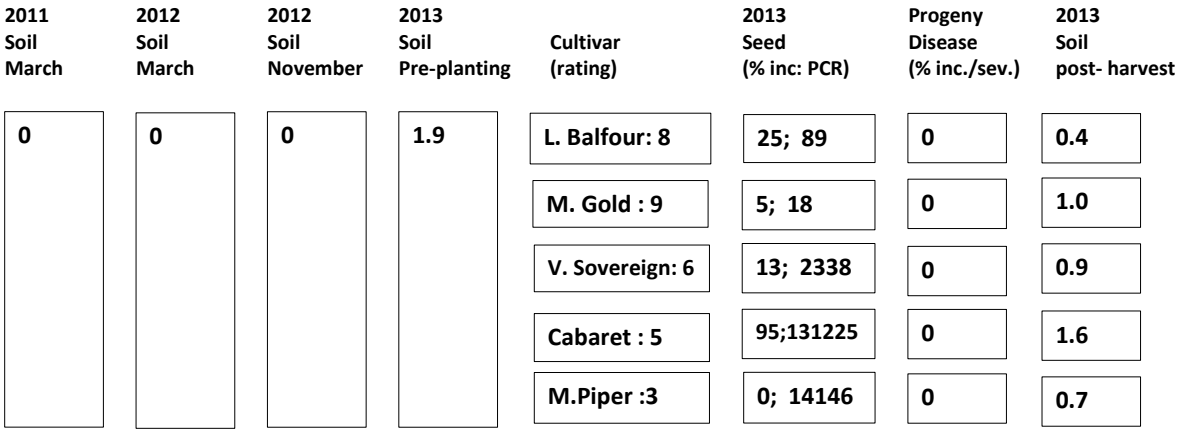

Figure 4. Monitoring of *S. subterranea* inoculum in soil (sporeballs / g soil) pre-planting and seed inoculum levels (powdery scab incidence (%) and *S. subterranea* contamination (sporeballs / g peel)) for seed stocks of five cultivars planted in Den-South Field in 2013. Disease (% incidence powdery scab) on progeny stocks and soil inoculum in the cultivar strips following harvest of the potato crop (November 2013) are shown.

DISCUSSION

With time we plan to establish a data set that will give us a greater understanding of what happens in the soil when diseased seed is planted, which factors prolong the persistence of a pathogen in the soil and thus the long-term impact of contaminating soil on future potato crops. We are already seeing interesting trends in the results, for example, all seed stocks planted in each year (2011-2013) had silver scurf. DNA on the seed stocks ranged from 1 to 240 ng DNA/g peel in 2011, 3 to 498 ng DNA / g peel in 2012 and just 1 to 20 ng DNA / g peel in 2013. The absence of detectable *H. solani* in the soil indicates that seed inoculum was the source of disease where it occurred. However there were marked seasonal variations in disease severity, no disease symptoms were found on progeny tubers in 2011. In 2012, all cultivars had silver scurf on the progeny and finally in 2013, three of the five cultivars had silver scurf on the progeny.

The seed sourced for planting in 2013 had powdery scab symptoms (particularly on cv. Cabaret), in addition, the soil into which potato was planted had detectable inoculum. However, despite both seed and soil-borne inoculum being present, the warm dry weather of 2013 reduced the risk of powdery scab, but conversely, all progeny stocks in 2013 had common scab, it being particularly severe in the susceptible cultivar Maris Piper (100% incidence and mean severity of 51%).

As the project develops, the effects of differences between the sustainable and conventional husbandry practices on seed and soil-borne pathogens of potato will be examined. For example, the use of municipal compost in the sustainable treatment may be a source of inoculum or the increased organic matter may have an impact on pathogen epidemiology in comparison to the conventional treatment. Additionally, the use of any seed treatments and in-furrow applications of azoxystrobin (Amistar) in the conventional treatment can be compared

to the sustainable treatments where these applications may be reduced or not used at all. In the future, pathogen and disease results will be related to other datasets that are being collected alongside to see if there are any relations with other components of the system. For example, there will be opportunities to see if there are any effects of soil chemistry and biophysics, weed abundance etc. We will also in the longer term be able to relate trends in pathogens to meteorological data that is being collected at the site.

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THE STATUS OF POTATO CYST NEMATODES IN SCOTLAND

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Summary: In the three years since 2010, when Directive 2007/33/EC on the control of PCN came into force, implementation of the new statutory measures, particularly the new harmonized soil sampling rate, has resulted in an over two-fold increase in the area of land recorded as infested with PCN. There has been a marked fall in the area of land that is now derecorded and thus cleared for potato production. Consequently, the area of land recorded as infested is now increasing at a much greater rate than prior to 2010. PCN, particularly *G. pallida*, is a specific problem in the traditional potato growing areas within Central Scotland, and a predominant one in Angus, where the incidence of positive PCN tests is at least five times greater than in Northern Scotland. It is likely that the greater intensity of potato production and cultural practices in Angus have resulted in a more 'advanced' PCN problem in this region. The higher sampling rate introduced with the new Directive has highlighted this problem. Overall loss of land to seed production in Angus due to the recording of new PCN infestations is in excess of 6% p.a., compared to less than 1% in Northern Scotland. Improved management practices are urgently required to maintain Scotland's health status in relation to PCN.

THE EU PCN DIRECTIVE (2007/33/EC)

PCN have been subject to controls under European legislation since 1969. A new PCN control Directive came into force on July 1, 2010, strengthening control measures against PCN, and harmonising approaches adopted across the EU, whilst taking account of changes in the understanding of the biology of the pest, its distribution across the EU and practices within the potato industry. The Directive is implemented in Scotland by the Plant Health (Scotland) Amendment Order 2010, which was laid before the Scottish Parliament on May 21, 2010. Under the Plant Health Fees (Scotland) Amendment Regulation 2010, fees are now in place for PCN testing.

Under the new Directive seed potatoes must only be planted on land which has been found to be free from PCN infestation following an official soil test. Failure to pass this test results in the land concerned being 'recorded' as infested with potato cyst nematodes. No seed potatoes may be grown in this land, either for inspection within the SPCS or for farm saved seed. Ware potatoes may be grown, but only if an Official Control Programme is in place. The land remains 'recorded' as infested until such time as a future official tests show that PCN are no longer present. Growers wishing to produce seed potatoes, either for classification or for farm-saved seed which will be planted other than at the place of production, are requested to make

an application for a soil test by 31 August of the preceding year. The new PCN Directive sets out a harmonised protocol for soil sampling for use by all EU Member States. Fields are sampled at a standard rate of 1500ml/ha or, if certain conditions are met which reduce the risk of PCN infestation, at a lower rate of 400ml/ha.

MATERIALS AND METHODS

Data and business management: All PCN tests applied for under the EU PCN Directive are now managed under SPUDS (Seed Potato Universal Data System). Fields to be tested are digitally mapped and sampling patterns and bar-coded labels are automatically generated.
Cyst extraction: PCN are extracted from dry soil samples using an automated cyst extraction system (carousel) based on traditional principles of sieving and flotation. The carousel has a cyst recovery rate of 93%.
PCN diagnostics: A novel high throughput polymerase chain reaction (PCR) method uses the entire 'float' (debris) from the carousel, eliminating the need for visual examination. A first PCR assay tests for the presence of *Globodera* spp. and a second assay provides diagnosis to species (Reid *et al*., 2010). All test results are recorded and collated using SPUDS.

RESULTS

Incidence of PCN: Impact of the new EU Directive

Figure 1. Area of land testing positive for either species of PCN, and for the individual species *Globodera pallida* and *Globodera rostochiensis* between 1998 and 2013. The total volume of soil sampled and tested per annum is also included.

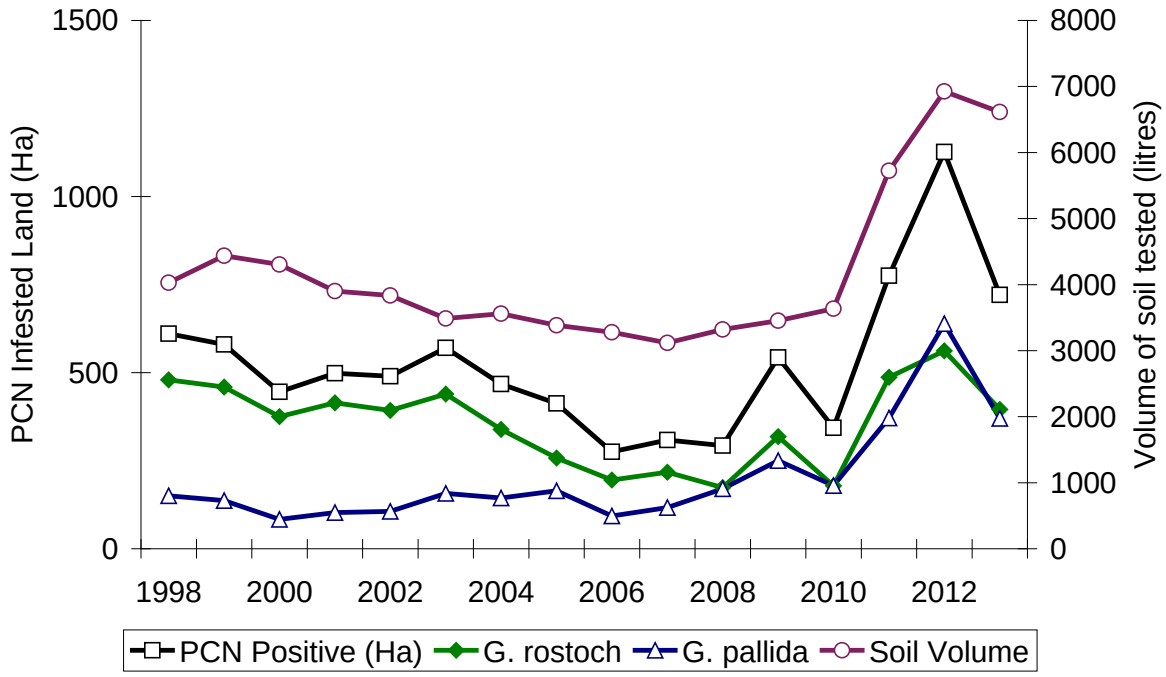

Figure 1 shows that the overall area recorded as infested with PCN has increased since the introduction of the new EU Directive in 2010 and this increase has generally been in proportion to the increase in the amount of soil sampled and tested as stipulated by the new Directive. The mean area of land tested p.a. over 2011-13 (16,400 ha) has declined by 12% when compared with the amount of land tested in the years prior to the introduction of the new Directive, possibly associated with a change in the Scottish Government policy regarding charging for PCN testing. Making comparisons over the same time periods, the area of land recorded as infested with PCN has increased by 122%, from 393 ha to 874 ha p.a.

PCN Testing: Geographical Breakdown 2008-13

Breaking down the area tested and the area found infested with PCN by counties over the last six years shows a highly consistent geographic pattern (Table 1). Data for 2008-10 were collated from results using the 'old' visual diagnostic method; whilst for 2011-13, the data come from the molecular diagnostic methodology introduced with the advent of the new Directive. Angus currently contributes 30% of the total area tested, followed by Perth (17%), Aberdeen (12%) and Kincardine (11%). Of the total area of tested land found infested with PCN, 68% of *G. pallida* infestations and 48% of *G. rostochiensis* infestations were in Angus. The only other counties with a disproportionately higher incidence of PCN (*G. rostochiensis* only) over the six year period were Kincardine and Fife. The incidence of both species was lower across the Grampian, Highland and Borders regions. There is no evidence to suggest that the introduction of the new EU Directive and the change in diagnostic method has affected the consistency of these results.

Table 1. County comparisons 2008-13 showing the area sampled and areas positive for both species of PCN as a percentage of the total area across the country for each category and years.

	Area Tested		**Area with *G. pallida***		**Area with *G. rostoch***	
County	**2008-10**	**2011-13**	**2008-10**	**2011-13**	**2008-10**	**2011-13**
ANGUS	30%	30%	65%	68%	47%	48%
PERTH	17%	17%	10%	10%	13%	17%
ABERDEEN	13%	12%	5%	1%	3%	3%
KINCARDINE	10%	11%	6%	7%	15%	11%
FIFE	6%	6%	4%	6%	7%	8%
ROSS	4%	5%	0%	0%	1%	1%
BANFF	5%	4%	3%	2%	4%	4%
MORAY	6%	4%	0%	2%	3%	3%
BERWICK	2%	3%	0%	1%	1%	0%
Others	7%	8%	6%	4%	7%	4%

Incidence of PCN: Angus compared to Northern Scotland

To examine the potential impact of the new PCN Directive, two regions of contrasting PCN incidence were chosen for illustration. Data were collated for Angus which makes up approximately 30% of the area tested (an average of 5,300 ha p.a. over 2008-13), and for 'Northern Scotland' (Orkney, Caithness, Sutherland, Inverness, Nairn, Moray, Banff and Aberdeen) covering a similar percentage of the area tested (an average of 5,000 ha p.a.). The data collected under the old Directive (2008-10) and under the new Directive (2011-13)

showed the overall incidence of PCN to be at least 5 times higher in Angus than in Northern Scotland (Table 2). The effect of the new Directive has been a 2.25-fold increase in the amount of land recorded as infested. This figure applies equally to the data covering overall PCN incidence as well as to infestations of the individual species. It also applies to the data for Angus and All Scotland, although for Northern Scotland the increases are below 2-fold. This 2.25-fold general increase exceeds the 1.85 fold increase in the quantity of soil sampled.

Table 2. Comparisons between Angus, Northern Scotland and all of Scotland showing the areas infested with both species of PCN in 2008-10 and 2011-13. Data presented as a percentage of the total area tested within each region.

	Area Infested		***G. pallida***		***G. rostochiensis***	
	2008-10	**2011-13**	**2008-10**	**2011-13**	**2008-10**	**2011-13**
Angus	4.5%	10.1%	2.7%	6.4%	2.1%	4.8%
Northern	0.9%	1.7%	0.5%	0.6%	0.7%	1.3%
All Scotland	2.4%	5.3%	1.3%	2.8%	1.4%	3.1%

Impact on Land Available for Seed Production

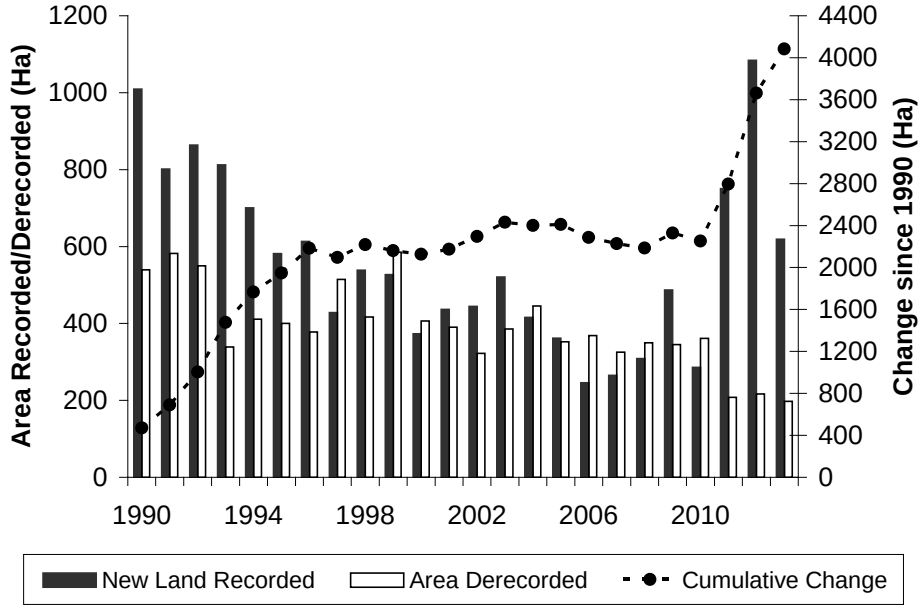

Figure 2. Area of land recorded and derecorded for PCN for each year between 1990 and 2013. The cumulative change in land recorded as infested since 1990 is included.

Changes in the area of land recorded as infested with PCN ('scheduled') and therefore prohibited from seed production are driven by the recording of new infestations and the derecording of previously infested land. Changes in infested land since 1990 across the whole

of Scotland are shown in Figure 2. Over the period 1996 to 2010 the rates of recording new land as infested and derecording previous infestations counteracted each other and the overall area of land recorded as infested showed minimal change. The higher sampling rate introduced with the new Directive has the effect of lowering the threshold population density at which PCN infestations are detected. Consequently, the rate at which land is now recorded as infested has increased. As a higher sampling rate of 1500ml/ha is now required for the derecording of infestations, the population has to decline to markedly lower levels to pass a derecording test. The sampling rate used under the previous Directive was 600ml per unit of up to 4ha (equivalent to as little as 150ml/ha). Consequently the 'success rate' for derecording fell from 88% under the old Directive to 72% under the new Directive.

Table 3. Comparisons between Angus, Northern Scotland and all of Scotland showing the new land recorded as infested with PCN, the area derecorded and the consequential change in the overall area of infested land for 2011-13. Percentages relate to the total area tested.

2011 - 13	**New Land Recorded (Ha)**	**New Land Recorded (%)**	**Area Derecorded (Ha)**	**Area Derecorded (%)**	**Change (Ha)**	**Change (%)**
Angus	1333	9.0%	348	2.3%	985	6.6%
Northern	182	1.3%	56	0.4%	126	0.9%
All Scotland	2476	5.0%	622	1.3%	1854	3.8%

Table 3 shows that the overall impact of the new Directive resulted in an extra 1854 ha recorded as infested with PCN and thus taken out of seed production over the three years since it was introduced (i.e. 618 ha p.a.). The area of land recorded as infested is expected to continue to increase. The incidence of new PCN infestations found across Scotland was 5.0% for 2011-13. New findings exclude the area of land submitted for derecording that subsequently tested positive, thus confirming the previous result. With 1.3% of the area tested derecorded, the overall change in land recorded as infested fell to 3.8%. For Angus, 9.0% of the previously clear land tested for PCN was recorded as infested. This was offset by derecording of 2.3% of the tested area, thus the overall change was 6.6%. For Northern Scotland, the overall change was 0.9%. Due to the minimum rotation period of 6 years for basic seed, this loss of land when expressed as a percentage is not compounded each year, but is compounded with each rotation period. Clearly, losses of 6.6% of the land tested in Angus every rotation period years present a far greater concern than compounding a change of 3.8% across all of Scotland, or even 0.9% across Northern Scotland, over a similar period.

Relaxation of restrictions on PCN infested land

The implementation of the new EU PCN Directive permitted the production of ware potatoes on any land recorded as infested with PCN providing that an approved control programme was adhered to. In 2011-13, applications from growers were accepted for the cultivation of ware potatoes to be grown on 1575 ha of infested land in Angus. Therefore the new Directive has provided a benefit in releasing land recorded as infested for ware production to offset the pressure on seed land. In Angus in 2011-13, whilst the area of land recorded as infested increased by 985 ha (Table 3); ware production was permitted on 1575 ha of PCN infested land

under control programmes. Therefore, whilst less land was available for seed potatoes, more was made available for ware. However, land cultivated under an official control programme is likely to be lost for seed production for the foreseeable future. No applications were received during 2011-13 to grow ware potatoes on land recorded as infested in Northern Scotland.

DISCUSSION

As 6.6% (328 ha) of the area of land in Angus tested prior to seed potato production returned positive PCN test results, this land has effectively been lost from seed production. This loss is also reflected by an estimated annual request from growers in Angus for ware potatoes to be grown on 525 ha of land recorded as infested. Such annual losses are clearly unsustainable if similar areas of seed potatoes are to continue to be grown. The problem has been previously recognized as a slowly developing epidemic of PCN, particularly of *G. pallida* and particularly in England (Trudgill *et al*., 2003). The problems of managing *G. pallida* in areas of intensive ware production in England are now being seen in areas of more intensive potato production in Scotland. Data collected by SASA show a steady increase in the incidence of *G. pallida* in statutory PCN tests over the past 25 years (from 4% to 50% of all PCN infestations). Over the same period there has been a steady decline in the incidence of *G. rostochiensis*. This change in the incidence of the two species appears to be closely linked with the availability of potato varieties resistant to either species. In 2012, 89% of the Scottish seed potato crop had no resistance to *G. pallida* and less than 1% was highly resistant. With many commercially successful varieties (e.g. Maris Piper) highly resistant to *G. rostochiensis*, 50% of the Scottish seed potato crop falls into this category. Varieties with high levels of resistance to *G. pallida* are now becoming available, largely through breeding programmes in the Netherlands. Much improved strategies for managing *G. pallida* in both seed and ware potato land are urgently required to provide sustainable options for seed potato production in Angus and the incorporation of highly resistant varieties into rotation programmes will play an important role in any successful strategy.

ACKNOWLEDGEMENTS

I acknowledge the support of all the RPID soil sampling and administrative staff for their input into the PCN soil sampling. I am extremely grateful for the technical assistance within SASA provided by the molecular diagnostic team and the input and discussions with staff within the Seed Potato Classification Scheme. Particular thanks are due to the Virology & Zoology lab staff whose perseverance and skill ensures that lab testing is completed in time for the growers to plant their crops each season.

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INCREASING SOIL TEMPERATURES WILL LIKELY BENEFIT POTATO CYST NEMATODES

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Summary: Across the UK, average soil temperatures differ significantly and soil temperatures are predicted to increase in the future due to the effects of climate change. This paper describes experiments in controlled environments to examine the impact of temperature on the life cycle of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* (PCN), persistent and economically important pests of potato. It also contributes to previous observations by showing that hatching in constant and fluctuating temperatures are not significantly different for both species of PCN. Our results indicate that both species of PCN are likely to increase more rapidly and in greater numbers with susceptible cultivars if soil temperatures increase in the future.

INTRODUCTION

The potato cyst nematodes (PCN) *Globodera rostochiensis* (Stone) and *Globodera pallida* (Woll) are economically important parasites of potatoes and other members of the *Solanaceae* family. They are quarantine organisms listed in the EU Plant Health Directive 2000/29/EC and are regulated by the European PCN Directive (2007/33/EC). In the UK, management of PCN relies on long rotations, nematicides and resistant cultivars, though for *G. pallida* there are very few commercially acceptable cultivars available with high levels of resistance. There are regional differences in the climatic conditions in potato growing regions around the UK and in addition there is a general trend towards increasing temperatures and changes in rainfall associated with climate change (Jones *et al*., 2007; Parker *et al*., 1992) both of which are likely to impact on the multiplication and damage caused by soil based plant parasitic nematodes. One of the most influential environmental factors affecting nematode development is temperature (Kakaire *et al*., 2012; Trudgill *et al*., 2005; Van der Waals *et al*., 2013). Nematodes, like the rest of ectothermic animals, depend on external heat sources to maintain their body temperature and as poikilothermic organisms, their body temperature is almost identical to that of their environment. Nematodes are adapted to particular temperature ranges and they can have different optimal temperatures for different stages in their life cycles such as feeding, hatching, reproduction and survival (Neilson & Boag, 1996).

The two species of PCN differ in their temperature responses. *G. pallida* populations are reported to hatch and reproduce at lower temperatures than *G. rostochiensis* populations, however *G. rostochiensis* is more successful than *G. pallida* at temperatures above 20º C (Franco, 1979). In northern Europe with temperate climates, there is usually one generation of potato cyst nematodes per year (Jones, 1950), although there are several studies which describe

the occurrence of a partial second generation and Jones (1950) observed that suitable soil temperatures may permit more than one generation of *Globodera* per year. In the Mediterranean region, Greco *et al*. (1988) recorded a completed second generation of *G. rostochiensis* at Avezzano in Italy and Jimenez-Perez *et al.* (2009) observed a second generation of *G. rostochiensis* at soil temperatures of 18˚C in Venezuela and a lack of entry into diapause. The occurrence of a second generation indicates that in some conditions PCN can evade the "obligatory" diapause requirement and thus can adapt to new environmental conditions.

The aim of our research is to investigate the relationship between soil temperature, the PCN life cycle and population multiplication in order to understand the risk to potato crops from PCN now and in the future, and to support the development of the Potato Council's PCN management model (Elliott *et al.*, 2004). This report focuses on the first part of the life cycle, hatching, to determine if hatching differs in constant versus comparable average fluctuating temperatures. We have previously shown that in constant temperatures the amount and rate of hatching is highly temperature dependent (Kaczmarek *et al.*, 2013). However, in field conditions temperatures continually fluctuate thus this report concerns a comparison of hatching at common average temperatures in constant and fluctuating temperature regimes.

MATERIALS AND METHODS

Nematode populations

Cysts from *G. rostochiensis* A (pathotype Ro1) and *G. pallida* E/Lindley (pathotype Pa2/3) populations from the James Hutton Institute PCN collection were stored at 4˚C for at least one year prior to use. Randomly selected cysts were used that had previously been size selected with a 250µm sieve to exclude small or damaged cysts. The *Solanum tuberosum* cultivar Desirée, which is susceptible to both species of PCN, was used for producing potato root diffusate (PRD) (Rawsthorne & Brodie, 1986).

Hatching experiments

Hatching experiments were performed on a thermal gradient table (Grant GRD 1, Camlab, Cambridge, UK). The metal gradient table was programmed to have a temperature switch after 12 hours and this created temperature conditions from constant temperatures ranging from ~8-17º or alternating temperatures as shown in Figure 1. The positions on the gradient table were defined by a plastic grid. Temperatures were recorded at the positions of the dishes with DS1920-F5 Temperature ibuttons (HomeChip Ltd., Milton Keynes, UK). Two experiments were performed, the first with *G. pallida* and the second with *G. rostochiensis*. Ten cysts were exposed to 2 ml of hatching agent, potato root diffusate (PRD) (Rawsthorne and Brodie 1986) in a 5cm Petri dish. The PRD was refreshed 8 times during the 33 day experiment, at three or four day intervals, each time juvenile nematodes (J2) were collected and counted. The accumulated number of hatched J2 was determined by removing the liquid from each petri dish into a 12 well multi-well plate and counting the juvenile nematodes using a stereomicroscope (Olympus S7-ST). To define the initial numbers of eggs, after finishing the experiment the cysts were collected from the multi-well dishes and crushed, and unhatched eggs and juveniles were counted.

Data analyses

The results obtained were analysed using GenStat Version 14.1 and Microsoft Excel Version 14.0.4760.1000. Boxplots of the curve parameters were examined after grouping the data according to whether the temperature was constant or fluctuating. Group 1 (constant) comprised the hatching data from the positions where the minimum temperature differs from the mean temperature by ≤2ºC and Group 2 (fluctuating) comprised the data at positions where the difference between the minimum temperature and the mean temperature is ≥2ºC.

Logistic curves were fitted to the cumulative proportions of eggs hatched and analysis of variance (ANOVA) was carried out on the parameters of the curves to test for differences in the hatching behaviour at the different temperatures of the 2 species. The cumulative proportions of hatched nematodes were used to estimate the parameters of a logistic curve describing the hatching rates using the equation:

$$Y=A + C/(1 + EXP(-B*(X - M)))$$

where Y is the proportion of hatched nematodes, A is the lower asymptote (an estimate of the number hatched at time zero), C is the maximum asymptote (an estimate of the proportion expected to hatch for at any given temperature). A+C is the upper asymptote, B is the slope of the intermediate part of the curve, M is the point of inflection (an estimate of the number of days until half of the eggs that are going to hatch have hatched). The fitted curves from the logistic model were used to calculate means and standard errors for the maximum rate of hatching.

RESULTS

Hatching in constant temperatures and with diurnal fluctuations in temperatures

In order to assess whether a diurnal temperature fluctuation influenced the hatching of PCN, a temperature gradient table was programmed to produce positions on the table with temperatures that changed every 12 hours and positions with constant temperatures (Figure 1). The average temperatures ranged from 8º to 17ºC. Both species had higher hatching rates at warmer temperatures. The percentage of total hatched nematodes for both PCN species at the average daily temperatures in the gradient is shown in a Figure 2. Due to the design of the gradient table, some temperature combinations had only one sample therefore estimating the variability for these was not possible.

Comparison of parameters from hatching in constant and fluctuating temperatures

It was previously reported by Kaczmarek *et al.,* 2013, that the total percentage hatching of PCN over a range of temperatures has a hyperbolic shape and that the parameters A, B, C and M of the logistic curve vary according to the mean temperature. Boxplots of the curve parameters are shown in Figure 3. As described above, Group 1 comprised the hatching data from constant temperatures and Group 2 comprised the data at positions where the temperature was fluctuating (diurnal). Figure 3 shows the curve parameters for *G. pallida* and *G. rostochiensis* split according to group. For both species, the figure shows that there are no differences in the medians for Group 1 and 2 (the constant and diurnal temperature regimes) for A, B, C and M, though there are differences in the variability and differences between the species.

12	13	14	15	16	16	17
12	13	13	14	15	15	16
11	12	13	14	14	15	16
11	11	12	13	13	14	15
10	11	11	12	13	13	14
9	10	11	11	12	11	13
8	9	9	10	10	11	12

Figure 1. Average temperatures at the 49 positions on the temperature gradient table at the positions where the Petri dishes were placed for the hatching test. The positions on the diagonal from bottom left to top right remained constant, the others changed every 12 hours with a maximum change from 8º to 17ºC.

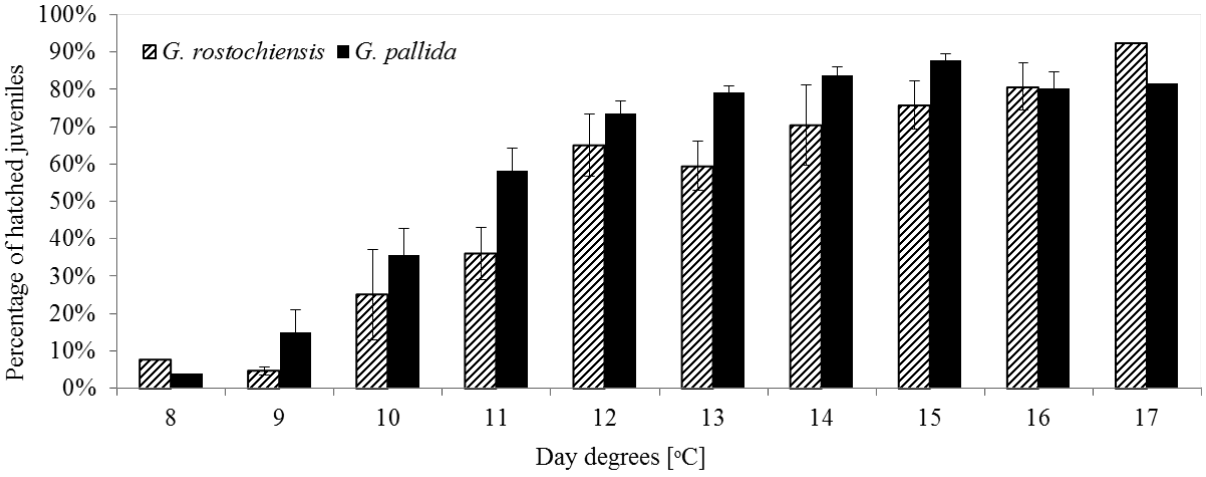

Figure 2. Total hatching of *G. rostochiensis* and *G. pallida* in potato root diffusate (PRD) after 33 days over a temperature gradient from 8º – 17ºC with constant or diurnal temperature fluctuations. Data are expressed as percentages of the total hatch per mean daily temperature (day degrees).

The ANOVA test comparing the responses over the combined diurnal and constant temperature regimes for *G. pallida* and *G. rostochiensis* for parameter M, which concerns the number of days until half of eggs that are going to hatch, have hatched, indicates that *G. rostochiensis* hatched earlier than *G. pallida* ($P<0.001$). The ANOVA for parameter B indicates that *G. rostochiensis* hatched at a faster rate than *G. pallida* ($P<0.001$). Parameter C showed a higher proportion of *G. pallida* eggs hatched overall than *G. rostochiensis.* The ANOVA for parameter C, confirmed that there are significant differences between the species in the means of the final proportion of hatched nematodes ($P=0.005$) and at different temperatures ($P<0.001$) however there was no significant difference in the overall PCN response to temperature ($P=0.218$).

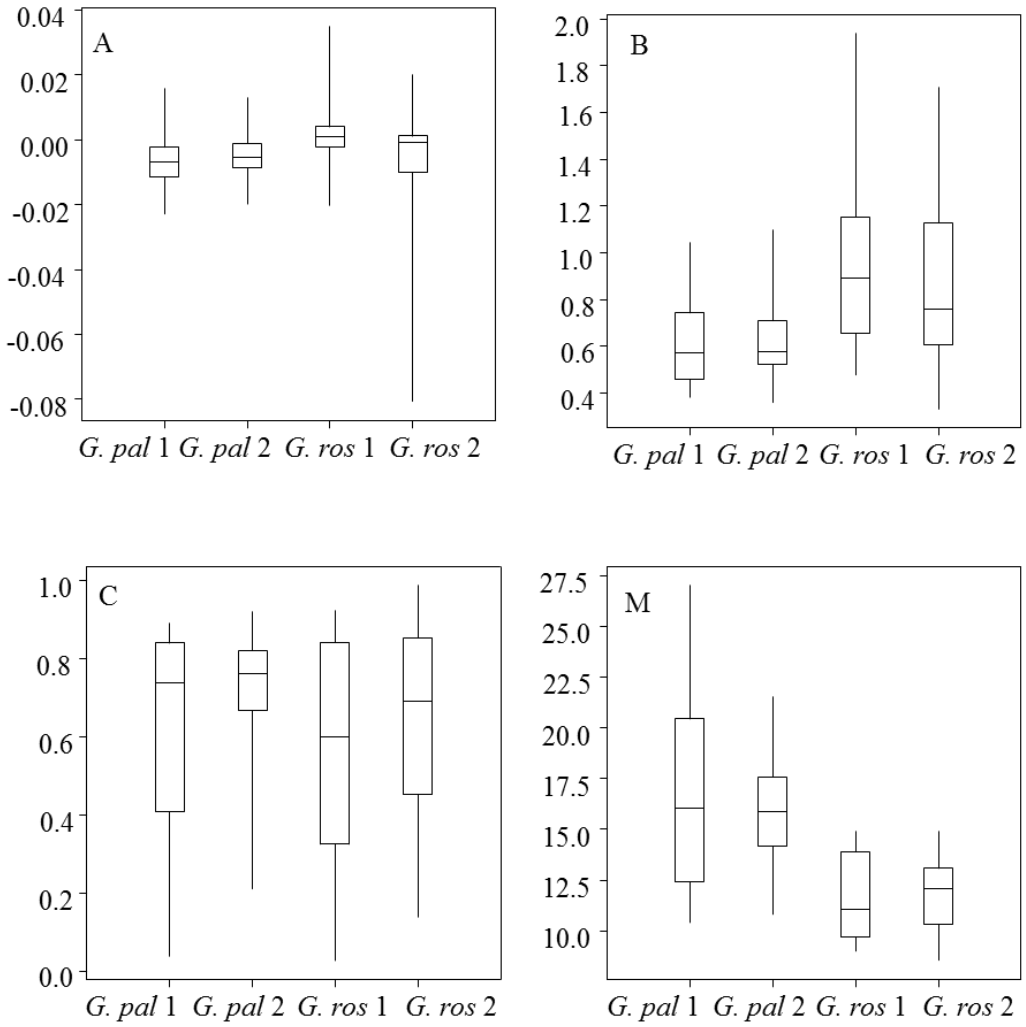

Figure 3. Boxplots of parameters A, B, C, M for the logistic curves fitted to the cumulative proportion of total hatch for *G. pallida* (*G. pal*) and *G. rostochiensis* (*G. ros*) grouped by (1) constant temperature regime or (2) fluctuating temperature regime.

DISCUSSION

The main goal of these experiments was to investigate whether there were differences in the hatching reaction of PCN in different temperature regimes. In the field, fluctuations in temperature occur. We wanted to determine if hatching rates at constant temperatures were equivalent to hatching rates at comparable average temperatures that were achieved with fluctuating temperatures. We found that hatching in constant and fluctuating temperatures were not significantly different for both species of PCN, though the species did differ in their hatching responses to temperature. These studies were performed with populations that have been cultured in the glasshouse for many generations and need to be confirmed with populations from the field. Previous findings (Kaczmarek *et. al*. 2013) indicated that low soil temperatures typically found shortly after potato planting in the UK are likely to favour *G. pallida* whereas warmer temperatures are likely to favour *G. rostochiensis*. This has implications for interspecific competition between the 2 species at different temperatures when

they occur as mixtures in the field, the host response to mixed infections and the composition of the final PCN populations. Our data also indicate that the hatching response is greater and faster at the higher temperatures tested and thus increases in soil temperatures due to regional climatic differences or climate change are likely to favour PCN multiplication.

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POTENTIAL USE OF POLYSULPHIDES IN THE CONTROL OF PCN

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Summary: Work with polysulphides in a granular formulation using a wood flour matrix centred on establishing the elution profile of the formulations and the resulting nematicidal properties of the collected elute. The granules generally showed a peak of polysulfide release in the first 2-6 elutions with an extended period of release at a lower level over the subsequent 20 elutions. In most cases juvenile mortality was greater than 80% for the initial period before declining to approximately 50%. A clear dose response curve was observed with diallyl trisulphide (DAS 3) and levels of greater than 15250 peak area resulted in 100% J2 mortality. Evaluation of four polysulphide formulations in a glasshouse trial showed levels of PCN control comparable to that of oxamyl were achieved regardless of formulation or dose. Work with cysts in soil in the absence of a host crop showed that high polysulphide treatment resulted in a decline in the viability of eggs and consequently reduced the J2 hatch.

INTRODUCTION

The potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis* are a serious and widespread pest of potato in the UK, present in approximately 64% of potato fields in England and Wales (Minnis *et al*. 2002) and causing losses in excess of £50 million annually (DEFRA 2004). Conventional control is achieved through an integrated approach using nematicides, resistant cultivars and crop rotation (Evans & Haydock 2000). However management of PCN is becoming progressively more challenging with increasing restrictions on the use of nematicides and an absence of new products becoming available. Plant extracts may provide alternative control agents as they have been shown to possess antimicrobial, insecticidal and nematicidal properties. Extracts from garlic have historically been thought to have a wide range of medicinal properties and more recently the sulphur-containing compounds including allicin and polysulphides, have been identified as being particularly biologically active (Anwar *et al*. 2009). Nematicidal properties of garlic extracts were originally reported by Auger *et al*. (2004). Subsequently, allicin and garlic essential oils have been shown to have nematicidal activity against *Meloidogyne incognita* (Gupta & Sharma 1993) and *Bursaphelenchus xylophilus* (Park *et al*. 2005) respectively. Garlic extract has been shown to exhibit nematicidal activity to PCN with a dose of 15 ml l^{-1} resulting in 100% J2 mortality after 24 hours and a dose of ≥550 µl l^{-1} reducing hatch from treated cysts (Danquah *et al*. 2011).

MATERIALS AND METHODS

Elution studies

Using just the barrel from a 60ml syringe, a 1cm layer of glass wool was placed on the base of the syringe on top of which 5g of a 50:50 mixture of the test granules and Celite was added prior to a further layer of glass wool on top. 15 ml of water was added to the tube and the elutriate collected into a clean tube for 15 minutes. This was repeated a further 19 times. After collection the elutriate was passed through a 0.45um PTFE filter and analysed using UPLC-PDA.

A number of *G. pallida* cysts were re-hydrated in tap water for four days after which the tap water was replaced by potato root exudate solution. Juveniles began hatching after a period of three to seven days. The elutriate was transferred into a six well culture plate into which batches of 30 J2 nematodes were added. After 48 hours each well was assessed using an inverted microscope with the number live and dead nematodes counted.

Efficacy Trial

The trial was conducted in an environmentally controlled glasshouse providing a 16 hour day length. One litre pots were filled with sterilize loam: horticulture sand (3:1) and planted with a single seed tuber of the susceptible cultivar Désirée. The potato cyst nematode inoculum (Pi) consisted of a total of five infective eggs and juveniles per ml of soil with the number of cysts required determined in a hatch experiment. The cyst inoculum was enclosed in a mesh "T bag" placed below the seed tuber. Each treatment was replicated seven times. Plants were maintained for 16 weeks prior to harvest. Prior to harvest, plant height and the weight of above ground tissue were recorded and all soil material retained for assessment of PCN multiplication.

According to the surface area of the pot, two dosage rates were calculated giving equivalent field rates of 30kg/ha and 60kg/ha. Treatments were randomised in seven blocks using the statistical programme Genstat. A duplicated set of unplanted pots were set up at the same time to examine the effects of the polysulphide granular treatments on the viability of eggs within a cyst. After 16 weeks the bags containing the cysts were retrieved from the soil and stimulated to hatch *In Vitro* in the presence of potato root exudates as described above.

RESULTS

The elution profiles of the four polysulphide granule formulations showed a peak release across DAS 1-6 over the first six collections after which there was a sustained release of DAS3 and to a lesser extent DAS2 over the remaining collections (Figure 1 & 2). The exception to this was granule 2 where lower levels of DAS 3 were observed. Incubation of newly hatched J2 PCN in elution's of known diallyl trisulfide concentration for 48 hours resulted in a significant dose response effect ($r^2 = 0.7864$) with concentrations of greater than 15250 peak area resulting in 100% mortality (Figure 3).

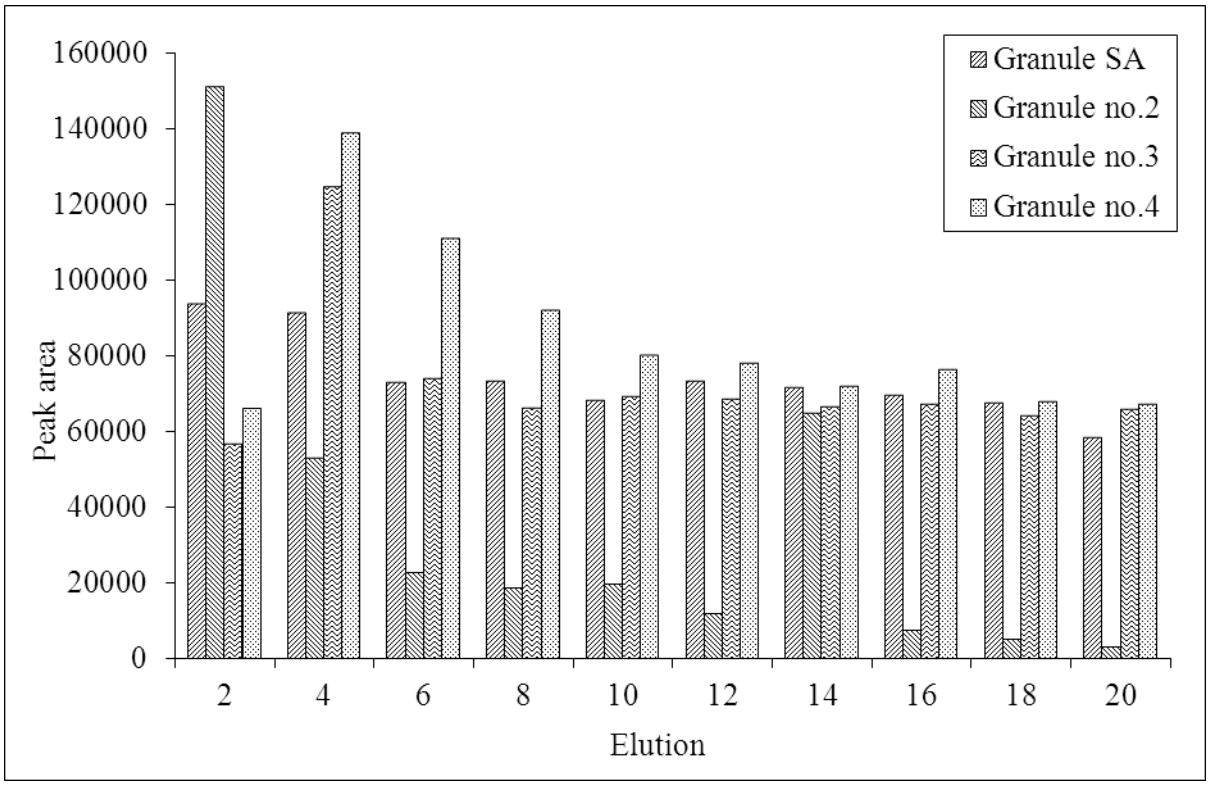

Figure 1. The total diallyl sulfide (DAS1-6) release of four granule formulations over 20 elution as measured using UPLC-PDA

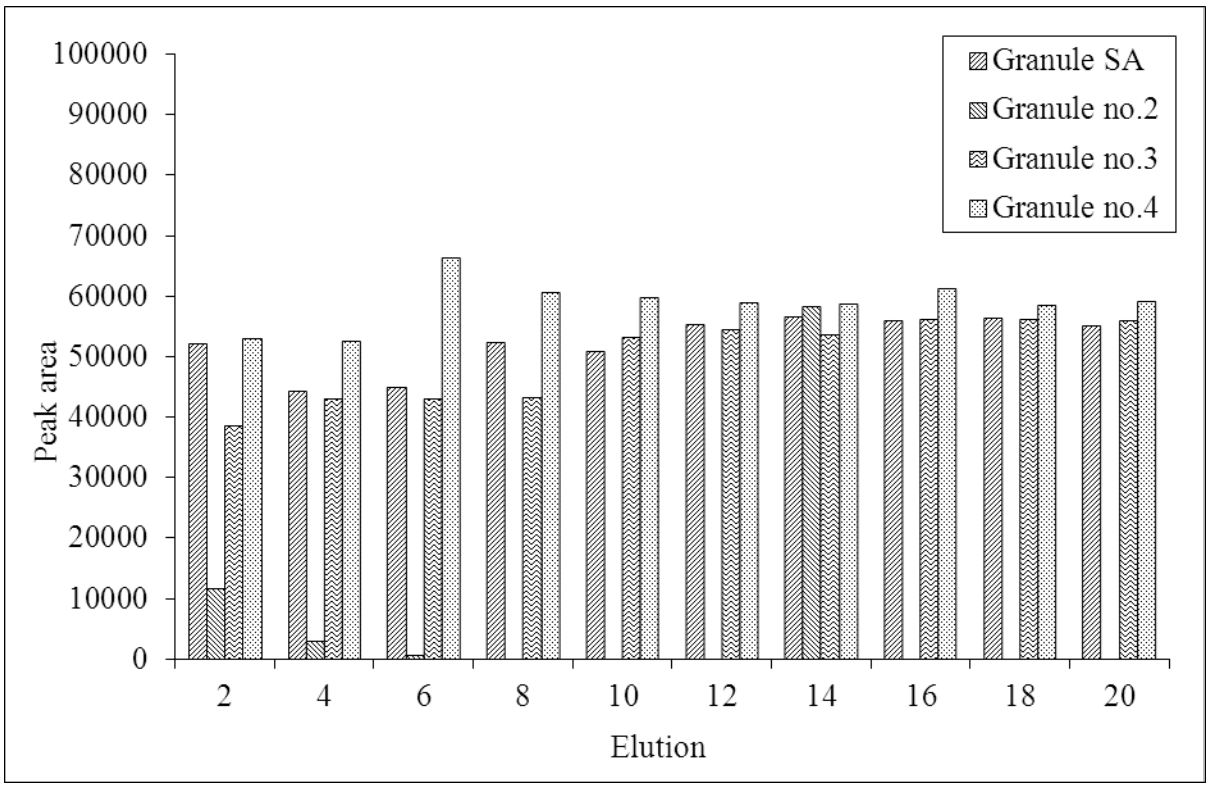

Figure 2. The diallyl sulfide 3 (DAS3) release of four granule formulations over 20 elution as measured using UPLC-PDA.

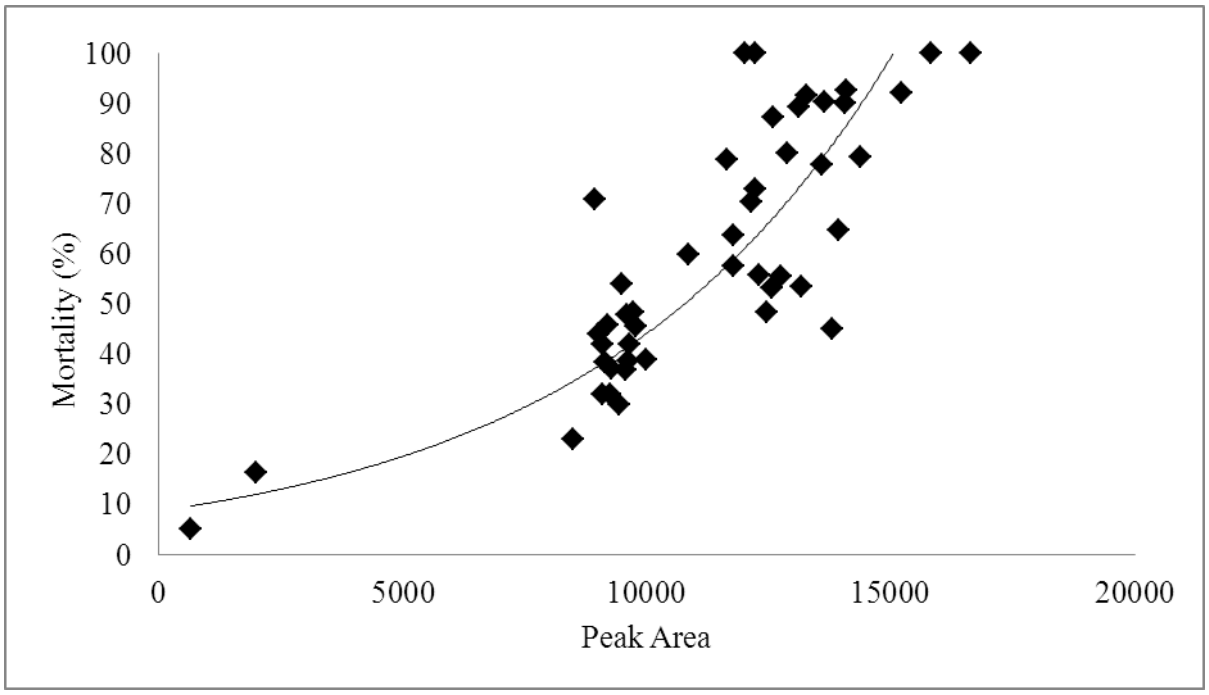

Figure 3. The effect of diallyl trisulfide (DAS3) on the mortality of PCN J2 after 48 hours of incubation.

Results from the efficacy trial showed that all treatments regardless of dose rate resulted in a significant reduction in the number of cysts recovered at the end of the trial compared to the untreated control and the majority of polysulphide treatments achieved levels of control similar to that of the oxamyl treatment (Figure 4).

Where cysts had been buried in treated soil in the absence of a host plant it was observed that there was a small but significant reduction in the number of J2 which emerged from the cysts (Figure 5).

DISCUSSION

Initial work with polysulphides in a granular formulation using a wood flour matrix centred on establishing the elution profile of the formulations and the resulting nematicidal properties of the collected elute. The granules generally showed a peak of release in elute 2-4 with an extended period of release over the subsequent 20-30 elutions. In most cases juvenile mortality was greater than 80% for the initial period before declining to approximately 50%. When elution profiles were repeated using a soil matrix the initial peak of polysulphide release was often lost but replaced with a higher level of polysulphide release in later elutions. This may be important in the control of later hatching cysts which under conventional control programmes would be unaffected due to the declining levels of active ingredient remaining in the soil.
Evaluation of selected formulations in two glasshouse trials showed levels of PCN control comparable to that of Vydate were achieved in some instances and at higher dose rates significantly greater control was achieved. Work with cysts in soil in the absence of a host crop showed that high polysulphide dosage resulted in a decline in the viability of eggs and consequently reduced the J2 hatch.

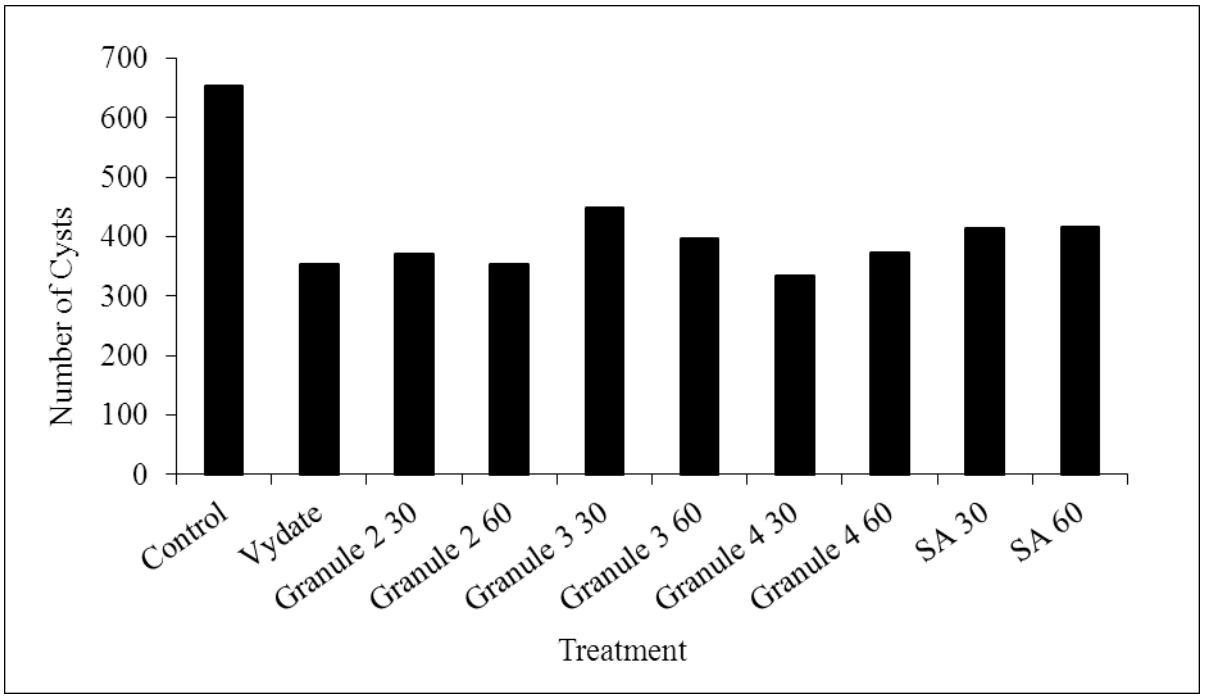

Figure 4. The effect of four polysulphide treatments at two dose rates on the number of PCN cysts per plant compared to an untreated control and Vydate.

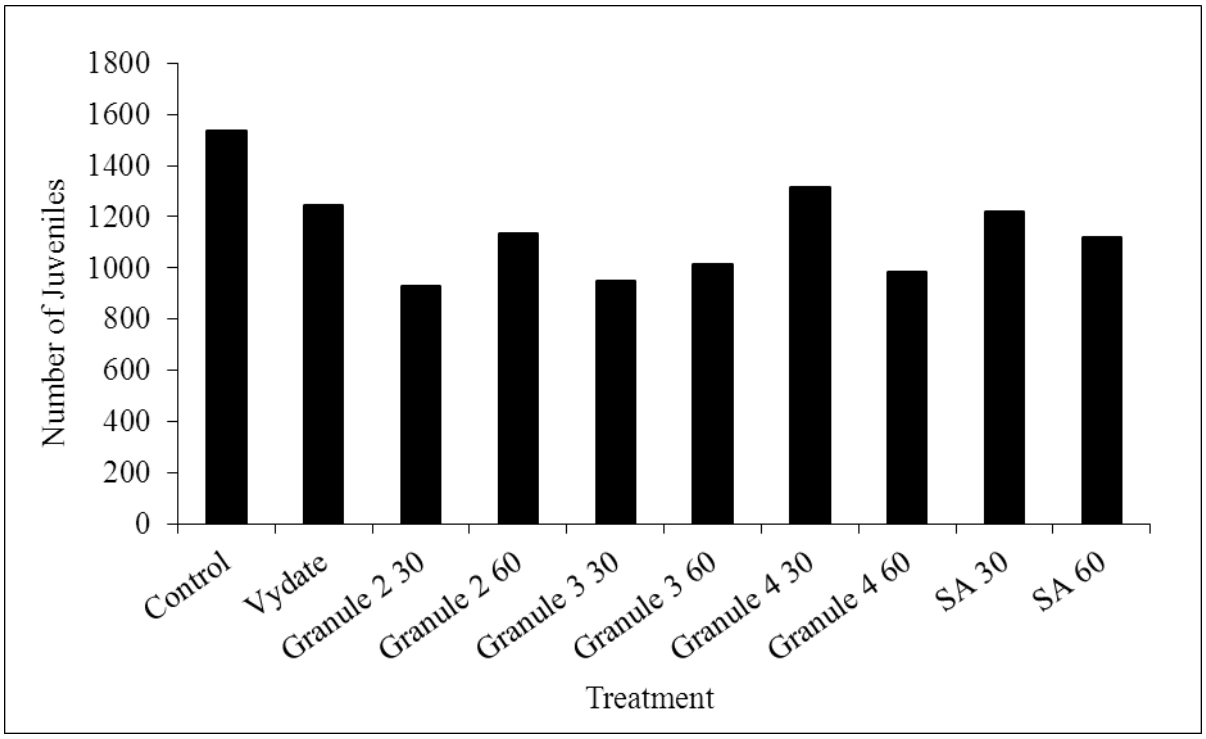

Figure 5. The effect of four polysulphide treatments at two dose rates on the number of viable juveniles per 5 PCN cysts compared to an untreated control and Vydate.

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IDENTIFICATION SOURCES OF RESISTANCE TO POTATO MOP-TOP VIRUS AND ITS VECTOR *SPONGOSPORA SUBTERRANEA*

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Summary: Thirty five *Solanum tuberosum* Group Phureja clones were evaluated to identify sources of resistance to *Potato mop-top virus* and its vector *Spongospora subterranea* (the causal agent of powdery scab). Visual disease assessments were made and TAS-ELISA, qPCR and qRT-PCR were used to assess infection. In the first year, eight clones showed good resistance to PMTV and these clones were tested in a second year in order to confirm their resistance. Clones PHU951 (901) and DB441 (2) had the least infection by PMTV and powdery scab on tubers. However, there were no significant differences between the eight clones and cv. Agria (the susceptible cultivar) for infection of roots by PMTV and *S. subterranea*.

INTRODUCTION

Potato mop-top virus (PMTV) is the type species of *Pomovirus* genus belonging to the family *Virgaviridae*. This virus causes various symptoms on potato including spraing (brown rings and necrotic arcs in infected tubers), yellow mottling on leaves and mopping (shortened internodes). PMTV is transmitted by *Spongospora subterranea*, the causal agent of powdery scab (Harrison & Jones 1970; Davey & Hons 2009). Both pathogens contribute to a reduction in tuber quality as a result of their tuber symptoms. Despite the employment of various approaches, to date there is no a reliable method to control these diseases. The most effective control method for most plant diseases, including potato diseases, is believed to be the use of resistant cultivars. Potato germplasm, including species such as *Solanum phureja* Juz.et Buk., is a potential source of host resistance to various diseases. This species is diploid (2n=2x=24) and cultivated in the Andean region of South America (Hawkes 1990). It grows in 3-4 months at low and high altitudes, producing tubers which lack a dormancy period (Hawkes,1990). Furthermore, it is able to hybridise with *S. tuberosum* leading to the transfer of valued characteristics, including disease resistance, into into tetraploid offspring (Ross, 1986). For example, hybridization between *S. phureja* and *S. tuberosum* produced *S. tuberosum* Group Phureja clones, some of which were identified as being resistant to PVY, PVA and PVV (Barbar, 2013). Additionally, a hybridization of *S. phureja* and *S. stenotomum* was shown to confer resistance to Erwinia-soft rot and Alternaria-leaf blight diseases (Hidalgo & Echandi 1982; Christ & Haynes 2001). The object of this study was to identify *S. tuberosum* Group Phureja clones resistant to PMTV and powdery scab.

MATERIALS AND METHODS

Field trial – year 1

Thirty five *S. tuberosum* Group Phureja clones, held at The James Hutton Institute, were tested in addition to cv. Agria (susceptible to powdery scab), cv. Nicola (sensitive to PMTV) and cv. Gladiator (resistant to powdery scab). In May 2011, the selected clones and cultivars were planted in a field known to be infested with *S. subterranea* and PMTV located at JHI (Invergowrie, Dundee, UK). Tubers were planted in a randomised complete block design with 3 replicates, with each plot containing three tubers of each clone spaced with 0.5m between tubers with a 1m path to separate the plots. Cultivar Maris Piper was planted in guard rows around of the field. During the growing season, irrigation, fertilization, pesticide and herbicide applications were made as per normal practice.

Six weeks after planting, three plants from each clone (one from each replicate) were collected to investigate *S. subterranea* and PMTV infection in roots. The root samples were visually scored for galls, caused by *S. subterranea* and nucleic acid was extracted from 10g of root samples, three plants from each clone. A qRT-PCR for detection of PMTV according to (Mumford *et al*., 2000) and qPCR for detection of *S. subterranea* according to van de Graaf *et al*. (2003) was conducted. The evaluation of infection level for both pathogens was dependant on the mean value of cycle threshold (Ct) in qPCR and qRT-PCR. In August 2011, leaf samples were collected from the remaining plants (6 plants per clone) and processed for TAS-ELISA testing according to Arli (1996) to detect PMTV. After harvest, tubers were washed and scored for severity of powdery scab symptoms using a standard scale (http://www.spongospora.ethz.ch/LaFretaz/scoringtable.htm). Subsequently, 25 tubers (25x3 rep.=75 per clone) were sampled randomly for each clone and sliced longitudinally three times to obtain six faces for assessing PMTV symptoms (Anon, 1976). Six of the 25 tubers were then sub-sampled for PMTV TAS-ELISA testing. Additionally, three tubers which were symptomless for PMTV and powdery scab were selected from all clones and tested for the presence of PMTV and *S. subterranea* using qRT-PCR and qPCR (Mumford *et al.*, 2000; van de Graff *et al*., 2003).

Field trial – year 2

The eight most resistant to PMTV and powder scab *S. tuberosum* Group Phureja clones, identified in the first year of trials (80.CP.23, DB.161 (10), DB.323 (3), DB.375 (1), DB.377 (4), DB.441 (2), PHU.950 (412), PHU.951 (901)) and control cultivars (cvs Gladiator and Agria), were planted in May 2012 in the same field as the first trial in a randomised complete block design with three replications, each comprising 12 plots. A plot was planted with three tubers of each clone spaced 0.5m apart with a 1m path to separate the plots. Cultivar Maris Piper cultivar was planted in guard rows surrounding the field. Irrigation, fertilization and other agricultural practices were applied as per normal practice.

Root samples of three plants per clone were collected after six weeks of planting and scored for root galls caused by *S. subterranea*. Nucleic acid extraction was carried out and samples tested using qRT-PCR assay to detect PMTV and qPCR to detect *S. subterranea* as in the first field trial. In August 2012, leaflet samples were collected from the remaining plants and tested for PMTV using TAS-ELISA assay according to (Arli 1996) . In October 2012, tubers were

harvested mechanically, washed and scored visually for severity of powdery scab using a same standard scale.

Subsequently, 25 tubers per plant (25x3 rep.=75 per clone) were selected randomly. They were sliced longitudinally to obtain six faces and scored for PMTV symptoms using Anon`s key (1976). After that, tissue core of six, sub-sampled from 25 tubers, were tested by PMTV TAS-ELISA. Furthermore, the nucleic acid was extracted from six PMTV and *S. subterranea* symptomless tubers and then processed by qRT-PCR assay to detect PMTV and qPCR for *S. subterranea*. The assessment of PMTV and *S. subterranea* was also based on the number of cycle threshold.

RESULTS

There were significant differences between clones for PMTV accumulation and severity of powdery scab symptoms. All clones of *S. tuberosum* Group Phureja had less disease than the susceptible cultivar Agria. Some clones had significantly less powdery scab symptoms than the resistant control cultivar Gladiator. The figures below show combined data for the 8 best performing clones in the two seasons, in addition to control cultivars.

All eight clones had less powdery scab than cv. Agria (the susceptible cultivar) Figure (1). Also, three clones: DB377(4), DB161.10 and DB441(2) displayed significantly less powdery scab than Gladiator (the resistant cultivar).

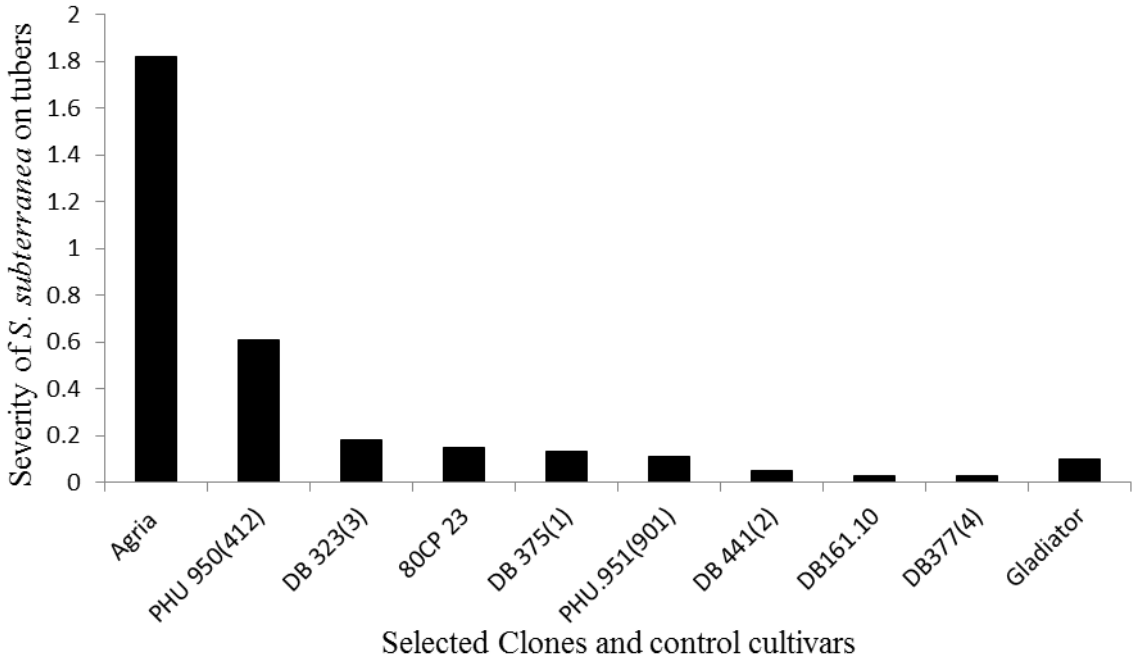

Figure 1. Mean of *S. subterranea* severity on tubers in two seasons.

The mean percentage (%) of tubers scoring positive by TAS-ELISA for the eight clones and control cultivars in two seasons is presented in Figure 2. PMTV was detected in 31.6% of tubers of cv. Agria and in 2.7-11.1% of tubers of the eight tested clones.

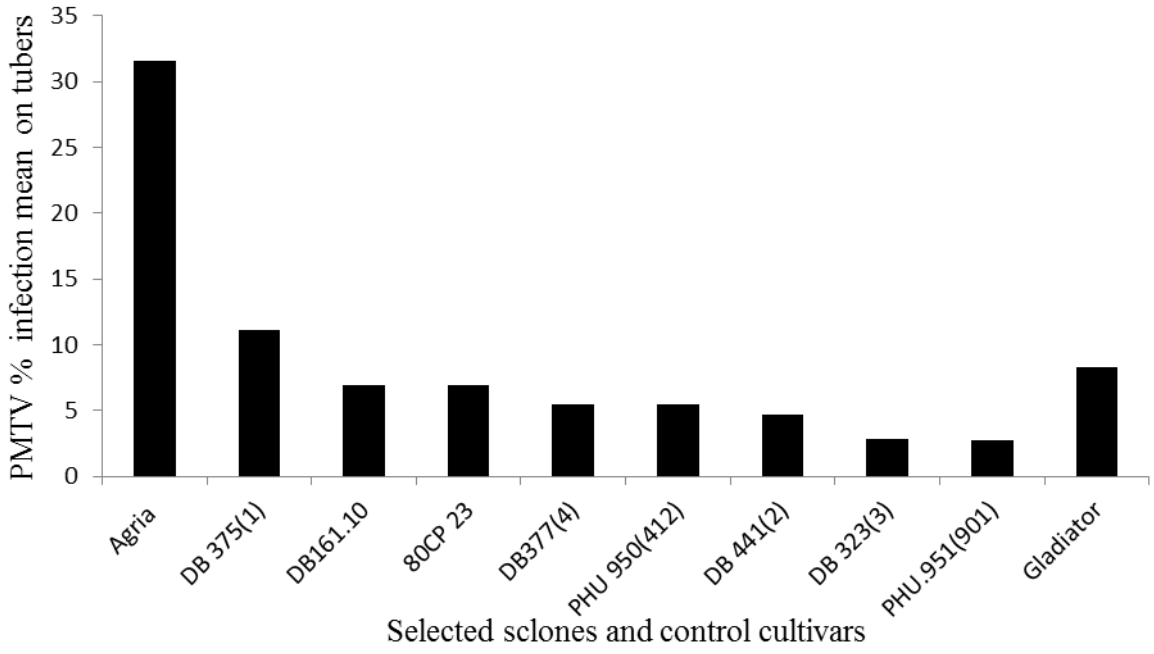

Figure 2. Mean of PMTV % on tubers in two seasons.

There were no significant differences between all clones and cv. Agria in *S. subterranea*-root infections (Figure 3). However, all tested clones displayed less infection in symptomless tubers than cv. Agria through detection the infection in later amplification cycles. Also, two clones (PHU951 (901) and DB441 (2)) had significantly less infection than cv. Gladiator (resistant control).

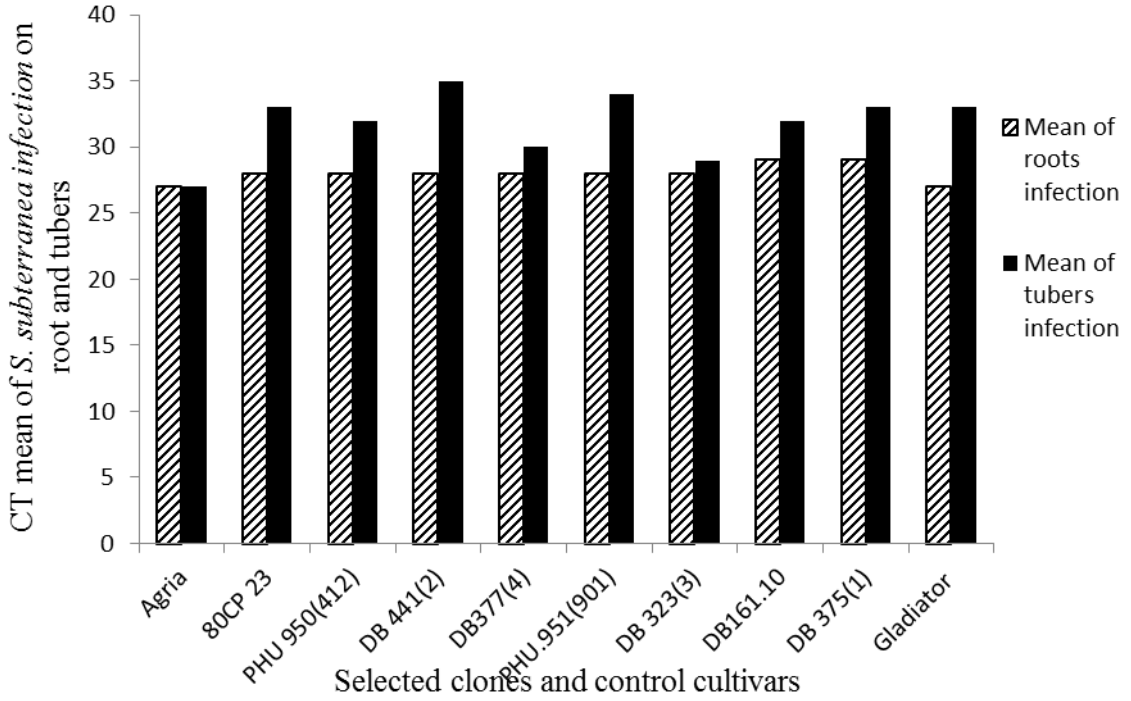

Figure 3. Mean of *S. subterranea* infection on roots and tubers in two seasons.

The mean of PMTV infection in roots and tubers, assessed by qRT-PCR and expressed as Ct, showed that the infection level detected in roots was similar in all clones and cultivars examined (Fig 4). All clones except DB161.10 had significantly less PMTV infection in tubers than cv. Agria and four clones showed less infection than cv. Gladiator.

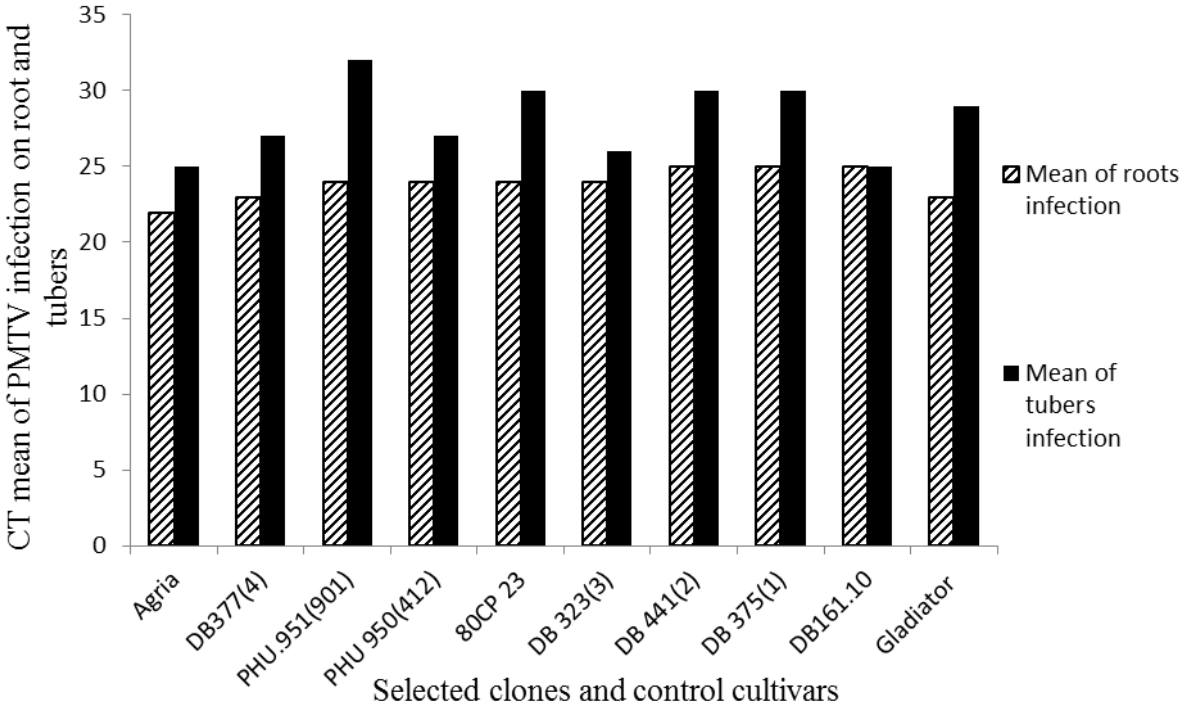

Figure 4. Mean of PMTV infection on roots and tubers in two seasons.

DISCUSSION

Our data shows that *S. tuberosum* Group Phureja is a useful source of resistance to *Spongospora subterranea* and PMTV. Field resistance was observed in tubers of eight clones. These clones had significantly less powdery scab and PMTV infection than the susceptible cultivar (Agria) and some of them had less powdery scab than the resistant cultivar (Gladiator). However, the level of *S. subterranea* and PMTV infection in roots samples of all selected clones was not significantly different than found in root infections of cv. Agria.

The significant reduction to PMTV and *S. subterranea* infection in the most resistant eight clones of *S. tuberosum* Group Phureja might be as a result of the presence a single gene or genes that could confer a resistance to these pathogens. This is in agreement with previous studies. For example, some clones of *S. .tuberosum* Group Phureja were resistant to *Streptomyces scabies*, *Potato leafroll virus* and *Erwinia carotovora* (De Maine *et al*., 1993; Lees *et al*., 2000).

The disparity in root and tuber susceptibility to *S. subterranea*, noticed in this study, is in agreement with previous studies that have shown no association between root and tuber infection *S. subterranea* (Merz *et al*.,2012;Tegg *et al*.,2013).

This difference is possible due to effect of the ontogenic host resistance. In this type of resistance, the host reacts with pathogens in four different ways: in the first one, the host becomes susceptible in the growth period and then converts to be resistant in the maturity period. In the second type, it is resistant during early stage of growth and turns to be susceptible in maturity period. The host in the third type of this resistance becomes resistant during growth period and early adult period. However, during maturation, it becomes susceptible. In the last reaction, the host is susceptible during juvenile and growth period then becomes resistant during the early adult stage and then turns to be susceptible after maturity period (Agrios, 2004). In our study, the reaction of the selected clones with PMTV and *S. subterranea* could be similar to the first type of the ontogenic host resistance. Additionally, it was suggested that the mechanism of tuber resistance to *S. subterranea* infection, is due to increased suberin content in lenticular and periderm tissues of tubers, provides an additional physical barrier to disrupt infection while root tissues lack this material (Tegg *et al*, 2013).

Overall, the clones that have excellent levels of field resistance to both pathogens can be used in potato breeding programmes as parents to introduce resistance into valuable cultivars.

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ABBREVIATIONS

The following abbreviations may be present, without definition, in the papers in this and previous editions of the Proceedings of the CPNB Conferences.

acid equivalent	a.e.	litres per hectare	litres/ha
active ingredient	a.i.	logarithm, common, base 10	log
approximately	c.	logarithm, natural	ln
body weight	b.w.	low volume	LV
boiling point	b.p.	maximum	max
centimetre(s)	cm	maximum residue level	MRL
coefficient of variation	CV	metre(s)	m
colony-forming unit(s)	cfu	metres per second	m/s
compare	cf	milligram(s)	mg
concentration x time product	ct	milligrams per kg	mg/kg
concentration required to kill 50% of test organisms	LC_{50}	millilitres(s)	ml
		millimetre(s)	mm
correlation coefficient	*r*	Minimum	min
cultivar	cv.	minimum harvest interval	MHI.
cultivars	cvs.	minute (time unit)	min
day(s)	d	moisture content	M.C.
days after treatment	DAT	molar concentration	M
degrees Celsius (centigrade)	DC	more than	>
degrees of freedom	df	no significant difference	NSD
dose required to kill 50% of test organisms	LD_{50}	not less than	|<
		not more than	|>
emulsifiable concentrate	EC	page	p.
enzyme-linked immuno-sorbant Assay	ELISA	pages	pp.
		parts per billion	ppb
European and Mediterranean Plant Protection Organization	EPPO		
fast-protein liquid chromatography	FPLC	parts per million	ppm
for example	e.g.	parts per trillion	ppt
freezing point	f.p.	pascal	Pa
gas chromatography-mass spectrometry	gc-ms	percentage	%
		polyacrylamide gel electrophoresis	PAGE
gas-liquid chromatography	glc		
genetically modified	GM	polymerase chain reaction	PCR
genetically modified organism	GMO	post-emergence	post-em.
gram(s)	g	pre-emergence	pre-em.
growth stage	GS	pre-plant incorporated	ppi
hectare(s)	ha	probability (statistical)	*p*
high performance (or pressure) liquid chromatography	hplc	relative humidity	r.h.
		revolutions per minute	rev/min
high volume	HV	second (time unit)	S
hour	h	standard error	SE
integrated crop management	ICM	standard error of the difference	SED
integrated pest management	IPM	standard error of the mean	SEM
kilogram(s)	kg	soluble powder	SP
kilogram(s) per hectare	kg/ha	species (singular)	sp.
kilometres per hour	km/h	species (plural)	spp.
least significant difference	LSD	square metre	m^2
less than	<	subspecies	ssp.
litre(s)	litre(s)	suspension concentrate	SC

systemic acquired resistance	SAR	mega	(x 10^6)	M
tandem mass spectrometry	MS-MS	kilo	(x10^3)	k
technical grade	tech.	milli	(x10^{-3})	m
temperature	temp.	micro	(x10^{-6})	μ
thin-layer chromatography	tlc	nano	(x10^{-9})	n
time for 50% loss; half life	DT_{50}	pico	(x10^{-12})	p
tonne(s)	t			
tonne(s) per hectare	t/ha			
ultralow volume	ULV			
vapour pressure	v.p.			
variety (wild plant use)	var.			
volume	**V**			
water dispersible granule	WG			
weight	*wt*			
weight by volume	*wt/v*			
weight by weight	*wt/wt*			
wettable powder	WP			