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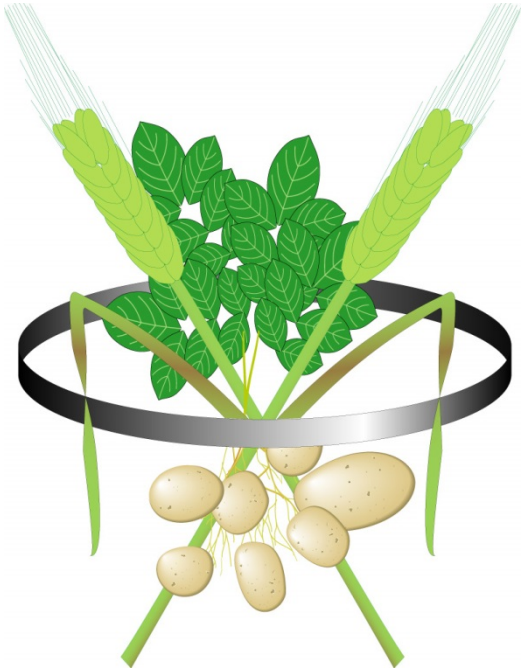

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THE GLOBAL MOVEMENT OF PLANT PESTS AND PATHOGENS: IMPLICATIONS FOR FOOD SECURITY

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Summary: Over the past centuries, crop diseases have led to the starvation of the people, the ruination of economies and the downfall of governments. Of the various challenges, the threat to plants of fungal (and oomycete) infection outstrips that posed by bacterial and viral diseases combined. Indeed, fungal and oomycete diseases have been increasing in severity and scale since the mid 20th Century and now pose a serious threat to global food security and ecosystem health.

We face a future blighted by known adversaries, by new variants of old foes and by new diseases. Modern agricultural intensification practices have heightened the challenge - the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance genes, and use of single target site antifungals has hastened emergence of new virulent and fungicide-resistant strains. Climate change compounds the saga as we see altered disease demographics - pathogens are on the move poleward in a warming world.

This presentation will highlight some current notable and persistent fungal diseases. It will consider the evolutionary “drivers” which underpin emergence of new diseases and manmade “accelerators” of spread. I will set these points in the context of four different recent disease modelling meta-analyses, which show the global distributions of crop pathogens; their predicted movement and crop disease saturation. The talk will include a Scotland-centric look at agriculture and its future under a changing climate, both with regards to crop demography and new pests and pathogens. I shall conclude with some thoughts on future threats and challenges, on fungal disease mitigation and of ways of enhancing global food security.

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CAN WE REDUCE DETRIMENTAL IMPACTS OF NITROGEN FERTILISATION BY CROP PLANT SELECTION?

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The green revolution has enabled large increases in crop yields which in turn have led to large increases in human population size. One aspect of this advance was the industrialisation of nitrogen fertiliser production through the Haber-Bosch process. It is becoming apparent that continued increases in application are not sustainable for a number of reasons including possible restriction in energy supplies in the future due to diminishing fossil fuel availability and negative environmental implications such as issues with nitrate leaching and the release of the potent greenhouse gas nitrous oxide. Perhaps the best marker of this is the fact that anthropogenic production recently overtook natural nitrogen fixation as the dominant source of reactive nitrogen compounds on the planet (Canfield *et al.*, 2010). The UK Department of Energy and Climate Change (DECC, 2016) have estimated that agriculture produces approximately 9% of the UKs greenhouse gas emissions with this figure not including emissions associated with fertiliser production which are extensive.

Thus there is a clear need to both control both application and losses of nitrogen from agricultural systems whilst maintaining or improving profitability of farming. This process is not simple as outlined in a recent United States Environmental Protection Agency report (US EPA, 2013). This report compares the major sources of greenhouse gases and estimates the mitigation option available both at no or increasing cost at a global level. Mitigation options for non-paddy agriculture include reduction in fertilisation, the application of nitrification inhibitors and conversion to reduced or no till systems. However, it is also estimated that even if all options are taken the mitigation potential is below 12% with the remaining emissions recalcitrant. Therefore there is a need to explore novel options for the reduction of emissions from agriculture. One of these options may be the selection or breeding of cultivars that control soil nitrogen cycling reducing the detrimental impacts associated with fertilisation.

The two main processes of the nitrogen cycle that are most relevant in agriculture are nitrification and denitrification. Nitrification is the conversion of ammonium to nitrate which occurs rapidly under aerobic conditions. This process is important for two main reasons: first nitrate is much more mobile in soil than ammonium and is therefore easily leached; second nitrous oxide is a by-product of conversion. Denitrification is the stepwise reduction of nitrogen oxides, including nitrate, which are used as alternative electron acceptors to maintain respiration under oxygen limiting conditions. A key intermediate, and common end point, in the pathway is nitrous oxide. Thus, in addition to both processes representing losses of nitrogen they also represent significant sources of greenhouse gas emissions.

The rhizosphere, the zone of plant root influence in soil, is one of if not the most active zone of soil. This is due to the fact that most soil organisms are limited by nutrient sources notably carbon due to the complexity of the soil system. Plant roots exude large amounts of nutrient into soil for a number of reasons which relieve this carbon limitation and allow proliferation of large populations of soil organisms. Distinct community structures are formed in the rhizosphere that are affected both by environmental conditions such as the soil type and the

species or cultivar of the host plant (Schlaeppi *et al.*, 2014). This selective structuring of the community and its associated activity provides an opportunity to engineer the system to benefit agricultural production through, for example, manipulation of nitrogen cycling.

Inhibition of nitrification by the plant, termed biological nitrification inhibition, studied most in pasture grasses where significant inhibition has been seen could possibly be exploited in crops (Subbarao *et al.* 2009). We have observed significant variation in inhibition levels of root exudates collected from a range of barley (*Hordeum vulgare*) cultivars and shown, in greenhouse experiments, that both exudates and plants significantly affect the community structures of ammonium oxidising bacteria (responsible for the first step of nitrification). Further, we have confirmed that a high inhibition cultivar significantly inhibits nitrification in a field experiment when fertilised with ammonium.

We have also screened barley cultivars to assess differences in nitrous oxide emission. This screen indicated significant variation emissions from soil supporting different lines. Further experimentation using a limited range of contrasting cultivars has demonstrated that these effects may be connected to root exudation although other mechanisms have not been ruled out and that both exudate composition and quantity may be important. This effect may be mediated through manipulation of soil microbial community dynamics as a combination of exudate effects and soil physical characteristics. Further work has sought to: 1. dissect the role of exudation quality and composition; 2. explore the interaction between soil aerobic status and denitrification flux and 3. assess links between soil community dynamics and shifts in nitrogen cycle flux including the end product of denitrification.

We believe that these findings provide evidence that in the near future we may be able to aid the development of crop cultivars that can reduce the negative environmental impacts of fertilisation and are now seeking to explore how cultivar selection together with agronomy can be applied to increase the efficiency of fertiliser uptake and reduce the recalcitrance of greenhouse gas emissions from arable agriculture.

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IPM, FROM RESEARCH INTO PRACTICE: MIND THE GAPS!

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Summary: New IPM tools for Scottish, UK and EU agriculture are being developed singly and in optimal combinations using the 'IPM toolbox' approach for several cropping systems including soft fruit, potato, cereals, and oilseed rape. These are being deployed initially at field scale, but increasingly at farm to regional scale ('area wide IPM') by more advanced IPM users. However, because IPM solutions are ecologically-based they are typically more complex and longer term than current pesticide-centred crop protection strategies. Farmers face 'day to day' and seasonally variable pest and disease problems that need fast acting solutions to meet quality standards. Many farmers are initially sceptical of the culinary equivalent of 'slow cooking' methods needed for IPM, using many new ingredients which need to be blended carefully and sampled regularly during the 'slow cook' process. One way forward is to work closely with more pioneering farmers and help them build up the IPM toolbox gradually, ensuring each IPM tool is used optimally and is compatible with the other tools in the toolbox, including the ongoing need to use selective pesticides when required. Precision pesticide use within the IPM toolbox should enable U.K. agriculture and horticulture sectors to cope in the transition, facing new and evolving pests (some with multiple resistance to pesticides), fewer pesticide active ingredients, the short and long term impacts of climate change and changing regulatory food and feed quality standards across the world.

In this paper the term 'pest' is used to collectively denote diseases, weeds and invertebrate pests. IPM (Integrated Pest Management) is a long term crop protection approach across multiple fields, farms and seasons that requires networks of researchers, farmers, crop protection advisors, policy makers, food manufacturers and consumers to interact positively (Lamichane *et al*., 2016). It aims to reduce current dependency on pesticides for crop protection, thereby reducing potential harm to consumers and the environment, including declining pollinators. All EU Member States have a NAP (National Action Plan) and Scotland has launched its own 'Integrated Pest Management Plan for Scottish Growers' (https://consult.gov.scot/cap-reform-and-crop-policy/9a1bb2d9/). This is designed to be a 'whole farm' approach to maximising efficiency of production whilst minimising negative effects on the environment. Underpinning IPM research, funded by Scottish Government's 'SEFARI' (https://sefariblog.wordpress.com/about/), links research at six world-leading Scottish research institutes. This collaborative research continuously drip feeds new innovations into the IPM toolboxes for soft fruit, potatoes, cereals and oilseed rape crops in Scotland, helping growers face rapidly changing pest challenges. In addition, The James Hutton Institute (JHI) has recently launched a web-based information and communication source alongside a new multi-disciplinary Research Centre called 'IPM@Hutton' (http://ipm.hutton.ac.uk/). This showcases our innovative and multidisciplinary IPM research and resultant practical farm-based applications across several cropping systems. This new IPM Centre at JHI represents the work of about 50 research scientists and demonstrates how we are combining research from plant molecular biology, crop genetics, pest and pathogen epidemiology, ecology, entomology,

pathology, social sciences and climate change studies to meet future crop protection challenges. The aim of 'IPM@Hutton' is to design and communicate more sustainable crop protection systems based on IPM, which utilise both ecological and biotechnological approaches to avoid 'boom and bust' pest cycles and reduce our reliance on a diminishing chemically-based arsenal of plant protection products (Birch, Begg and Squire, 2011). By working closely with scientists at SRUC, with farmers and with the crop protection industry we ensure that the research is translated into practical farming solutions.

Many challenges still need to be addressed before IPM can become 'mainstream' in Scotland and U.K., particularly for arable crops grown under more variable conditions than protected fruit and vegetables. Each IPM toolbox needs to be crop and region specific, but at the same time remain flexible enough to cope with changing pest pressures, co-evolving pest populations, new invasive threats and changes in crop management systems.

CASE STUDY: SCOTTISH SOFT FRUIT IPM STUDIES ON FARMS

The Scottish soft fruit industry serves as a useful case study of these challenges, since it is a high value crop which is usually eaten fresh, soon after harvest. In 2016, Scottish strawberries had an output value of £84 million and raspberries £12 million, from a combined area of about 1300 ha.

Modern production methods using polytunnels, precision use of micronutrients and irrigation, new varieties with modified chilling requirements, soil-free growing media, together with mass release of commercially reared pollinators and biocontrol agents have enabled both production and value to rise over the past decade by 144% and 74% for Scottish strawberries and raspberries respectively (http://www.gov.scot/Topics/Statistics/Browse/Agriculture-Fisheries/agritopics/Horticulture).

Moving from open field to protected cultivation comes with its own problems in terms of pest pressure, reflected in a doubling of percentage crop area sprayed with pesticides on protected versus field grown strawberries (SASA, Pesticide use in Scotland, Soft Fruit Crops 2016). During this period the threat from existing and new pests like Spotted Wing Drosophila (SWD) has increased, because protected cropping for extended season production also provides 'green bridges' for overwintering and habitats for pests that can be hard to detect in low numbers. Often, few IPM tools have been developed or are commercially available yet, other than monitoring traps (Birch, Begg and Squire, 2011). Pests such as aphids are now present all year round and some species have resistance to one or more classes of insecticide (Foster *et al.*, 2002). Additionally, aphid species which were formerly not prevalent in open field raspberry plantations, such as *Macrosiphum euphorbiae,* have now reached pest status on protected soft fruit crops, adding further complexity to the design of IPM strategies against multiple aphid pest species. Each species has differing migration patterns, temporal dynamics and behaviours, particularly involving interactions with key natural enemies (predators, parasitoids and pathogenic fungi). Conservatively, even if aphids only reduce crop production by 1-2% in Scotland, this still amounts to c. £1-1.5 million per year losses in output value (£115 million overall value in 2016 for Scottish soft fruit). In some years the value of fruit losses caused by aphids can be significantly higher, particularly if applied insecticide sprays are ineffective (Fountain, 2015; AHDB SF 140, Final Report).

Growers in Scotland are demonstrating an awareness and uptake of IPM which is resulting in reduced pesticide usage, equivalent to 82% of sampled growers in Scotland implementing some IPM tools. According to SASA survey figures, the amount of pesticides applied (by weight) has decreased by 42% since 2014 and 38% since 2011/12 (Reay *et al*., 2016). Concurrently, Scottish growers have increased use of biological control and biopesticides by

28% since 2014, accounting for 30% of the total soft fruit area in Scotland. However, biopesticides still only account for 3% of pesticides applied to soft fruit when characterised by type and mode of action, which is a decline of 58% since 2014. This indicates a gap in grower confidence and/or lack of available products that meet their current needs. From recent IPM trials on protected raspberries at JHI, biopesticides can be as effective as conventional synthetic insecticides such as the neonicotinoid 'Calypso', but require more frequent application, careful timing, specialised spray equipment and augmentation with release of commercially reared mix of several parasitoid wasp species ('SCEPTRE' Sustainable Crop and Environment Protection-Target Research for Edibles project final report 2015, DEFRA/AHDB https://horticulture.ahdb.org.uk/sites/default/files/research_papers/CP%20077_Report_Final_All%20years.pdf; http://ipm.hutton.ac.uk/toolboxes/soft-fruit-ipm-toolbox).

Seasonal variation in pest pressure, often driven by climatic conditions affecting over-wintering and Spring-Summer migrations, are likely to be a key factor also affecting pesticide use on soft fruit. Cooler climatic conditions slow down pest build up on protected crops during the growing season, whereas milder winters can facilitate increased local over-wintering of aphids on either the crop or weeds that serve as hosts to polyphagous aphids such as *M. euphorbiae* (CABI Invasive Species Compendium; https://www.cabi.org/isc/datasheet/32154). Overwintering of pests such as aphids on protected crops or on nearby weeds creates new problems for growers switching to biocontrol, and presents another research gap. Overwintering pests are usually cold adapted, locally abundant over the non-growing season and can start to build up earlier than aphids migrating longer distances in the spring or summer. These early season pest build ups are often in localised 'hotspots' that are hard to detect by scouting or monitoring. Commercially available biocontrol agents are generally not well adapted to operate at lower temperatures found in Scottish early spring. This results in the balance between pests and natural enemies being out of phase and so over-wintering pests can get a head start in the crop. To fill this gap, new research at JHI is testing 'smart cameras' to detect pest 'hotspots' before they build up to damaging or economic levels. In the future it should be possible to link 'smart cameras' to either tractor-based sprayers or to drones, so that pest 'hotspots' can be treated with IPM-compatible pesticides or biopesticides earlier in the season, saving pesticide use and facilitating the later use of biocontrol agents if further pest outbreaks are detected. This 'precision' monitoring (e.g. traps enhanced with pest-specific host plant attractants) combined with 'hotspot' treatment approach has already been shown to successfully reduce pesticide use against raspberry beetle on commercial farms by up to 40% compared with standard pesticide regimes (Birch, Begg & Squire, 2011). This reduction in pesticide dependence also has benefits for on-farm biodiversity, particularly pollinators which are under threat globally. Soft fruit growers invest heavily in commercially reared bumblebees to promote uniform pollination in polytunnels. It makes commercial and environmental sense to reduce the risk of harming these augmented pollinators, and also endemic pollinators such as hoverflies, by cutting down on pesticide use on fresh fruit. Given that multiple pesticide residues have recently been detected on fresh fruit and vegetables (excluding soft fruit) included in school children's '5 a Day' supplied diet (Cooke, 2016) there is ongoing effort in the food industry to keep pesticide residues below MRL levels, particularly for children.

In conclusion, there is still much work needed to translate existing research outputs into practical IPM tools for growers and farmers. These IPM 'toolboxes' need to be regularly updated and improved, because existing pests adapt quickly (e.g. pesticide resistance, cold hardiness, over-wintering ability) and new invasive pests can make existing IPM systems ineffective in a short period of time. Fastest progress can be made by 'co-innovation' involving close and regular collaboration between researchers, farmers, advisors, consumers and industry stakeholders.

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IPM PRACTICES ON ARABLE FARMS IN THE UK AND IRELAND

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Summary: In 2017, a short survey was conducted in the UK and Ireland to establish baselines for the perception and adoption of Integrated Pest Management (IPM) strategies. The survey targeted a representative sample of arable farmers from each country. The survey asked for information on uptake of specific IPM practices, the farm, the farmer and their farm business. Preliminary data suggests that there are some growers who are not familiar with the term IPM, despite them being aware of, and adopting, various IPM practices on their farms. This survey is part of a wider study aimed at improving farmer awareness and adoption of IPM in the UK and Ireland.

INTRODUCTION

Integrated Pest Management is the use of an optimal mix of pest (predominantly weeds, diseases or insects) control techniques and tools, taking into account factors including profit, risk, sustainability, humans and environmental safety. In 2009, the EU set rules for the sustainable use of pesticides (Directive 2009/128/ EC) and, as a result, all partner countries must set objectives to reduce risks associated with pesticide usage. In order for realistic objectives to be set, and an effective plan to be designed to meet those objectives, we must first attempt to understand the current perception and adoption of IPM amongst farmers.

MATERIALS AND METHODS

In 2017 a short survey was conducted in the UK and Ireland to establish baselines for IPM. Research institutes/universities in Scotland, England, Ireland and Northern Ireland used an identical questionnaire in the four countries, which was administered on-farm or by post. Approximately 250 arable farmers were surveyed. Data were collected on familiarity of the term IPM, the use of the arable rotation, crop and variety selection, tillage regime, and measures they use to control the introduction and spread of weeds, diseases and invertebrate pests on their farms. A final section of the questionnaire enabled the collection of a range of contextual information on the farm business, the farmer and their situation and the financial performance of their farm business. This combination of data will allow for rigorous statistical analysis of the links between IPM uptake and socio-demographic situation of the farmers. This information and understanding is key to the focusing of knowledge exchange activities

required to raise IPM uptake and fully exploit the agronomic research currently being conducted in this area.

RESULTS AND DISCUSSION

Initial findings indicate that around 1/3 of growers are unfamiliar with the term IPM despite them being aware of the pest control techniques which, when combined in a meaningful way, constitute IPM practice (Figure 1).

Figure 1. Survey respondents were asked to state how familiar they are with IPM on a 1 to 5 scale. Chart represents % of sample selecting each option (initial responses from S. England and Scotland only).

Each country in the European Union is required to show that they are implementing National Action Plans to improve IPM uptake. The fact that some growers are unfamiliar with IPM must be remedied through research and knowledge transfer efforts in the future. In our survey collaborating farmers provided data on what they viewed as being the most valuable sources of pest management advice (Table 1). The findings indicate that knowledge exchange of best practice advice should involve all practitioners of IPM.

Table 1. Ranking of the most important sources of pest management advice (initial responses from S. England and Scotland only).

Information Source	% Sample
Past experience	80
Independent agronomist	56
Open days/crop walks	38
Merchant agronomist	30

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INTRODUCING PARTRIDGE: A EUROPEAN NETWORK OF DEMONSTRATION SITES TO HIGHLIGHT HOW AGRI-ENVIRONMENT SCHEMES CAN BE IMPROVED

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Summary: The PARTRIDGE project is a new collaboration supported by the North Sea Region Interreg fund. It aims to show, on 10 demonstration sites across northern Europe, that Grey Partridge populations, and those of associated farmland wildlife, can increase by at least 30% in three years if at least 7% of the farmland is converted to quality Grey Partridge habitats. It is hoped that this will lead to improvements in agri-policy across the region to better support biodiversity measures in local agri-environment schemes. This paper introduces the project and describes some preliminary successes.

INTRODUCTION

The Grey Partridge *Perdix perdix* was once a very abundant gamebird with a sustainable harvest of around two million birds taken annually at the turn of the 20th century (Tapper, 1999), suggesting the current population in the UK is less than 1% of what it must have been at that time. Furthermore, the Grey Partridge has long been considered an indicator species whose fortunes reflect that of many others (Potts, 2012) and indeed many farmland specialists have experienced similar long-term trends, such as the Tree Sparrow *Passer montanus* and Corn Bunting *Emberiza calandra* with declines of 96% and 87% respectively between 1967 and 2014. These population trends are closely linked to changes in agricultural practices (Newton, 2004) and similar trends are evident across Europe where agriculture has also advanced to increase food production (Donald *et al*., 2001).

The principle tool available to land managers across Europe, by which it is theoretically possible to counter the changes to the environment that have led to the declines in farmland wildlife, is the agri-environment scheme. These schemes use CAP Pillar 2 funds to pay farmers to include a variety of measures in their management plans that are thought to benefit wildlife. Despite such measures being available in various guises since the late 1980s, the EU's "State of Nature in the EU" report (2015), suggested that biodiversity was still threatened, with, for example, agriculture identified as the greatest pressure impacting on EU bird populations.

There are many possible explanations for this, including that agri-environment schemes aren't able to support sympathetic management on a large enough scale at the farm level, that perhaps they don't support key measures that are required, or perhaps the changes they do support don't amount to enough at the landscape-scale. This paper introduces the new PARTRIDGE (Protecting the Areas Resources Through Researched Innovative Demonstration of Good Examples) project, part-funded by the Interreg North Sea Region programme, which aims to:

1) Show how farmland can be managed to support increasing or stable populations of Grey Partridge, across 10 demonstration sites in five EU countries,
2) Demonstrate how this impacts on other farmland wildlife, with a prediction that populations will increase by at least 30% over the period,
3) Investigate farmers' decision-making processes across the partner countries, especially with regards to participation in agri-environment schemes, and
4) Use this information to attempt to improve agri-environment schemes and agricultural policy generally for the betterment of biodiversity.

We will also present illustrative Grey Partridge results from one site in Scotland.

MATERIALS AND METHODS

The PARTRIDGE project began in early 2017 and will finish at the end of 2020. There are two demonstration sites, each of at least 500ha in size, in Scotland, England, The Netherlands, Belgium and Germany (Figure 1), with each demonstration site paired with a comparable reference area nearby but at least 4km away. On all the sites we began a programme of monitoring Grey Partridge, other farmland birds and Brown Hare *Lepus europaeus* in 2017 – before most major changes to habitats were made. All monitoring protocols are consistent across the partner countries.

Grey Partridge are counted by driving round sites in a 4x4 vehicle in spring and autumn using all available access points and stubble fields in autumn, and surveying fields with the help of binoculars. This is done from sunrise until about 3-4 hours afterwards and in the last 2-3 hours before sunset. Spring counts reveal pair locations, autumn counts indicate breeding success.
All other birds on the sites are monitored using mapping techniques to reveal the number of territories and maximum count. Sites are surveyed on foot along a fixed route, from sunrise until approximately five hours afterwards. All birds seen or heard are recorded on a tablet using the app "Avimap".

Each demonstration site is adding habitats suitable for Grey Partridge to bring coverage up to at least 7% of the land area, which is thought to be the minimum required for populations to stabilize or increase (*c.f.* Aebischer & Ewald, 2004, Ewald *et al*., 2012). This is focusing on cover crops (aka game crops, wild bird crops, etc.) which provide year-round resources for the birds and are simple for farmers to manage. The exact seed-mix used varies from country to country to be locally optimal, but comprises annual, biennial and perennial plants. Farmers at the demonstration sites are being encouraged to establish large blocks (minimum of 1ha) or wide strips (minimum 24m) of cover crops, with the whole area sown in one spring. Then, in the following spring, half of each plot is cut and the soil-surface is disturbed with a harrow or disc, to create short, open vegetation which is ideal for foraging Grey Partridge chicks. The uncut half is good for nesting cover and escape cover from predators, especially in the winter when most agricultural landscapes lack such a resource. In subsequent years, the tall vegetation of each plot is cut such that there is always short brood-rearing and taller nesting/escape cover available in each plot.

One of the demonstration sites in Scotland, Balgonie Estate, Fife, began implementing beneficial habitats for Grey Partridge independently of the PARTRIDGE study in 2015 – marginally earlier than the other sites. In this case the habitats were narrow (4m) strips of cover crops comprising Chicory *Cichorium intybus* with either Kale *Brassica oleracea* or Brown Mustard *Brassica juncea,* totalling approx. 7km in length. This was also designed to be a potential long-term crop with minimal intervention required after establishment, as the Chicory is a perennial plant.

RESULTS

Progress with PARTRIDGE

The 10 demonstration sites and their paired reference sites are all well established and surveys on them have begun. New habitats have been created and progress has been made towards the target of 7% coverage on all demonstration sites.

Response to early habitat changes at Balgonie

Grey Partridge productivity, adult survival during the breeding season and total autumn density increased on the estate after the new Chicory habitats were introduced (Table 1).

DISCUSSION

The new PARTRIDGE project offers an opportunity to share best practise in farmland habitat management across northern Europe, to show what can be done to enhance farmland biodiversity. The Grey Partridge is a very well-studied farmland bird whose population trend of late mirrors that of several other species. Much is known about the management requirements of the Grey Partridge following decades of research and measures such as beetle banks, conservation headlands and appropriate hedgerow management are all proven to benefit this species (Potts, 2012).

The project partners predict that the habitat improvements and other measures being undertaken will result in a 30% increase in Grey Partridge, other farmland birds and Brown Hare by 2020 when the project ends. The preliminary data from Balgonie suggest this will be possible and this is in line with the findings of some case-studies (e.g. Potts, 2012, Draycott, 2012). PARTRIDGE would be the first demonstration of this on such an international scale. This information could then lead to enhanced guidelines for agri-environment schemes across the North Sea Region with subsequent benefits for wildlife on a huge scale.

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Table 1. Changes in Grey Partridge populations at Balgonie after habitat improvements in spring 2015.

Year	Spring density (/100ha)	Young:Old ratio	Autumn density (/100ha)
2014	4.3	1.02	14.1
2015	4.4	1.81	25.3
2016	4.5	2.01	30.2

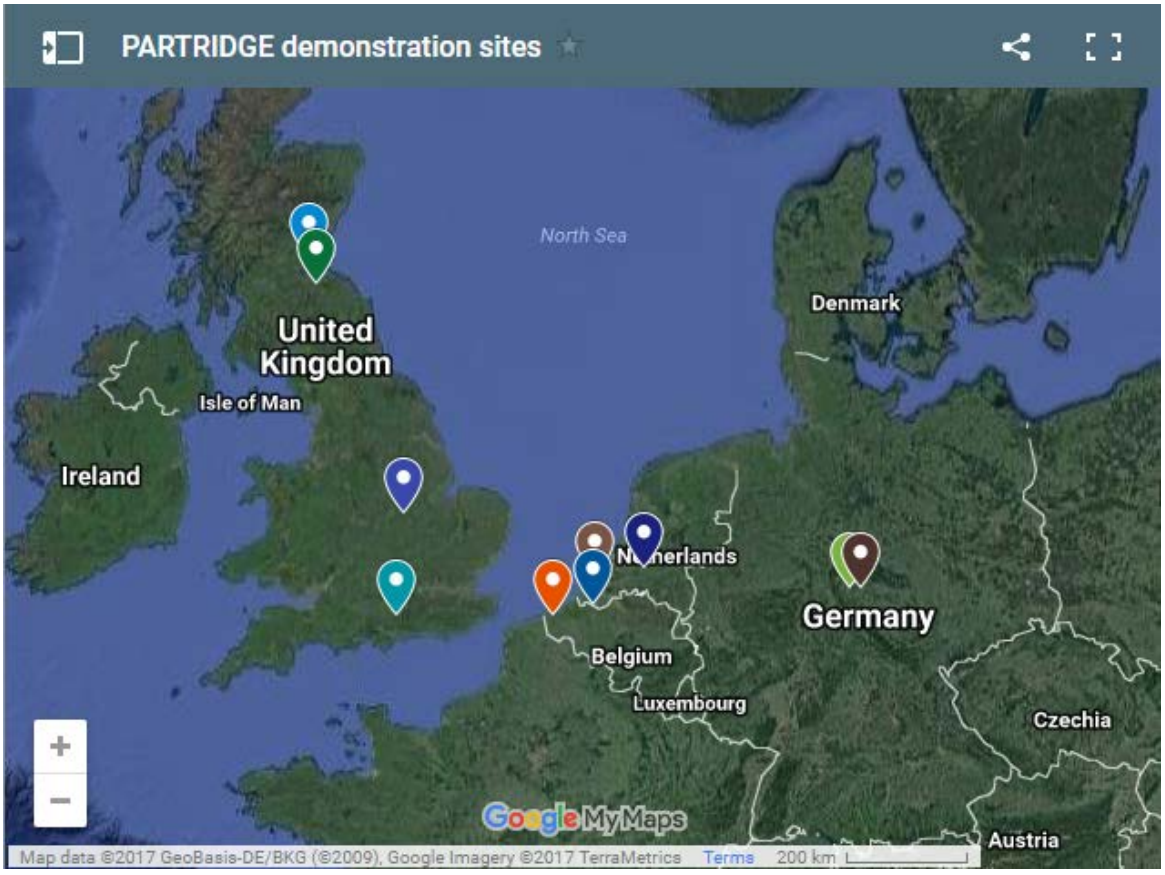

Figure 1. Location of all demonstration sites in the PARTRIDGE project.

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HEALTHY SOILS FOR CROP PRODUCTION

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Summary: Globally, soils are under severe pressure from both anthropogenic and environmental sources, thus protection of soils is of paramount importance. Soils underpin civilization but as rates of soil formation are in the order of 1 cm per 100 years, they represent an essentially non-renewable resource. The complexity of soil systems may be a driver of non-engagement by the agricultural community regarding soil health management. For soil protection to maintain (or improve) soil health to be successful the generation of rapid monitoring tools that provide simple understandable outputs is essential. As soil biota have fundamental roles in the mediation of soil ecosystem services, they are logical candidates as effective proxy indicators of soil health and quality. We outline an industry-academic partnership that is assessing biological, chemical and physical parameters of agricultural soils from across the UK to identify the appropriate parameters that provide a robust indication of soil health.

The maintenance of food security delivered by a sustainable intensification of agriculture (Tilman *et al*., 2010) is arguably the greatest global challenge (Godfray *et al*., 2010), exacerbated by a decreasing land resource (Lambin & Meyfroidt, 2011) that is degraded (Banwart, 2011), impacted by a changing climate (IPCC, 2014), compounded by a recognised and increasing yield gap (Van Ittersum et al., 2013) and underpinned by volatile food prices (UNEP, 2009). In the EU there is a further constraint driven by legislation to reduce application of synthetic actives (91/414/EEC; 128/2009/CE).

Soil is a key asset of natural capital, providing goods and services that sustain life through the support of food production but with impacts beyond agricultural systems such as provision and promotion of biodiversity, carbon sequestration and greenhouse gas mitigation. It is therefore perplexing that soils have been significantly undervalued as an asset (Panagos *et al*., 2016).

As a consequence of past agricultural intensification, degradation threats to soils are numerous (Powlson *et al*. 2011). Compounded with an ever burgeoning global population, the area of soil usable for cultivation has declined from 0.32 to 0.25 ha per capita between 1975 and 2000. A reduction of soil fertility has contributed to crop yields stagnating since 1996. Sustainable intensification of agriculture aims to minimise pressures on the environment in terms of water-, energy- and fertilizer-use, and on maintaining soil as a sustainable resource, each balanced against the necessity of maintaining, or increasing, crop productivity. Success in meeting these objectives will require better understanding and management of the biological processes and interactions that underpin the functioning of plant-soil systems. With approximately 40% of agricultural soils already suffering degradation (Doran & Zeiss, 2000), protection of soil health is clearly critical, the alternative being further conversion of natural ecosystems for cultivation.

The definition of soil health varies but Kibblewhite *et al*. (2008) describe it as the ability to maintain production while providing other essential ecosystem services and maintaining biodiversity. In Europe, there is an increasing list of legislation relating to the protection of soils

as a result of the Common Agricultural Policy (CAP) reform (Creamer *et al*. 2010). Thus, there is a policy driver for effective monitoring of soils at local-, regional- and national-scales. This view was underpinned by the EIP-AGRI group on soil-borne pathogens that recommended a soil health strategy should combine the following elements: monitoring, prevention (in the context of pest and pathogens), crop rotation and additional measures (e.g. application of organic matter). Soil changes slowly, and our understanding of the biological, chemical and physical components that determines its quality is incomplete. An absence of an integrated approach to soil health and soil quality can exacerbate the impact of soil-borne diseases. Soil-borne pathogens typically represent a minor component of a soil system but are diverse and include fungi, bacteria, insects, protozoa and nematodes. Thus, development of a soil health strategy should not focus on an individual pest/pathogen but encompass the whole soil system, i.e. the biological, chemical and physical aspects of soil. The promotion of soil health, including the development of management strategies to remediate degraded soils, is critical. The perception of the complexity of soil systems which are difficult to manage may have prevented the engagement of the agricultural industry with soil health management.

Current commercial practice is to provide agronomic advice based on a limited number of chemical parameters such as pH, C, P, K and Mg with biological measures lacking. However, soil assessment often ignores the physical aspects of soil, i.e. bulk density, which is used as a proxy indicator of compaction, and rooting impedance, both of which impact crop growth and yield. Due to the inherent complexity of soil processes, biological indicators have been suggested as a sensitive proxy measure that integrate key soil parameters such as nutrient cycling, quality and resilience. Thus, only analysing chemical and/or physical attributes of soils, whilst ignoring the biological component, is inadequate as soil biota drive many soil processes, e.g. nutrient cycles. Given that biota play such fundamental roles in many soil ecosystem services, biological properties are logical candidates as effective indicators, to complement soil physico-chemical properties (Ritz *et al*., 2009). Biotic indices provide a long-term integrated measure of soil health contrasting with a shorter-term snapshot provided by current measures of soil chemistry and physics. Soil biological, chemical and physical parameters are dependent upon the various interactions of the soil food web that, for example, directly affects plant productivity through root herbivory, parasitism and mutualism; impacts nutrient cycling (through organic matter decomposition and nutrient mineralisation) and influences formation and structure of soil.

Nematodes are a key component of the soil food web, occurring at different trophic levels and forming links between plants, bacteria, fungi and other soil fauna (de Ruiter *et al*. 1993; Traunspurger 2000). Their abundance, diversity and rapid generation times as well as relative ease of extraction from soil (Ritz & Trudgill 1999) make nematodes an ideal biological group to use as a potential proxy measure of soil health. Inhabiting the film of water surrounding soil particles, the cuticle is in constant contact with the soil environment, and because most species spend their entire life cycle in the soil, nematodes are constantly affected by the surrounding soil conditions (Ritz & Trudgill 1999). Furthermore, their range of responsiveness to toxins and stresses such as desiccation make them valuable indicators in disturbed systems (Neher 2001).

Historically nematodes have been viewed through a prism of a crop pathogen that costs global agriculture an estimated $125 Billion per annum to manage (Chitwood, 2003). However, beneficial nematodes predominate in soils, even those soils with known high abundances of Potato Cyst and pathogenic Free-Living nematodes. Nematodes can be classified into feeding guilds (Yeates *et al*. 1993) such as bacterivores, fungivores, herbivores, omnivores and predators thus function maybe inferred according to taxa. Through analysing nematode communities a picture of the entire soil community is gained, e.g. we can ascertain if soils are dominated by bacteria (i.e. disturbed or maturing soils, nutrient replete with low C:N) or fungi (i.e. degraded soil, depleted nutrients with high C:N). Recent advances in technology has

facilitated the development and subsequent deployment of high-throughput DNA-based methods to characterise nematode communities at functional level (Donn *et al*., 2012; Griffiths *et al*., 2012; Chen *et al*., 2014; Wiesel *et al*., 2015). Thus, soil monitoring at national scale generating thousands of soil samples for soil health assessment is now a realistic proposition.

We will outline a unique and novel industry-academic partnership funded by InnovateUK that is assessing biological, chemical and physical parameters of agricultural soils from across the UK to identify the appropriate parameters that provide a robust indication of soil health.

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MEASURING AND MANAGING SOIL HEALTH IN FIELD HORTICULTURAL CROPS – OUTCOMES FROM THE GREATsoils PROJECT

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Summary: This paper summarises the objectives and achievements of the horticulture-specific component of AHDBs GREATsoils programme. It defines soil health and summarises the main chemical, physical and biological methods used for measuring it in UK agricultural soils. It explains the importance of linking results obtained from soil health testing to the development of management strategies to improve soil health and provides links to the outputs from the GREATsoils project.

INTRODUCTION

Soil health is critically important to growers because there is a direct relationship between functional, healthy soils and reliable good yields of quality crops. It therefore has a clear impact on profit margins. As margins are squeezed, growers are keen to ensure they can get consistently good yields of marketable crops, yet many have become aware that their soils have suffered following years of intensive cultivations with little or no organic matter returned to the soil. Increasing numbers of growers now see the value in measuring soil health in order to gain a better understanding of their soils and to help them choose appropriate management strategies to improve them.

Aspects of soil health (in particular soil carbon content) are also important in relation to ecosystem services (such as maintenance of water quality) and climate change mitigation and thus it is important to be able to measure soil health effectively in order to determine the impact of management strategies. Governments and policy makers are increasingly recognising the importance of soil health and for this reason, several UK projects have been commissioned to look at aspects of soil health and soil management in soil-based agricultural and horticultural systems.

One of these, the GREATsoils programme (Growing resilient, efficient and thriving soils), is funded by AHDB. The first part of the programme (CP107b) began in April 2015 and aimed to inspire and support fruit, vegetable and salad growers to develop the abilities and confidence to assess the health of their soils and take practical action to improve management strategies. This paper defines soil health in practical terms, describes the most appropriate soil health measurement techniques for growers and summarises key outputs from the project.

SOIL HEALTH DEFINED

Soil health can be defined in many ways. A broadly useful definition might be: “the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant

and animal health" (Doran *et al.*, 1996). The components of soil health include physical parameters such as soil structure; chemical parameters such as crop nutrient indices and soil organic matter content and biological parameters such as earthworm numbers. These can all be affected by the ways in which we manage our soils. The term soil quality is sometimes used interchangeably with soil health, but it is more usually thought to include parameters that cannot be changed, such as soil depth and soil texture (the relative percentages of sand, silt and clay).

METHODS FOR ASSESSING AND MEASURING SOIL HEALTH

Soil health cannot be adequately assessed by looking at a single variable such as crop yield, water quality, soil compaction or earthworm activity. Although some parameters are arguably more useful than others, it is generally recognised that measurement of several parameters is likely to be necessary in order to give a useful indication of the health of a soil. Techniques can be broadly be divided into those that measure chemical soil characteristics (such as pH, P, K and Mg index [or status in Scotland] and organic matter content), physical characteristics (such as water infiltration, soil compaction and soil structure) and biological characteristics (such as soil respiration and earthworm numbers).

A useful suite of parameters for use in assessing a UK soil should:

- be easy (and ideally relatively inexpensive) to measure;
- measure changes in soil functions;
- encompass chemical, physical and biological properties;
- be accessible to many users;
- be applicable across a wide range of soil types and UK climate areas;
- be sensitive to variations in climate and management.

There is a wide variety of soil assessment methods and techniques available, but up until now there was a lack of clear guidance for UK growers to help them choose methods that are most appropriate to them. A scientific literature review was conducted as part of the GREATsoils project in 2015. It assessed the relevance of a wide range of soil testing and assessment methods used worldwide and the results were presented at a series of four regional grower meetings (Scotland, East Midlands, West Midlands and Southeast England). The main methods were presented to growers (and many of the practical techniques were demonstrated in the field). Each method was discussed with reference to cost, ease of use, usefulness of results and evidence of merit. The methods were then rated by the growers and project team members in terms of their potential for use in UK soil-based horticultural production systems. Following extensive testing in GREATsoils field trials during the project, further guidance and case studies have been published on the most appropriate methods for testing aspects of soil health. All are available on the GREATsoils web page (www.ahdb.org.uk/greatsoils). Key methods currently used in the UK and the usefulness and effectiveness of each are briefly summarised in Tables 1 and 2. They have been discussed in more detail, along with their grower ratings in AHDB (2016).

Some of the chemical test methods, such as routine analysis for pH and extractable major nutrients are long established and widely used, but evidence obtained during the GREATsoils project strongly suggests that there is scope for more frequent testing in some high value cropping systems. Others are fairly widely used (for example soil organic matter testing using the loss-on-ignition method) but there is often some doubt amongst farmers about how to interpret the results for their own soil types and farming system.

Although many farmers are investigating soil physical characteristics in their fields, few interviewed during the GREATsoils project were formalising these assessments or recording detailed information. Many were looking for guidance to do such assessments better.

Table 1. Key chemical and physical soil health assessment methods[1]

Chemical test	Skill needed by grower	Time input	Cost	Comments
Soil pH	none, lab does it	small	low	Vitally important in terms of soil health and crop productivity. Results easy to interpret based on extensive scientific evidence.
Extractable P, K, Mg	none, lab does it	small	low	As above
Base cation saturation ratio	none, lab does it	can be high	high	Particularly popular with organic growers; limited scientific evidence to prove its worth.
Soil trace elements	none, lab does it	small	moderate	Crop availability strongly linked to soil pH. Worth testing where evidence of deficiencies/toxicities or with high value crops. Results easy to interpret based on extensive scientific evidence.
Soil organic matter (Loss on ignition)	none, lab does it	small	low	Worth testing, though expect changes to be slow where attempts are being made to increase it. Other methods can give indication of impact of management practices on long-term changes in soil organic matter content, but these are either not commercially available or there is limited scientific evidence to prove their worth.
Physical test/evaluation				
Soil compaction test	low skill	small	low	Can be done using a spade, knife or penetrometer. Gives quick, useful indications of problem areas within fields.
Infiltration test	low skill	moderate	low	Give indication of speed at which water can flow down through soil. Useful quick indicator of one aspect of soil health.
Visual evaluation of soil structure	moderate skill	moderate	low	Takes longer than above but gives more detailed and more useful information. Several good published techniques available[2].
Full soil profile investigation	high skill	moderate	high (if paid help needed)	Takes longer than simple structural evaluations, but gives more detailed and more useful information. Essential in order to make the right decisions on field drainage and sub-soiling. Should ideally be done prior to making decisions on implementing new cropping systems. Help from soil management specialists often required.

[1]Soil health, and methods used to test it are discussed in more detail in Wood and Litterick, 2017 and numerous publications from the GREATsoils project, all of which are or will be available from the website (www.ahdb.org.uk/greatsoils); [2]A good example of a technique suitable for use in cultivated soils is Ball *et al.,* 2012.

Table 2. Key Biological soil health assessment methods[1]

Technique/ test	Skill needed by grower	Time input	Cost	Comments
Plant health monitoring	high skill	small	low	Can assess visible appearance and incidence of pests/diseases on both crops and weeds. General acceptance that the healthier soil is, the less crops and weeds will suffer nutritional disorders and pest and disease attack.
Soil respiration	None or small	small	moderate	Can send to lab or purchase Solvita® tests to do on-farm. The premise is that healthy soils have higher respiration rates, but limited evidence as yet to prove this.
Earthworm counts	Low skill	high	low	Gives broad indication of soil health; numbers linked to soil texture and rainfall as well as to soil organic matter content and management system.
Soil foodweb	None, lab does it	small	high	Measures microorganism numbers (named types, e.g. fungi, bacteria and protozoans); particularly popular with organic growers; limited scientific evidence to prove its worth.

[1]Soil health, and methods used to test it are discussed in more detail in Wood and Litterick, 2017 and numerous publications from the GREATsoils project, all of which are or will be available from the website (www.ahdb.org.uk/greatsoils).

A relatively small number of farmers are using more novel soil health measurement methods (such as base cation saturation ratio testing, earthworm counts, soil respiration testing and soil foodweb assessments) but there is currently limited scientific evidence as to the value of these methods and little information on how to use the results in order to improve soil health.

STRATEGIES FOR MAINTAINING AND IMPROVING SOIL HEALTH

Soil health measurement is only useful in commercial agriculture/horticulture when the measurements obtained from it can be easily interpreted, then used to develop and implement strategies for the improvement of soil health. Some of these strategies (such as the use of organic manures and long-term grass/clover leys to build soil organic matter levels) have been well understood and practised for decades, though perhaps they are used less in intensive arable horticultural systems. Others, such as the use of minimum tillage and the use of cover crops and green manures have been less used by conventional growers until very recently and their impact on soil health parameters is somewhat less well understood.

Some of the best known, well-proven strategies for improving soil health are the least easy to implement in intensive horticultural systems. For example there are few livestock producers left in some of the more intensive horticultural areas in the UK and limited tonnages of off-farm bulky organic materials (such as composts) are available, so the amendment of soils with organic amendments is costly or impossible. Further work is required to develop appropriate methods for improving the health of soils in these intensive systems, particularly since there is

evidence that it is they which are suffering the greatest losses in terms of soil health, ease of cultivations and crop productivity.

The main methods by which farmers and growers can maintain and improve soil health include the following:

- Maintenance of soil pH at an appropriate level for the rotation.
- Maintenance of soils at target index (or status in Scotland) for P, K and Mg.
- Regular, efficient maintenance of field drains.
- Subsoiling where necessary (only when conditions are right to do so).
- Applications of bulky organic manures (e.g. animal manures and composts).
- Ploughing in of crop residues and straw
- Sowing green manures and cover crops between crops or between/around rows/beds.
- Minimisation of the number and duration of periods in which the soil is bare.
- Minimisation of tillage in some or all of the rotation.
- Increasing diversity in the rotation (different crops with different rooting patterns).

Some high value crop growers are investigating more novel methods for soil health improvement including:

- Bringing animals back onto holdings which have had no livestock for decades;
- Growing biomass in the form of grass, hedges or trees (for chipping, mulching or composting on the farm where no sources of bulky organic matter exist locally).

Many of these methods require the development of skills and techniques which are to a degree specific to farms/farmers, farming systems, soils and climate. Although the government and levy-board funded research which has helped and is continuing to develop such methods is vital and useful, the importance of simple, farmer-driven field trials on working farms cannot be overestimated, particularly when these are linked to active, entrepreneurial grower groups. The Innovative Farmers Programme is particularly important in funding a large number of small projects on numerous topics (including many related to soils and soil health) throughout the UK (https://www.soilassociation.org/our-campaigns/better-food/transforming-the-way-we-farm/innovative-farmers/).

FARMER TRIALS AND FIELD LABS

A key aim of the first GREATsoils project has been to engage with growers throughout, in order to collect information on how soil health measurement techniques and tests are being used in practice and to determine how useful each method is found to be. To this end, following completion of the initial grower consultations and literature review, a series of six field trials were set up with grower groups based in different areas of the UK under the GREATsoils project, and an additional four trials linked to the project were set up under the auspices of the Innovative Farmers (IF) Programme (part-funded by AHDB and the IF Programme). The topics for study were chosen by the grower groups. Most chose to test several soil health parameters before and after using a new (to them) management option being undertaken with the aim of improving soil health. The grower groups, their location and the topics being studied are summarised in Table 3. The ten field trials are due to finish in the early part of 2018 and the findings will be available on the GREATsoils web page (www.ahdb.org.uk/greatsoils).

PROJECT OUTPUTS AND CONCLUSIONS

A comprehensive knowledge exchange programme has ensured that findings from the AHDB Horticulture-funded GREATsoils project CP107b have been made available to growers throughout the UK. The main project outputs (literature from which is (or will be made) available on the GREATsoils website (www.ahdb.org.uk/greatsoils).will include:

- A literature review and critical appraisal of techniques to measure aspects of soil health;
- Over 30 articles in the horticultural trade press;
- Around 30 practical grower meetings held in association with the field trials;
- Four advisor training workshops;
- Twelve grower workshops on aspects of soil health measurement/management;
- Six grower case studies on aspects of soil health measurement/management;
- Six guidance notes on aspects of soil health measurement/management.
- Four webinars on aspects of soil health measurement/management;
- Two YOUtube videos on aspects of soil health management;
- Five grower blogs on their opinions and practical experiences concerning soil health monitoring and management
- GREATsoils bulletin – made available to project followers.

Project CP107b wasshowcased at 25 conferences and sector events in its 3 year duration. From project inception to date, the project has a network of 670 members and 1370 followers on Twitter.

CURRENT RELEVANT WORK

AHDB is continuing work under the GREATsoils banner, with the aim of helping farmers to develop an improved understanding of their soils, through a range of projects including a new, industry-wide research programme, which aims to improve on-farm understanding of soil health and management. The five-year “Soil Biology and Soil Health Partnership” is co-funded by the British Beet Research Organisation (BBRO) and led by Elizabeth Stockdale (Head of Farming Systems at NIAB). The Soil Biology and Health Research and Knowledge Exchange Partnership is working over the next 4 years to develop a “soil health toolkit” which will link new and existing measures of soil chemical, physical and biological properties, together with an interpretation framework and guidance on options for farm management to improve soil health.To find out more, visit www.ahdb.org.uk/greatsoils.

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We acknowledge the financial support of AHDB (https://ahdb.org.uk/) in funding the GREATsoils project and Innovative farmers (https://www.innovativefarmers.org/) in funding the linked Field Labs. We also thank the farmers and growers too numerous to mention for their enthusiastic support of field work and project meetings.

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FARMING & WATER SCOTLAND – HELPING FARMERS KEEP ON THE RIGHT SIDE OF THE REGULATIONS

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Summary: Scotland is widely recognised as having good water quality. However, despite the introduction of the Diffuse Pollution General Binding Rules in 2008, some catchments within Scotland are still struggling to achieve water quality objectives. In 2016, Scottish Government commissioned SAC Consulting and SEPA to work in partnership to develop and attend a programme of agricultural shows and events across Scotland branded as Farming and Water Scotland. The initiative aimed to help raise the awareness of the Diffuse Pollution General Binding Rules and provide practical ideas to help farmers and land managers reduce pollution risks, comply with legislation and also benefit the farm business.

This paper highlights some of the key findings from the Farming and Water Scotland agricultural shows programme.

INTRODUCTION

The environmental quality of Scotland's lochs, rivers, wetlands and seas is world renowned. As natural assets, these features help to support the health and wellbeing of our population, our tourism industry, our food and drink industry and our wildlife. Maintaining these features in a favourable condition will contribute to the economic success and well-being in Scotland (SEPA, 2015).

In 2014, 66% of the 3169 rivers, lochs, estuaries, coastal waters and Groundwaters identified in the Scotland river basin district were recorded as being in good or better condition (SEPA, 2014).

In rural environments one of the largest risks to good water quality in Scotland is rural diffuse pollution from agriculture and forestry land use sources (SEPA, 2007).

The mechanisms of diffuse pollution have been well documented (Novotny & Olem, 1994). Sediment, nutrient and faecal bacteria being transported from the land into the water environment, often during times of rainfall, have been shown to impact to water quality.

With the introduction of the Diffuse Pollution General Binding Rules (DP GBRs) in 2008, the Scottish Rural Development Programme 2007-2013 and the start of the first cycle of Priority Catchment work by SEPA, the need to produce regulatory guidance for the rural land use sectors was identified. This was taken forward by the Diffuse Pollution Management Group (DPMAG) as part of the national awareness raising campaign (SEPA, 2015).

In the first phase of Priority Catchment work, SEPA identified a low level of regulatory compliance on agricultural land (Field, 2013). To increase information to the sector about the Diffuse Pollution GBRs, regulators and industry bodies worked in partnership to develop the

Farming and Water Scotland guidance ‘Know the Rules’ and the tractor cab sticker ‘Mind the Gap’. These guides, published in 2014, aimed to help farmers and land managers keep on the right side of the regulations, reduce the diffuse pollution risk and benefit the farm business.

Diffuse pollution messages were routinely promoted at Scottish Government funded Veterinary and Advisory Service meetings, held by SAC Consulting and attended by SEPA (SRUC, 2016), but it was noted that this method would not reach all farmers.

In 2015 the Scottish Government and DPMAG stakeholders identified a need to take Farming and Water Scotland initiative out into the rural community in a way that would promote and encourage behaviour change (Scottish Government, 2010).

Under the umbrella of Farming and Water Scotland (SAC, 2016) the Partnership between SRUC and SEPA began a 3 year campaign to attend 30 – 50 agricultural shows and events each year. The project was co-managed by SRUC and SEPA. The events had to enable engagement with land managers, be geographically spread, provide a way to contact the ‘hard to reach’ audience and cover a range of diffuse pollution topics. Alongside the events calendar, the partnership has managed a website and social media campaign to maximise promotion of the initiative to the target audience.

MATERIALS AND METHODS

A number of agricultural shows and events were visited to review the kind of equipment used at agricultural shows.

After discussion with the SRUC Events team and other traders at agricultural shows, a professional ‘gazebo’ type shelter was purchased, along with a range of information boards and demonstration kit. A small transit-type van was leased for the duration of the project and this was liveried in the project colours showing the Farming and Water Scotland (FWS) logo, web and social media links. The van also supported an enlarged copy of the Mind the Gap sticker.

The agricultural events calendar was reviewed for 2016 and pitches at around 30 shows and events were booked. A second shelter and demonstration kit was purchased to allow coverage of more than one show per weekend.

Rotas were developed to provide adequate staff coverage for each show and support to set up the equipment the night before the show. Each show would have a stand manager and a member of staff from both SAC Consulting and SEPA in attendance at some stage in the day.

Knowledge transfer and exchange materials were developed around the main themes for promotion by FWS under the following headings:

- Nutrient management and application
- Soil health and management
- Alternative watering for livestock
- Cultivated land
- Pesticide applications
- Sheep dipping
- Ditching, dredging & river bank protection

Topics were promoted using a range of photo boards, demonstration equipment, videos, guest speakers and advisory staff, competitions, case studies, technical guidance notes and information on grant funding. Demonstration kit on the stand included various alternative watering systems, growing cover crops, a weed wiper and pesticide sprayer plus a variety of sprayer nozzles. Free branded merchandise was also available to promote the partnership.

RESULTS

In years one and two of the initiative, FWS covered around 80 agricultural shows and events speaking to over 3000 farmers and land managers in Scotland about diffuse pollution risks and mitigation measures.

DISCUSSION

Experienced SAC Consulting Agricultural Consultants and SEPA regulators were available on the stand to talk to land managers about farm scale diffuse pollution risk, give technical guidance and highlight the added business benefits that have been gained from using pollution reduction methods, drawing from both their own and other farmers' experiences. Interactions with land managers were positive and productive. Hosting local SAC Consulting staff on the stand often provided a familiar face to some of the audience which was a good way to initiate dialogue.

The range of demonstration equipment further encouraged dialogue and discussion. The demonstration equipment was tailored to the events and predominant farm type in each geographical area, making the stand topical and engaging for the anticipated audience. The initiative provided relevant information and experiences, technical knowledge and a neutral 'space' to discuss the protection of water quality within the rural community.

The topics covered at shows varied greatly, as questions from land managers and the general public could be difficult to predict. Examples included: initial awareness of diffuse pollution risks; local water quality concerns; septic tanks; nutrient management; constructed farm wetlands; soils health; pesticide training courses; alternative livestock watering options; fencing water margins; woodland grazing; providing grass buffer strips and funding opportunities.

After the first year of the shows, the figures revealed that whilst some large shows were providing useful engagement, FWS reached a much larger percentage of the audience at smaller shows.

For some of the audience at the smaller shows, especially those out with Priority Catchments, the message was 'new' to the audience. Where the event was in a Priority Catchment area, it was found that engagement included more farm specific, developed guidance. The availability of knowledgeable and technical staff from both SRUC and SEPA made this possible for FWS to deliver useful and practical advice to land managers with a local context.

There were a number of unexpected outcomes from the shows programme that hadn't been anticipated at the start of the project. Once the shows programme was underway, it quickly became apparent that there were a number of networking opportunities with trade stands. Sales staff are often quick to spot an opportunity to gain an edge over their competitors and are keen to make sure that they design and produce equipment that can help farmers to comply with the rules. Making sales staff aware of the diffuse pollution GBRs also means that they can pass this information on to their clients.

Enabling staff from SAC Consulting and SEPA to work together on the stand fostered an understanding of the roles of each organisation and an introduction to local staff contacts. It provided a platform for them to work together and to discuss diffuse pollution risks, regulation, compliance and funding in a positive, sector focused way.

Agricultural shows attract a wide audience including the general public as well as active land managers. Whilst Farming and Water Scotland's primary role is awareness raising within the agricultural sector, being able to show how Scotland's farmers have been addressing the challenges of diffuse pollution and water quality for public health and well-being was of interest to non-farming public and has added value to the project.

At the end of the three-year project there is expected to be a summary produced highlighting key achievements and the lessons learned from this approach.

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EVIDENCE FROM TARGETED PESTICIDE MONITORING TO IDENTIFY PRIORITIES UNDER THE WATER FRAMEWORK DIRECTIVE

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Summary: SEPA are adopting a tiered monitoring programme for some pesticides that uses different levels and types of standardised monitoring and analytical techniques not yet widely adopted by regulators. The aim of this programme is to enable us to target our resources to environmental concerns rather than standard compliance monitoring. We exemplify this approach using a survey of glyphosate in Scottish Rivers. We found low levels of glyphosate in the majority of samples taken indicating there is a very low risk of breaches of the Environmental Quality Standard in Scotland. Consequently, based on its current ecotoxicity assessment, there is a very low risk of environmental harm from glyphosate leaching into surface waters. Low cost screening methods such as ELISA can provide sufficiently nuanced data for assessing environmental risk in the real world.

INTRODUCTION

Pesticides are an important component in intensive agriculture systems. They are used over a wide area and due to their properties have the potential for causing unintended environmental impacts. The Water Framework Directive (WFD) aims to protect and enhance the water environment in Member States of the European Union. The WFD required the achievement of good ecological status in all waters by 2015. Good ecological status is quantified by comparing the biological community present with what might be expected with minimal anthropogenic impact. Good ecological status requires assessments relating to biodiversity, hydrological and chemical characteristics of a water body in relation to the "Reference Conditions".

Substances that pose a risk across the EU are identified as Priority Substances (PS) under the WFD. Specific Pollutants (SP) are substances that pose a risk to the water environment in only a limited number of Member States, generally from one to less than four, and are identified by individual member states. These chemicals are formally known as river basin specific pollutants (RBSPs). So far 33 PSs and 28 SPs have been identified. These include a range of industrial chemicals, plant protection products and the compounds of metals. About half of these are (or were) pesticides.

Good chemical status is defined as compliance with all of the quality standards established for substances at a European Level. This ensures a minimum chemical quality everywhere in the European Community. The WFD uses Environmental Quality Standards (EQS) to protect the environment. The EQS is based on ecotoxicology data so that when it is met there should be no environmental degradation from that chemical. For PSs the EQS values are listed in Directive 2008/105/EC. A water body will fail the good chemical status test when the EQS for any of these substances are exceeded. In the event of a failure, there is a requirement to reduce the quantities of the substance reaching the environment through a reduction in discharges, emissions and losses until good chemical status is achieved. A similar approach

is followed nationally for RBSPs. For PHSs, there is also a requirement to stop these substances reaching the environment through the cessation and/or phasing out of inputs.
In 2014 the UK designated Glyphosate as a specific pollutant under the Water Framework Directive (WFD) in part at least due to its high use. The Environmental Quality Standard (EQS) is 196 µg/l (annual average). This reflects the commonly held view of a relatively benign ecotoxicological profile. Glyphosate was under consideration as a potential candidate for the first watch list, an investigative monitoring programme under the auspices of the WFD. However, the monitoring data available led to its removal from the final watch list (Carvalho *et al.*, 2015).

Glyphosate has been used for more than 40 years and is the most widely used pesticide in the world. It is the active ingredient (a.i.) in Monsanto's Roundup® (Battaglin *et al.*, 2014). In the United Kingdom (UK), glyphosate is approved in over 400 products for use in the agricultural, amenity and home-garden sectors.

The work reported here was part of a targeted monitoring campaign aimed at establishing environmental concentrations in a number of Scottish rivers during periods of high use. The low cost Enzyme Linked Immunosorbent Assay (ELISA) method was chosen to allow SEPA to investigate how these novel techniques can be used to augment the traditional analytical tools in the regulator's analytical arsenal.

MATERIALS AND METHODS

All samples were collected in 50 ml plastic centrifuge tubes by SEPA's dedicated sampling team. The samples were collected from the main flow of the river ensuring river bed or floating material was not introduced into the sample. To prevent floating surface material entering the bottle, the sample bottles were filled with the mouth facing downstream and with the bottle mouth held just below the surface. Samples were collected upstream of the sampler. Once sampled, samples were stored in a chilled dark environment. They were either transported to the laboratory on the day of collection in chilled transport crates or stored overnight in a fridge before transport to the laboratory by courier. Once transferred to the laboratory, samples were stored in a freezer in the dark for up to one month prior to analysis. Figure 1 provide details of the locations sampled. Immediately prior to analysis the samples were removed from the freezer and allowed to defrost and reach room temperature.

Glyphosate ELISA analysis and derivatisation kits were purchased from Abraxis Inc. (Warminster, PA 18974, USA). The kits have been evaluated in comparison to more traditional analytical approaches (Rubio *et al.*, 2003; Byer *et al.*, 2008). Samples were firstly derivatised with the Abraxis Glyphosate derivatisation kit (Abraxis Inc. PN 500087) and then analysed using the Abraxis Glyphosate plate kit (Abraxis Inc. PN 500086). The final incubation with the colour solution was fixed at 30 minutes for all analyses after which the stopping solution was added to stop the reaction. The plates were read within 15 minutes of adding the stopping solution using a Multiskan FC microplate photometer fitted with a 450 nm filter (Thermo Scientific, Hemel Hempstead, UK). The plate was shaken for 5 seconds at a slow speed prior to the reading of the absorbance values for each well. The performance of the plate reader was confirmed using a Multiskan Verification plate (Thermo Scientific Hemel Hempstead, UK).

All standards and samples were analysed in duplicate wells and the average of the wells used in calculations. As recommended by the manufacturer, the %B/Bo was calculated by dividing the mean absorbance of each standard or sample by the mean absorbance of the zero standard. This was then used to produce a standard curve for the calibration standards using a log/logit curve. The concentrations of standards and samples were then calculated from the curve parameters. Any concentrations calculated as less than 0.05µg/l were reported as <0.05µg/l. Less than results were multiplied by 0.5 for use in calculations.

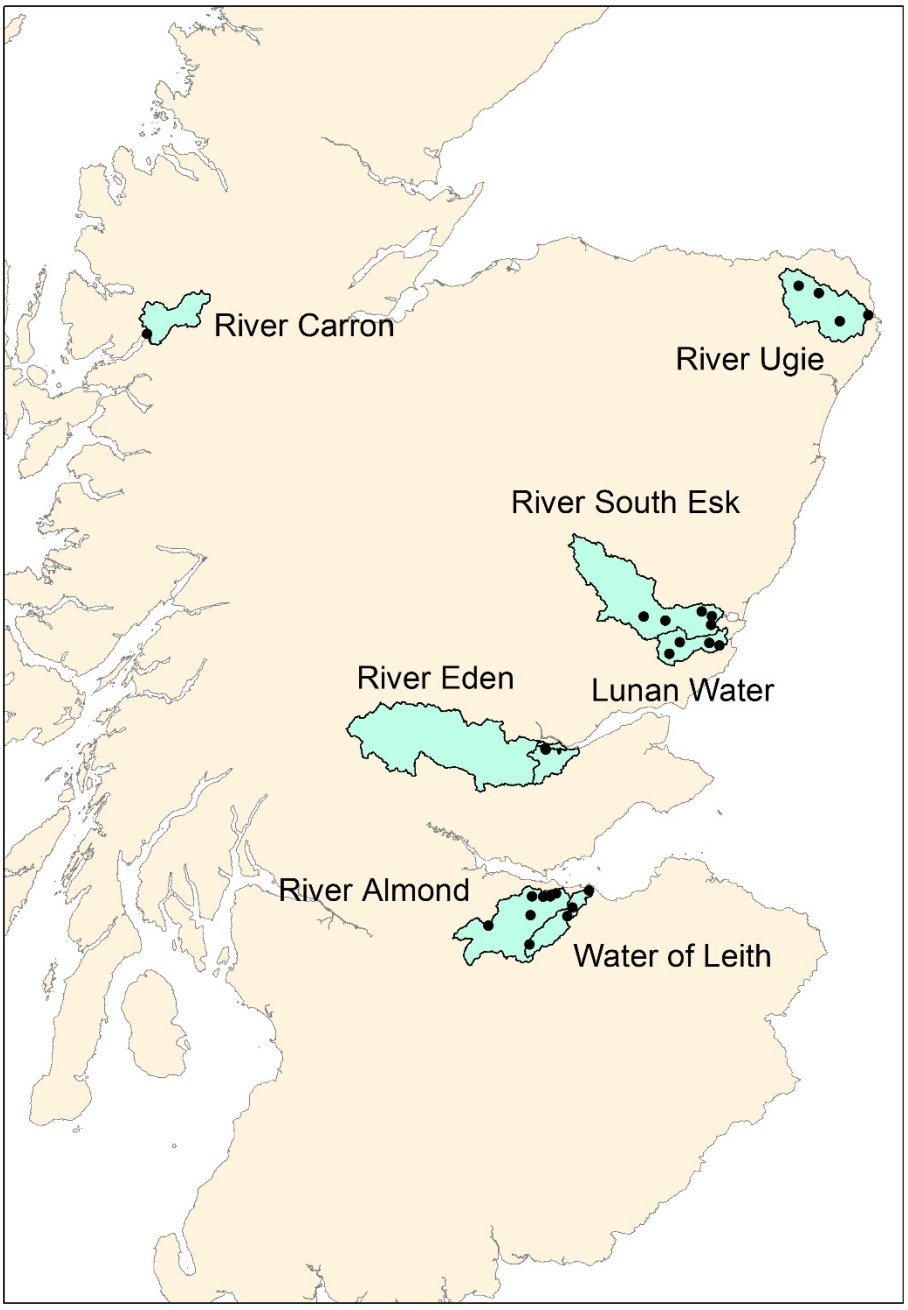

Figure 1. Catchments and monitoring points for analysing the concentrations of glyphosate in Scottish Rivers.

RESULTS

As can be seen more than 50 % of all of the results were above the limit of detection but less than 0.275 µg/l. About a quarter of all samples had a concentration below the limit of detection (0.025 µg/l in Figure 2). The results are similar to those reported for streams and rivers in the USA (Battaglin *et al.*, 2014) and elsewhere in Europe (Horth & Blackmore, 2009).

The results show that there is no significant risk of breaching the annual average EQS in any

of the sites monitored (Figure 2). The highest value observed was 3.3 µg/l from the North Ugie Water from a sample taken in September. This is less than 2 % of the annual average EQS which is 196 µg/l. The catchments monitored and the times of sampling include intensively farmed areas and the periods of maximum use in Scotland. We are therefore confident that we have made a reasonable assessment of the overall risks for EQS compliance.

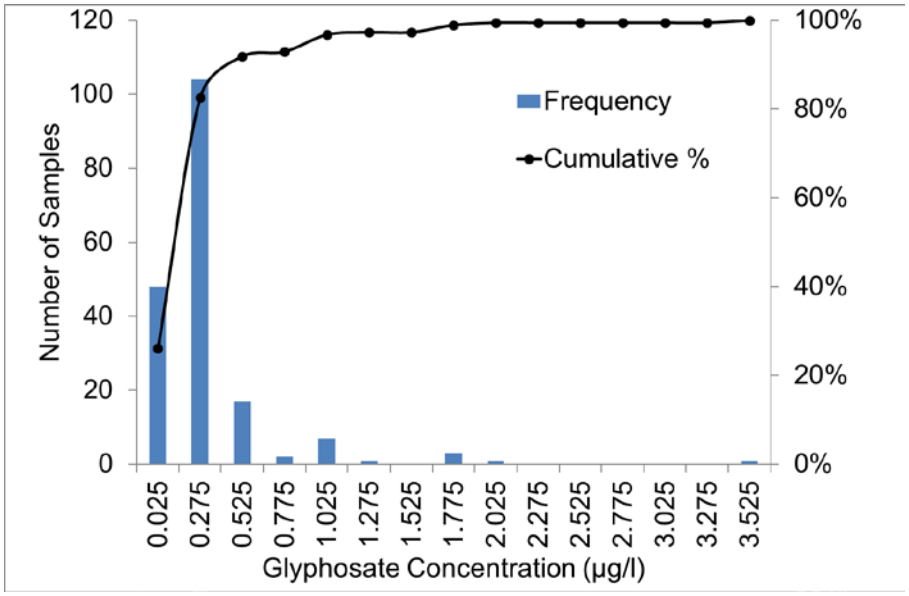

Figure 2. Frequency distribution showing the concentrations of glyphosate in Scottish Rivers.

In the Almond catchment there is a strong correlation (r-sq. 0.80) with % arable land cover and average glyphosate concentration (Figure 3). In other catchments, there is no relationship or as in the case of the Ugie catchment a weak negative correlation with arable land cover and average glyphosate concentrations (Figure 4).

DISCUSSION

We have found low levels of glyphosate in the majority of samples taken (75 % > LOD). The majority of samples had a concentration above the limit of detection but less than 0.5 µg/l. The EQS has been set at 196 µg/l as the annual average concentration. The risk of breaching the EQS is therefore remote based on the fact that we have monitored in catchments with a high arable land cover and also the peak use period for Scotland.

The relationship with arable land cover is complex as might be expected. The data shown for the Almond and Ugie catchments reveal that it is not the case that monitoring points that drain areas with higher arable land cover have correspondingly higher concentrations of glyphosate. Other factors affecting leaching from soils are also important including soil type, the timing of pesticide applications relative to rainfall patterns and when the sample was collected.

The speed with which we can deploy screening methods like ELISA kits together with their low cost is seen as a useful way of tackling emerging concerns where the risk to the environment is unknown. This allows the targeting of responses to areas of highest concern. Following the work reported here, we are confident that the risk to the environment has been adequately characterised. The cost to SEPA of developing, validating and accrediting a new analytical

method is in the region of £50,000 not including capital costs associated with instrumentation. This survey cost around 10 per cent of this initial cost of development. In the current financial climate this is seen as a significant saving and offers us a chance to better investigate these kind of issues as we can avoid the initial high start-up costs.

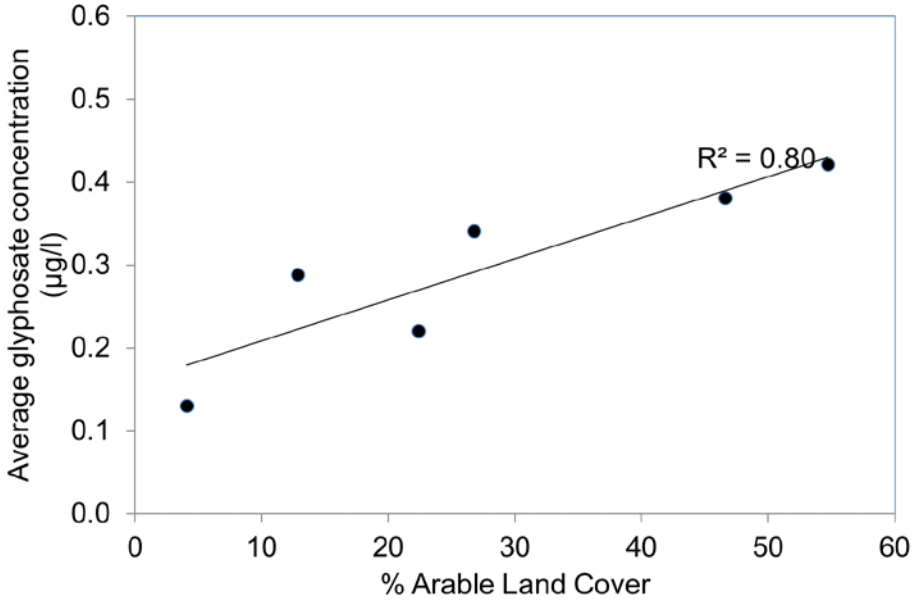

Figure 3. Correlation between percentage arable land cover and the average concentration of glyphosate in the River Almond catchment.

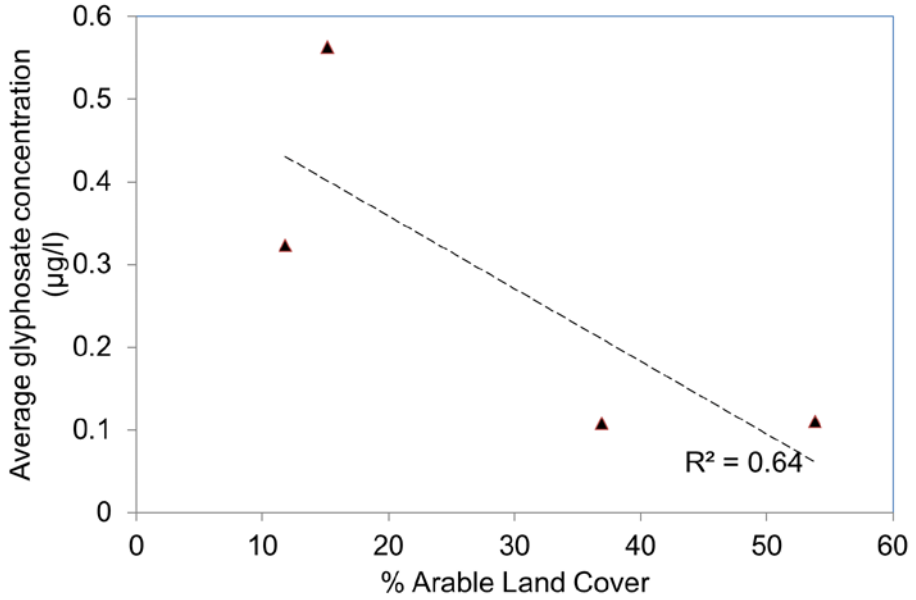

Figure 4. Correlation between percentage arable land cover and the average concentration of glyphosate in the River Ugie catchment.

In summary, low levels of glyphosate were found in the majority of samples taken with 75 % of samples being above the limit of detection. Despite the widespread occurrence in the rivers monitored there is a very low risk of EQS failures in Scotland. Consequently, based on its current ecotoxicity assessment, there is a very low risk of environmental harm from glyphosate

leaching into surface waters. The relationship between arable land cover and the observed arable glyphosate concentrations is complex and to some extent is catchment specific. Further analysis of these data is required to better understand the intra and inter catchment variation. Low cost methods such as ELISA can provide sufficiently nuanced data for environmental risk assessment.

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"THE BEES' NEEDS": USING MOLECULAR ANALYSIS OF BEE COLLECTED POLLEN TO UNDERSTAND WHICH PLANTS PLAY AN IMPORTANT ROLE IN HONEY BEE FORAGE

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Summary: Honey bees and other pollinators provide essential pollination services to agriculture and the environment; however they are under increasing pressure from changes in land management, disease and climate change. Current mitigation places emphasis on establishing flower meadows to improve nutritional diversity, but preserving what is already in place is also of importance. 'CSI Pollen' was a recent European citizen science project coordinated by COLOSS, investigating the diversity of pollen collected by honey bees in many countries across Europe. Volunteer beekeepers sampled pollen from colonies every three weeks during the foraging season over a two to three year period, creating a huge collection of data and samples. A selection of samples collected from 14 Scottish sites during the second year of study in 2015 were analysed by DNA fingerprinting to identify pollen gathered by honey bees at critical points of the colony's life cycle; some results and potential implications for land use are discussed here.

INTRODUCTION

Pollinator decline has been well documented in scientific and mainstream press (Potts *et al*, 2010). Factors affecting pollinator success include land management (urbanisation, habitat fragmentation and agricultural practices including pesticide use), disease and climate change (IPBES, 2016). Initiatives such as the Pollinator Strategy for Scotland (SNH, 2017) aim to address these factors but require high quality information to better inform decision making, improve advice and develop appropriate agri-environment incentives.

Current guidelines encourage establishing new forage sources for bees, such as flower meadows. Whilst nutritional diversity may be critical for pollinator health, our understanding of the specific nutritional needs of pollinators is still in development (Filipiak *et al*, 2017, Di Pasquale *et al*, 2013). Understanding and preserving the modern landscape features which are currently used by pollinators may also be an important aspect of supporting their health.
European honey bees (*Apis mellifera*) are social insects, living in colonies of approximately 20-40,000 individuals. A colony of honey bees, much like other pollinators, requires a balance of nectar and pollen to feed the various life stages of the colony. Honey bees use collective decision making to utilise the most profitable forage sources in the local environment (Seeley *et al*, 2009). By studying the identity of pollen balls collected from the legs of incoming foragers we can gain insight into these decisions and the nutritional availability within their local environment.

COLOSS is an international scientific research association which studies honey bee colony losses and works to improve the well-being of bees, particularly *A. mellifera*, at a global level

(http://www.coloss.org/). A citizen science project known as 'CSI Pollen' was devised and co-ordinated by members of COLOSS during 2014-16 to investigate the diversity of pollen available across Europe. Pollen was collected across the foraging season, sorted by colour, part-identified and results related to local land use. Results are still being analysed but this paper aims to highlight the potential of this and similar studies to better understand nutritional availability for honey bees and other pollinators in the modern environment.

MATERIALS AND METHODS

Scottish beekeepers were contacted through beekeeping literature and networks in early 2014 and suitable volunteers were recruited, with sites (apiaries) located across Scotland; three colonies from each were sampled. Hives were adapted to allow attachment of pollen traps to the entrance of colonies during sampling periods. When attached, these traps removed pollen balls from the legs of foraging bees as they returned to the colony. Sampling occurred every three weeks during the active foraging season; if a colony was not considered to be in a state to allow sampling to take place (for instance it had low food stores) or the beekeeper was unavailable, then the colony was not included in the sampling event. Excess pollen was removed from samples weighing over approximately 20g (a 'jar lid'); the remainder was dried and then stored at -20C.

Following analysis as part of CSI Pollen (Gray *et al*, in preparation), available samples collected from Scottish colonies during 2015 (Figure 1) and 2016 were sent to SASA. In total 389 samples were received at SASA (235 samples collected in 2015 and 134 in 2016).

Figure 1. Location of 14 Scottish apiaries sampled as part of CSI Pollen in 2015.

As limited resources were available for analysis, pollen was identified from selected samples from the largest dataset (2015) chosen to reflect important periods in the seasonality of honey bee colonies. April samples were used to investigate nutritional availability during colony build up and June samples tested as beekeepers often report a lack of forage availability during this month. Samples tested are highlighted in Table 1.

Table 1. 2015 samples collected – Y indicates sampling occurred; highlighted boxes indicate samples taken for analysis.

Location	April 1	April 2	May	June	June/ July	July	Aug 1	Aug 2	Sep
Orkney		Y	Y	Y	Y	Y			
Tain			Y	Y		Y	Y	Y	
Inverness		Y	Y	Y	Y	Y	Y	Y	
Banchory	Y	Y	Y	Y	Y	Y	Y		Y
Cairndow	Y	Y	Y	Y			Y		Y
Oban 1			Y	Y		Y		Y	
Oban 2				Y	Y	Y	Y		Y
Comrie	Y	Y		Y		Y		Y	Y
Dunblane	Y	Y	Y	Y		Y	Y	Y	Y
Edinburgh				Y		Y			
Peebles				Y	Y	Y	Y	Y	Y
Wemyss Bay	Y	Y	Y	Y	Y	Y	Y	Y	Y
Kilmarnock	Y		Y	Y	Y	Y	Y	Y	Y
Dumfries	Y	Y	Y	Y	Y	Y	Y	Y	

A sub-sample of 24 pollen balls was taken from each of the selected samples. Pollen was identified using DNA fingerprinting methods adapted from Fazekas *et al*, 2012 and Taberlet *et al* 1991(Reid *et al*, in preparation).

To determine the accuracy of molecular identifications, a further 96 pollen ball samples were split in two; one part analysed using molecular methods and the other identified by microscopy using methods described in Maurer, 2012.

RESULTS

Microscopic confirmation of molecular identifications

Microscopic analysis confirmed the molecular identification of pollen balls to genus or family in all but three samples (97% accuracy); although in 15 samples (16%) the pollen identified by molecular methods was not the predominant species present. An average of 98% of all the pollen contained within a pollen ball was from the predominant species.

April samples

24 pollen balls were selected from samples collected in April 2015 from each of the participating apiary sites and identified by molecular analysis (Table 2). Gorse (*Ulex europaeus*) was the predominant pollen collected from every apiary sampled; flowering cherry

(*Prunus spp*.) and willow (*Salix spp*.) were also commonly found at most sites (8/9 sites and 6/9 sites respectively).

Table 2. Identification of randomly selected pollen balls from samples collected in April 2015. Site locations listed by latitude from north to south.

Location	*Ulex sp.*	*Prunus. spp.*	*Salix spp.*	*Skimmia spp.*	OTHERS	TOTAL
Orkney	11	0	0	0	10	21
Inverness	8	13	0	0	3	24
Banchory	18	4	1	0	1	24
Comrie	17	7	0	0	0	24
Dunblane	8	8	7	0	0	23
Cairndow	11	5	3	1	4	24
Wemyss Bay	10	1	5	7	1	24
Kilmarnock	13	1	7	0	0	21
Dalry	6	5	5	3	3	22
TOTAL	102	44	28	11	22	207

June samples

Up to 24 pollen balls were selected from 14 colonies and each ball was identified by molecular analysis. Results are tabulated in Table 3. Tree pollens, predominantly Acer (*Acer spp.)* and Rowan (*Sorbus spp*.), were identified as the main source of pollen (33%); shrubs and hedgerow plants, predominantly Broom (*Cytisus sp.)* and Hawthorn (*Crataegus sp.)*, were present in 30% of pollen balls tested.

Table 3. Identification of selected pollen balls from samples collected in June 2015. Site locations listed by latitude from north to south.

	Acer spp.	*Cytisus sp.*	*Sorbus spp.*	*Crataegus sp.*	Other	TOTAL
Orkney	7	0	0	0	13	20
Tain	9	4	2	0	6	21
Inverness	1	6	2	2	5	16
Banchory	4	9	5	0	5	23
Oban 1	1	0	0	0	9	10
Oban 2	1	1	4	2	12	20
Comrie	0	1	2	1	12	16
Dunblane	4	2	2	2	8	18
Cairndow	4	2	4	5	9	24
Wemyss Bay	0	0	0	1	9	10
Edinburgh	0	0	0	2	13	15
Kilmarnock	6	0	3	5	3	17
Peebles	2	3	3	2	7	17
Dalry	0	3	0	4	11	18
TOTAL	39	31	27	26	122	245

Pollen diversity

Only 13 plant species were identified from the 9 sites during April sampling. Gorse was identified in almost half of the 207 samples analysed. Although not directly comparable, June samples were more diverse, with 32 species identified from 14 sites and the predominant species (Acer) making up just 16% of the samples analysed.

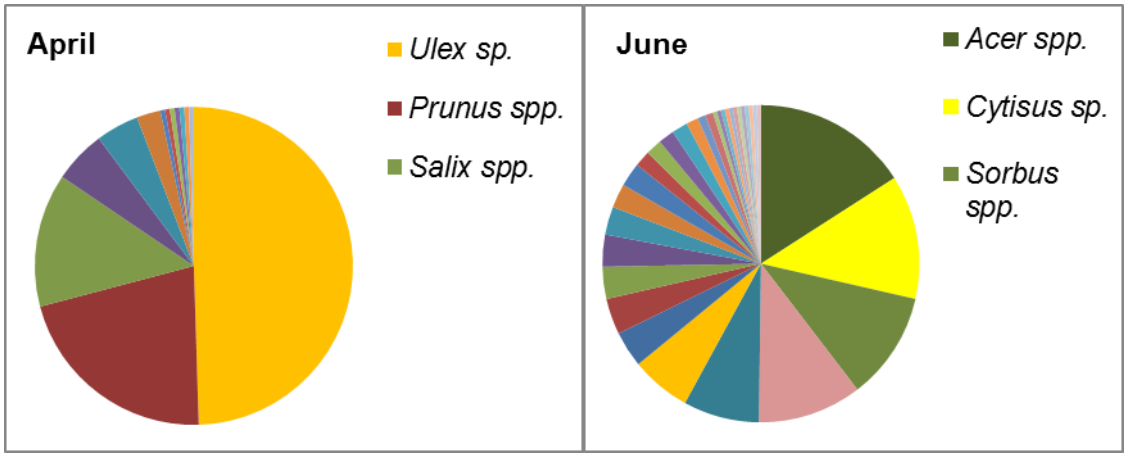

Figure 3. Identification of pollen balls collected from Scottish apiaries during April 2015 (n=207) and June 2015 (n=245).

DISCUSSION

This cursory glance into the full set of samples collected already gives us some insight into the foraging choices made by honey bees in Scotland. This may indicate nutritional choices made by the bees or simply what is currently available in the local environment.

Pollen identified from April samples indicated a strong preference for gorse, a shrub freely available across the Scottish landscape in spring which can provide a good nutritional source (Filipiak *et al*, 2017) and may be resilient in the face of climate change due to its extended flowering season. Current agricultural policy encourages the removal of dense gorse coverage to prevent incursion into grazing areas, however this plant may play an important role in pollinator nutrition in Scotland.

During both sampling periods, trees, shrubs and hedgerow plants made up a large amount of the pollens identified within samples. These plants provide large volumes of pollen and nectar but may be under-recorded in the type of flower visitation studies generally used to ascertain pollinator foraging choices (Fowler *et al*, 2016). Trees and shrubs may again provide some nutritional resilience during periods of heavy rainfall or drought and may be of considerable importance to honey bees and other pollinators.

Although plants providing large volumes of pollen and nectar were common in the pollen analysed and only a small part of the sample was analysed, it is important to note that no sample tested was homologous. Even when a single nearby plantation or crop could provide the volume of food required by a honey bee colony, they seek nutritional diversity on a daily basis.

A depth of understanding of specific nutritional requirements for honey bees and native pollinators is required to fully inform environmental improvements for pollinators. However,

even this brief glimpse into the foraging behaviour of honey bees highlights the importance of maintaining trees and shrubs as well as improving nutritional diversity. Land managers are faced with many conflicting priorities but preserving what is already present in our natural environment may be a first step to land management with pollinators in mind.

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THE EXPLOITATION OF LOW RABBIT NUMBERS TO CONTROL DAMAGE IN THE LONG TERM

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Summary: Rabbit numbers have decreased by over 95% in Scotland in the last 10 years. The decrease has probably boosted farm incomes by £millions by increasing yields and lowering the costs of implementing control measures. However experience gained after the introduction of myxomatosis in the 1950s suggests that rabbit numbers may again rise to damaging proportions with resulting loss of revenue. A similar reduction in rabbit populations to that which was caused by myxomatosis has now occurred due to a new disease, Rabbit Haemorrhagic Disease (RHDV) introduced in Britain in 1994 and Rabbit Haemorrhagic Disease Virus variant 2 (RHDV2) which was first recorded in Scotland in 2014. It would be expeditious for farmers to continue controlling even low numbers of rabbits so that their populations cannot again rise to damaging levels.

INTRODUCTION and BACKGROUND

The wild rabbit (*Oryctolagus cuniculus*) has, in the past been considered the major agricultural pest in Great Britain costing the industry £100m a year (Meikle, 2010, Pimenntel *et a*l., 2001). In 1954 rabbit numbers were reduced when the viral disease myxomatosis decreased their numbers by an estimated 99% (Thompson & Worden, 1956) and farmers reaped the benefits of increased yields and the elimination of the costs of controlling the rabbits mainly by gassing and fencing the crops. However the control effort lapsed and rabbit numbers increased (Trout *et al*., 1986; Kolb 1994). Fenner in 1953 observed that some rabbits were surviving myxomatosis due to the virus becoming less virulent (Fenner & Marshall, 1957) and the rabbits developing resistance to the disease (Marshall & Fenner 1958). By 1992 it was estimated that rabbit populations had risen to a third of that seen before myxomatosis occurred (Flowedew *et al*., 1992) and by 1997 rabbit numbers in Scotland were estimated to be 9.5 million (Battersby, 2005). By 2010 myxomatosis was still sporadically occurring in wild populations in Scotland but mortality was extremely low (Boag *et al*., 2013).

In 1994 a new virus disease Rabbit Haemorrhagic Disease Virus (RHDV) was recorded in wild rabbits in Britain which was probably responsible for the 97% reduction rabbit numbers in marginal land in Scotland between 1995 and 2009 (Aebischer *et al*. in 2012). The existence of a non-pathogenic strain had possibly mitigated the impact of RHDV by imparting some immunity to some rabbit populations (Forrester *et al*., 2009). However another more pathogenic strain of RHDV named Rabbit Haemorrhagic Disease Virus variant 2 (RHDV2) was recorded in Scotland in 2014 (Bailly *et al*., 2014). This is probably now responsible for rabbits in some areas decreasing even further to extremely low levels (below 95% of their levels found in 2009 (Boag *et al.,* 2016).

The purpose of this paper is to review control measures and how they have changed over the years and try and outline those which could be used to maintain low rabbit numbers on agricultural land in the future.

CONTROL

Control methods have changed over the years and the most appropriate depends upon individual situations i.e. topography, vegetation, time of year and density of rabbits.

Snaring

In the past snaring was widely used to control rabbits in Scotland but the Wildlife and Natural Environment Act Scotland 2011 (WANE act 2011) introduced from 1st April 2013 much stricter regulations which included all snares having an identification number, a stop on the snare at 13 cm etc. All the operators using snares had to attend a course before operating them. These regulations are so strict that from personal experience some gamekeepers and farmers have given up snaring all together.

Gassing

Gassing is still permitted but strict health and safety regulations now apply (HSE, 2012). All operators need to have had training so usually only contractors do this work and not farmers which used to be the case. Gassing is considered an effective means of controlling rabbits reducing populations by 80% (DEFRA, 2010). The only commercially available are fumigant formulations that produce phosphine gas on contact with moisture but carbon monoxide is also used in some cases and a mixture of propane and oxygen ignited electronically is an option used in the USA against Gophers. Some rabbit populations e.g. those in sand dunes may never use burrows so gassing is of little use where this occurs (Kolb, 1991).

Fencing

Wire netting is still widely used but initially expensive especially if used to encompass whole fields to protect the crops. An investigation into a range of wire netting designs was found to be approximately 80% efficient over a 6 year period (McKillop *et al*., 1986). However when populations of rabbits are low they tend to restrict themselves to fewer warrens where it may be more cost effective fence round these areas rather than protect the crop. After myxomatosis tree guards used to protect individual saplings were not required but by the 1990s they were commonly used throughout the country.

Electric fencing

Initially portable electric fencing costs less than traditional wire fencing but there are continuing running costs which are greater than with wire fencing for example more frequent inspection of the fencing is necessary and more herbicide is needed to keep vegetation down below the fence. Efficiency was similar to that of traditional wire fencing when costed over a 6 year period (McKillop *et al*., 1986). The advice given as to what type of fence should be used depended upon the individual situation pertaining at the time.

Traps

There are two types of traps used for rabbits. Cage traps when baited will catch wild rabbits which can be humanely dispatched and no-target animals released unharmed (Shephard *et al*., 1978).

Drop traps, sometimes referred to as rabbit boxes, installed in an effective fence situated between a rabbit warren and a crop can be extremely efficient with multiple rabbit captures being quite common. It has also the advantage that non-target animals and birds e.g. hedgehogs and pheasants can be released unharmed. The drawback is that, for humanitarian reasons, all traps must be inspected within a 24 hour period.

Ferreting

This form of control has been found to be effective but is labour intensive. Anecdotal evidence suggests that although it is used throughout the country little information is available to indicate just how often it is used and its impact on rabbit populations.

Shooting

When densities of rabbits are high then shooting can be generally ineffective even if lamping at night and expensive if a shot gun is used. Most often rabbits are shot with a .22 rifle with a silencer is but even at night rabbits can become lamp shy if repeatedly hunted. Hunting efficiency with an experienced marksman was found, using this method, to be 79% (Hampton et al., 2015).The use of thermal imaging sights, which are expensive, could overcome this drawback.

Warren ripping

This procedure is used but uncommon in Britain and entails collapsing burrows using a plough or JCB digger and is very effective in destroying warrens and hindering recolonization (McPhee & Butler, 2010). It is probably only justified when rabbit numbers are high (Mutze, 1991). In Britain for humanitarian reasons warren ripping must be preceded by gassing which is an added expense.

Repellents

Effective repellents against rabbits have been developed (Morgan & Woodhouse, 1995; Boag & Mlotkiewicz, 1994) but never used to control rabbits on farms. Their use is therefore limited to gardens and horticultural establishments.

Changes in agricultural practices and land use

Rabbit densities are related to their habitat and major contributors are soil type, aspect, cover and access to a plentiful food supply (Lombardi et al., 2003).To stop the recolonisation of rabbit prone areas experience has shown that the removal of refugia and reducing the cover used by rabbits e.g. gorse significantly reduced the number of active warrens and rabbit numbers (Boag, 1987). Changes in agricultural practices e.g. changing from extensive to intensive agricultural systems (increasing stocking rate and replacing heather by grassland) also significantly contributed to the long term reduction in rabbit populations. (Boag, 1987).

DISCUSSION and CONCLUSION

A delicate balance is required between reducing rabbit populations for economic reasons and maintaining rabbits in sufficient large numbers since they are considered a “multifunctional keystone species” (Boag *et al*., 2017). They are major source of food for a number of our iconic mammal e.g. fox (*Vulpes vulpes*) and bird species e.g. buzzards (*Buteo buteo*) as well as maintaining a unique type of vegetation favoured by some plants and rarer invertebrates e.g. Adonis blue butterfly (*Polyommatus bellagus*) (O’ Connor *et al*., 2014) . This subjective

balance will vary depending upon where priorities lie but farmers have lost £millions to rabbits in the past and now are benefiting from low numbers brought about by the introduction of RHDV2. They do not want to see a resurgence of rabbit numbers similar to that which occurred after myxomatosis curtailed rabbit populations in the early 1950s. If resistance to RHDV2 occurs similar to that which happened after myxomatosis by *circa*. 2040 farmers may again be faced with a multi-million bill to control rabbits. It would therefore be prudent to take advantage of the low numbers which exist at present and, with relatively little expense, continue to keep rabbit populations low on agricultural land. Restrictions on the use of both gassing and snaring probably mean that these control measures are less likely to be used by farmers than in the past. Warren ripping is only warranted when populations are high and repellents not effective for long periods of time. Although difficult to predict rabbit numbers are likely to be controlled in the future by using fencing, trapping and shooting. The choice of fencing will depend upon each individual case e.g. if the crop is transient then electric fencing would be that chosen. Traps (both cage and drop) have the advantage that they are humane while shooting is also considered a humane form of control if carried out properly.

Probably the best long term solution to stop or at least slow the resurgence of rabbit populations is to deny rabbits the environment where they multiply i.e. wild areas on farms where rabbits can set up warrens close to an abundant supply of food. If these areas are relatively small then it may mean that farmer's most economically option would be to fence off these areas rather than fencing around crops while the rabbit populations are low.

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IDENTIFYING HOW TO IMPROVE KNOWLEDGE TRANSFER RELATING TO INTEGRATED PEST MANAGEMENT (IPM) ON IRISH ARABLE FARMS

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Summary: The combination of the EU directive on the sustainable use of pesticides (Directive 2009/128/EC), the decreasing availability of pesticides through increasingly stringent regulations and the development of pesticide resistance in pests have all increased the need for integrated pest management (IPM) at farm level. The success of such practices and/or means of improving them are dependent on understanding current grower perceptions, as well as, adoption and sources of pest management advice and information. To achieve this a detailed survey of Irish arable growers focusing on pest management (weeds, diseases, insects & molluscs) perception and practice was conducted in spring 2017. This work will also focus on the next steps required for knowledge transfer (KT) agents (and industry actors) to achieve improved awareness and adoption of IPM on Irish arable farms.

INTRODUCTION

Integrated Pest Management (IPM) is the use of an optimal mix of pest (weeds, diseases, insects & molluscs) control techniques and tools, taking into account factors including profit, risk, sustainability, humans and environmental safety. This study aims to identify how to improve Knowledge Transfer (KT) of IPM to arable farmers. The availability of information has a significant role to play in IPM awareness and implementation at farm-level. This is due to the knowledge intensive and complex nature of IPM strategies. IPM is not a single adoption of an innovation but rather the integration of a number of existing and novel pest management practices in the first instance including cultural, physical and biological with chemical solutions considered as a last resort. A baseline understanding of grower's current perceptions, knowledge, attitudes and practices implemented relative to IPM were assessed.

MATERIALS AND METHODS

Farmer Survey

A survey instrument developed as part of a wider study on Establishing a Platform for Integrated Pest Management in Irish Crops (EPIC) was used to gather information on arable farmers relating to IPM perceptions and practice. The survey was a non-probability sample of arable farmers in Ireland and was distributed using a mixed mode methodology which included a paper and an online version. The paper version of the survey was distributed at a number of arable related events and meetings which took place during spring 2017 in Ireland. The online version was promoted through social media, websites and the farming press.

Best Practice Score

The best practice score is being developed as a means by which to quantify best arable farming practice relative to IPM in collaboration with the UK and a number of European countries as part of the wider EPIC study. Survey questions focusing on practice were selected to quantify IPM best practice. These questions were associated with:

- Rotation
- Influences on variety selection
- Preventative measures relating to the control of weeds, diseases, insects & molluscs
- Pest management planning
- Membership of an agronomy/crop discussion group

Initial weightings for this score were developed at a stakeholder workshop held in Ireland in June 2017. Stakeholders were asked to score each survey question (and options within questions) relating to IPM practice. By creating an IPM scoring system, scores can be applied to responses and each respondent can be rated in terms of their IPM activity. At the workshop, agronomic and IPM issues relating to key survey questions were discussed in detail to capture the contribution (%) of each question to the overall IPM score. Separate scores for options within questions were applied on a 1 to 5 scale (from 1 being not at all important to IPM to 5 being very important). Final scores were determined based on discussion within the group and a vote was taken if a clear consensus was not reached. Survey respondent's selection of options to each of the questions determines their overall IPM score (0-100%).

Survey Analysis

All survey responses were collated, coded and inputted into a statistical package for social scientists (SPSS) computer programme for analysis. Respondents who did not declare any arable land in the survey were removed from the sample. Responses were analysed for correlations between variables using Pearson's correlation and the relationship between certain variables was tested for significance.

Farmer Categorisation

The IPM Best Practice Score weightings developed at the stakeholder workshop were applied to the survey data in order to categorise growers based on their current adoption of practices. Based on the outcomes of the survey as well as the IPM Best Practice Score, growers will be identified and selected for further investigation through semi-structured interviews.

Semi-structured Interviews

Semi-structured interviews will be carried out with growers on a one-to-one basis to assess the effectiveness of the current KT chain for disseminating IPM best practice messages. This will include investigating information sources and KT supports used for pest management. The interviews will also delve deeper into grower's pest management practices, knowledge of IPM, motivations, attitude towards IPM, relationship with agronomist/crop protection advisor and barriers to IPM adoption.

INITIAL RESULTS

Farmer Survey Results

227 farmers with arable land completed the survey. 187 respondents self-reported that they were specialist arable farmers. The average arable area of respondents was 124 ha. 33% of respondents were <40 years old whilst 67% were >40 years old.

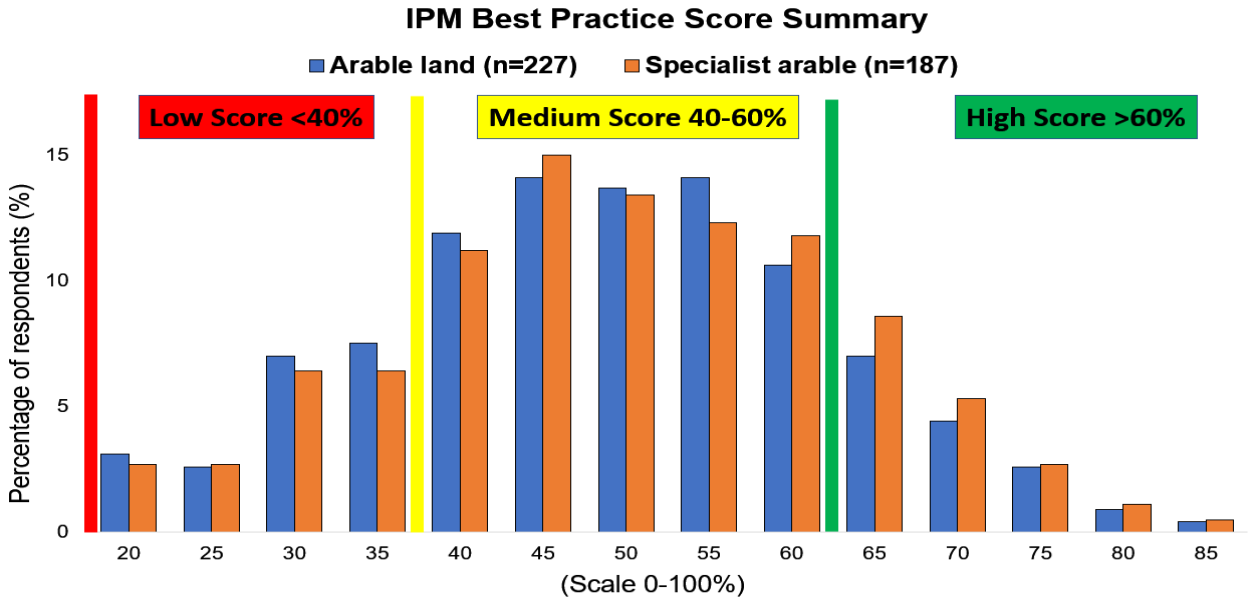

Figure 1. Best Practice IPM score (0-100%) summary for growers who declared arable land and those who self-reported themselves as specialist arable. Three groups: Low, Medium and High.

Self-reported specialist arable farmers

There was a total of 187 self-reported specialist arable farmer respondents. Low group (n=55), medium group (n=98), high group (n=34). 98% of respondents use a crop protection advisor which highlights the important role of the advisor in pest management decision making. Farmers in the high category are generally utilising more on farm information sources. 88% are using crop walking data from the previous season to assess the performance of various control measures. This compares with 63% in the medium group and 33% in the low group. Farmers in the high category also appear to be more likely to use a pest management plan. 97% are using one compared with 87% in the medium group and 62% in the low. Generally, farmers in the high category had a lower proportion of their land in continuous cereal production. Only 6% of farmers in the high group had 75-100% of their land in continuous cereal production compared with 20% in the medium group and 40% in the low group. Farmers in the high category were also more likely to be members of a discussion group. 82% were members of a discussion group compared to 76% of farmers in the medium group and 67% in the low group.

Relationship Between Variables

SPSS was used to identify the relationship between variables and determine if these relationships were significant. The analysis of respondents with arable land (n=227) indicated that there was a significant relationship between attitude towards IPM and the IPM Best Practice Score. This means that respondents who had a more positive attitude towards IPM scored higher. Self-reported familiarity of IPM was also significantly related to the IPM Best

Practice Score meaning that respondents who regarded themselves as being more familiar with IPM scored higher. Education and arable farm size were significantly related to self-reported familiarity of IPM meaning those with a higher level of education and a larger arable area regarded themselves as being more familiar with IPM. However, age, education and arable farm size showed no significant relationship with the IPM Best Practice Score. These relationships are outlined in Figure 2.

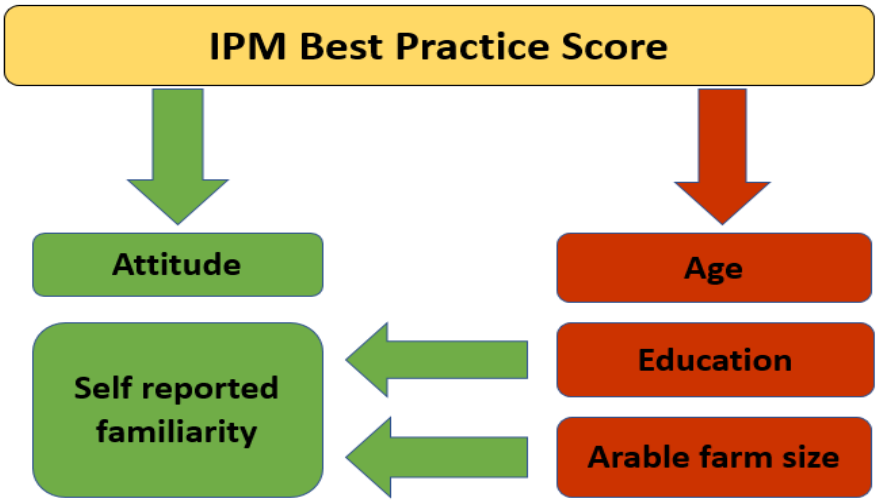

Figure 2. Significant relationships between variables for survey respondents (n=227). Green = significant relationship. Red = no relationship.

CONCLUSIONS & RECOMMENDATIONS

The results show that farmers are adopting IPM practices but there are differences in the rate of adoption. Initial results indicate that increased adoption of IPM based approaches is significantly related to farmer's attitude towards IPM and their self-reported familiarity of IPM. Further investigation, through semi-structured interviews, is required to assess the effectiveness of the current KT chain for dissemination of IPM best practice messages to growers. There is a need to better understand these differences in IPM practice adoption, and the role of KT within that amongst self-reported specialist arable farmers. The data suggests that the advisor's role in crop protection is very important. However, further investigation is required to assess this role along with farmers level of IPM knowledge. There is a need to identify the key actors involved in transferring information to growers particularly amongst the identified groups. Knowledge transfer is defined as being a unidirectional process which does not cope well with complexities (Manning, 2013). On the other-hand knowledge exchange or knowledge networks are multidirectional exchanges enabling the generation of knowledge and local solutions within a supportive social environment (Ingram, 2010). There is a need to empower farmers with the ability to utilise on farm information sources such as crop walking data and records for pest management purposes. The social environment the farmer operates in needs to support this approach which will enable them to take the initiative themselves to gather and use information that is specific to their farm and their locality as a basis for evidence based decision making.

ACKNOWLEDGEMENTS

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IPM TOOLS FOR PEST AND DISEASE MANAGEMENT IN RASPBERRY PLANTATIONS

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Summary: Pests and diseases in cane fruit plantations are estimated to cause yield losses of 5-20% annually and these losses could be exacerbated as pesticides are withdrawn before suitable alternatives are identified. To address this in raspberry (*Rubus idaeus*), research at the James Hutton Institute has generated and characterised germplasm and developed a suite of tools for monitoring and controlling the major pest and disease organisms in raspberry plantations. These crop management tools can be combined with germplasm expressing agronomic traits that confer resistance, along with habitat design to promote pest and disease suppression, to devise successful IPM approaches. The aim of our research is to provide novel methods that enable growers to produce soft fruit using IPM with fewer chemical inputs.

INTRODUCTION

Pests and diseases in cane fruit plantations are estimated to cause yield losses of 5-20% annually and these losses could be exacerbated as pesticides are withdrawn before suitable alternatives are identified. Raspberry pest and disease pressures are changing due to increased protected cropping and milder winter conditions, creating a need for year-round IPM. The pressure on growers to produce soft fruit with reduced chemical inputs has created a need for novel methods of pest and disease control in plantations. Successful approaches may require a combination of germplasm with agronomic traits that confer resistance, crop management tools for monitoring and controlling pests and diseases, and habitat design to promote pest and disease suppression. To address this in raspberry (*Rubus idaeus*), research at the James Hutton Institute focussed on understanding the biology of pest and disease organisms that are most destructive to raspberry plantations and developing tools for controlling the damage they cause.

METHODS AND RESULTS

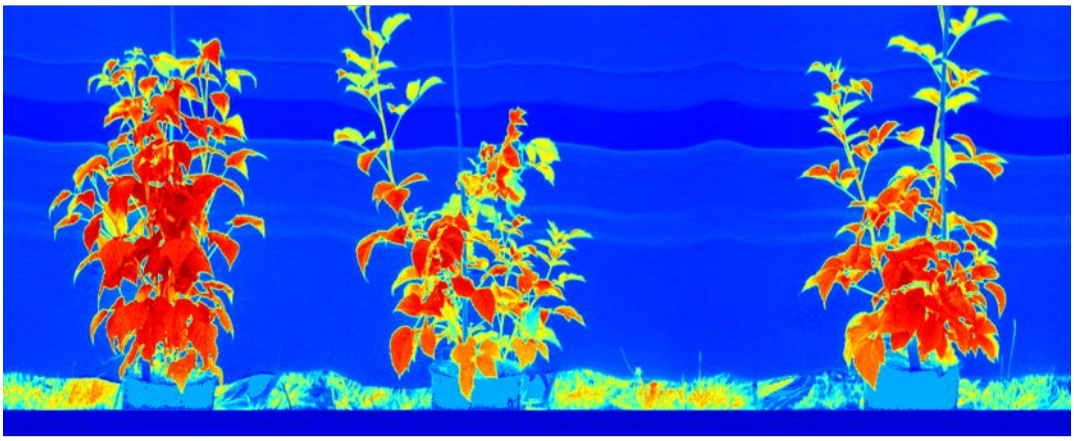
Research at the James Hutton Institute has focussed on a number of key areas for developing IPM tools:

Rubus genetics

Exploiting genetic variation in the ability of plants to resist or tolerate attack by insect herbivores and disease infection has long been a focus of crop breeding programmes, although the genetic basis for these traits is often poorly-understood. Technological advances

in large-scale plant genotyping, complemented by studies to phenotype plant-insect or plant-pathogen interactions, are now providing vital information about the genetic control of plant traits that regulate susceptibility (reviewed in Mitchell *et al*., 2016). Our recent research has identified genetic markers linked to shoot and root anatomical traits (Graham *et al*., 2014) thought to confer resistance to, or tolerance of, root disease (root rot, *Phytophthora rubi*), root-feeding pests (vine weevil, *Otiorhynchus sulcatus*) and shoot pests (spider mite, *Tetranychus urticae*; and raspberry aphid, *Amphorophora idaeus*;) (Karley *et al.,* 2015). Current work focusses on developing high throughput imaging methods to improve the efficiency of large-scale plant phenotyping for pest and disease resistance (Figure 1; Williams *et al*., 2017).

True colour image generated from VNIR camera



NDVI map generated from VNIR camera

Figure 1. Image collected from raspberry plants using a hyperspectral camera to generate spectral data across a range of wavelengths that can provide information on plant traits and responses to biotic stresses.

Raspberry breeding

The raspberry breeding programme was established in the 1950s and today the Raspberry Breeding Consortium comprises 20 industry partners, Scottish Government and the Agriculture and Horticulture Development Board (AHDB). The breeding programme selects floricane and primocane types for the fresh and processing industries, aiming to produce productive cultivars with high fruit quality that are suitable for low input production. Marker-

assisted breeding has become a crucial tool to identify important traits rapidly, such as *Phytophthora* resistance, fruit shelf-life and fruit size, with recent emphasis on the latter trait to reduce labour costs.

Pest and disease biology

Complementary research focusses on understanding the biology of arthropod pests as disease vectors, particularly emerging pests and diseases. This includes development of molecular tools to investigate the role of leaf and bud mite (*Phyllocoptes gracilis*) in transmission of the newly emerging Raspberry Leaf Blotch Virus and to identify raspberry genotypes that are less susceptible to the disease. Another recent invasive pest of soft and stone fruit is Spotted Wing Drosophila (*D. suzukii*). The James Hutton Institute leads the monitoring programme for this pest in Scotland and has been assessing seasonal and annual trends in pest abundance at a number of sites.

Raspberry plant health

The institute uses a range of biological and molecular methods to test pre-basic stock plants every year for approximately 30 viruses and two oomycetes, and is the sole source in the UK of pathogen-free planting material for entry in the UK Plant Health Certification Scheme, adhering to Scottish, UK and EPPO guidelines. This resource provides fruit producers and propagators with planting material of a known health standard and purity, and thus prevents the spread of pests and diseases.

Lure-enhanced traps

Pest traps can aid regulation of pest populations as tools to monitor and/or kill insect pests. Previous research has identified volatile plant chemicals that have been used successfully in lure-and-kill traps for raspberry beetle (*Byturus tomentosus*). More recently, plant volatiles have been identified that have potential as vine weevil attractants or deterrents, and their efficacy is currently being verified.

Biocontrol organisms

An effective tool for pest regulation is through management options that promote the abundance of natural enemies. One approach involves inundation of pest populations by releasing high abundances of natural enemies (e.g. parasitoid wasps) in optimised combinations, which has been used successfully for control of aphid infestations on raspberry, in combination with compatible biopesticides.

Pest-suppressive landscapes

Another approach to optimise the efficacy of biocontrol organisms examines the interaction of pest and beneficial species with the crop and surrounding semi-natural vegetation. The aim is to understand how increasing the diversity of vegetation associated with crops can reduce the extent of crop damage by insect pests and promote pest predation. Identifying the processes underlying this phenomenon will allow ecological engineers to design pest-suppressive agricultural landscapes with improved pest biocontrol that can be applied to soft fruit plantations.

DISCUSSION

Raspberry pest and disease pressures are changing with the increased use of protected cropping, and growers have fewer conventional pesticides at their disposal. To date, research at JHI has generated and characterised crop germplasm expressing desirable traits, developed appropriate pest management tools for IPM in raspberry plantations and examined pest-suppressive habitat designs for robust pest and disease management. These tools can be combined and tested as IPM toolboxes that reduce reliance on pesticides with the aim of identifying sustainable methods for pest and disease control.

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CAN PLANT GROWTH-PROMOTING RHIZOBACTERIA BE USED TO CONTROL BACTERIAL PATHOGENS ON HORTICULTURAL PRODUCE?

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Summary: There are few control options for bacteria pathogens on horticultural produce. We have previously demonstrated successful control of phytopathogens on cabbage and red onion in field trials by manipulation of the plant host response with the application of elicitors. In a similar vein, inoculation of crops with plant growth promoting rhizobacteria (PGPR) can provide a protective role by stimulating the host response and out-compete pathogenic microbes. We are currently investigating a role for PGPR in reducing the incidence of food-borne pathogens on horticultural produce, and determining the mechanism of any protective effect. The work is being carried out for *Escherichia coli* O157:H7 and *Salmonella enterica* on lettuce, broccoli and tomato.

INTRODUCTION

Food-borne outbreaks of verocytotoxigenic *Escherichia coli* VTEC) and non-typhoidal *Salmonella enterica* are often linked to the consumption of fresh produce, such as lettuce and tomato. Alternative strategies reduce the risk of infection from ready to eat (RTE) horticultural products, include biocontrol agents. Plant growth promoting rhizobacteria (PGPR) naturally reside on plants and can provide protection from stress, such as drought and disease. They have the potential to competitively exclude or inhibit colonisation of pathogens on plants, by secretion of antimicrobial metabolites. Commercial PGPR are typically from the *Bacillus* genus or *Pseudomonas fluorescens* species and sold as 'Biological Fungicides'. While some are authorised in the UK & EU as plant protection products e.g. Companion® (Growth Products) Serenade ® (BASF), Proradix (Sourcon Padena), others are sold as plant growth supplements, predominantly in Asia e.g. Bio-Cure-B™ (Stanes), Blight-End™ (AgriNaturals), Sheathguard™ (Agriguard). We have previously shown that VTEC and salmonellae preferentially colonise plant roots, where a sub-population can invade plant tissue and become endophytic (K. M. Wright et al., 2013). We have also shown that the application of elicitors, which induce a host defence response, can reduce the incidence of plant pathogens and associated disease symptoms (Adu *et al.*, 2016). Together, this suggests that manipulation of the plant defence system may be extended to food-borne pathogens to control their numbers on vegetable crops, and reduce the risk of food-borne illness. Therefore, we tested whether application of commercially available PGPR treatments could reduce colonisation of edible crops, by *E. coli* O157:H7 strain Sakai (VTEC) and *S. enterica* serovar Senftenberg. There is little data on the impact of application of PGPRs to horticultural produce in Scotland, therefore, we established field trials to assess yield and disease impacts of PGPRs on lettuce and broccoli in East Scotland.

MATERIALS AND METHODS

Bacterial strains and culture media

Commercially available PGPR applications used in this study were: Serenade® (*Bacillus amyloliquefaciens* QST713) (Bayer CropScience, UK); Companion® (*B. amyloliquefaciens* GB03) (Growth Products, USA); Subtilex NG® (*B. amyloliquefaciens* MG1600) (Becker Underwood, USA); BioNutrients™ Soluble AG 8-1-9 (*B. amyloliquefaciens* GB03 + 1 other, *B. lichenformis, B. fumilis, B. subtilis,* and *Saccharomyces cerevisiae*) (Growth Products, USA). Bacterial isolates were: *Pseudomonas fluorescens* SBW25 (Jackson *et al*., 2005); *Ps. putida* JHI-5304 (this study); *Escherichia coli* O157:H7 Sakai Stx- (Hayashi *et al.*, 2001) and *Salmonella enterica* sv Senftenberg, nalidixic acid resistant mutant (this study). Commercial treatments consisting of a single bacterial isolate from Companion, Serenade and Subtilex were purified to single colonies and used for laboratory and glasshouse experiments. BioNutrients™ was used at the manufacturer recommended formulation of 2 g/L. *Bacillus* and *Pseudomonas* spp. were grown in lysogeny broth (LB) at 27 °C, 200 rpm for 16 hours. MSgg medium (Branda *et al*., 2004) was used to induce biofilm production in *Bacillus* spp. VTEC Sakai (Shiga-toxin negative) and *S. enterica* sv Senftenberg (Ssf) were grown in LB or rich defined MOPS (RD MOPS) (Neidhardt *et al.*, 1974), supplemented with 0.2% glucose, thiamine, and essential and non-essential amino acids. Antibiotics were included where necessary for plasmid maintenance or for selection: 50 μgml^{-1} kanamycin (Kan), 30 μgml^{-1} nalidixic acid (NA), 100 μgml^{-1} ampicillin (Amp).

In vitro competition assay

Competition assays were performed as described in (Decoin *et al.*, 2015) with modifications. PGPR were grown in LB overnight at 27 °C, 200 rpm. To test the role of biofilm production, *Bacillus* PGPR were sub-cultured from LB culture 1:100 into MSgg media, at 27°C. VTEC Sakai transformed with pUC19 plasmid was grown in LB + Amp overnight at 37 °C and sub-cultured 1:100 into RD MOPS + Amp at 18 °C or 37 °C, 200 rpm. The culture density of the PGPR and VTEC Sakai was adjusted to 0.4 (OD_{600nm}) in PBS, mixed 1:1 and 10 µl spotted onto LB or MSgg + 32µg/ml X-galactosidase (XGal) agar plates and incubated at 27°C. Each experiment was repeated at least three times, with single isolates as controls. Blue colonies indicated *E. coli* from lactose fermentation, while white colonies indicated PGPR.

Glasshouse competition assay

Lettuce (*Lactuca sativa* var. Little Gem) and tomato (*Solanum lycopersicum* var. Moneymaker) were grown in compost in individual pots in a glasshouse at 22 °C (16 h light, 8 h dark) with 130 –150 µmol m^2 s^{-1} light intensity and 40% humidity. PGPR were applied to lettuce or tomato seedlings at the transplant stage by soaking the seedling roots for five minutes in a bacterial suspension (GBO3 ≡ ~ 10^6 cfu/ml; JHI_5304 ≡ ~10^7 cfu/ml). A second dose of 5 ml PGPR suspension was applied to the base of the plant after seven days. The plants were challenged with 10^7 cfu/ml VTEC Sakai or Ssf 14 days after transplant, by soaking the pot bases in 1 litre bacterial suspension for 1 hour (day 0). Colonisation of VTEC Sakai on lettuce or Ssf on tomato was measured after seven days by viable count on selective media: MacConkey agar + Kan for VTEC Sakai or XLT-4 + NA for Ssf. Each condition had 6 replicate plants and the experiment repeated 3 times.

Experimental Field trials

Experimental field trials for lettuce (Little Gem) and broccoli (var. Parthenon) were established at commercial sites in Fife, Scotland. Two treatment trials were set up: treatments were applied immediately prior to transplant in a non-randomised plot design, or immediately post-transplant in a randomised plot design. In the pre-planting treatment, a modular tray of each seedling (176 lettuce; 345 broccoli) was soaked with 1.5 litre of treatment before transplant

and in the post-planting treatment, treatments were applied in seven replicate plots of 24 plants for broccoli and 40 plants for lettuce. Subsequent treatments were applied at the plant base (Table 1). Treatments were applied with a backpack sprayer (Hozelock, 5 litre) at: Companion, 4.62ml/l; BioNutrients™, 2.9g/l; *Ps. putida* JHI-5304, 1 x 10^6 cfu/ml. Plants were assessed visually for disease symptoms at the time of harvest and yield measurements of the cumulative weight of 10 plants per plot for lettuce, and individual head diameters of 20 plants per plot for broccoli.

Table 1. Experimental field trial treatments and schedule

Crop	**Timing**	**Treatment Trials**	
		post-planting	pre-planting
Broccoli (var Parthenon) (planted 16/06/17)	Treatment 1: day 0	300ml/plot	1.5 litre/tray
	Treatment 2: day 14	300ml/plot	3.5 litre/plot
	Treatment 3: day 35	300ml/plot	3.5 litre/plot
	Assessment: day 81	20 plants/plot	60 plants/plot
Lettuce (Little Gem) (planted 05/07/17)	Treatment 1: day 0	250ml/plot	1.5 litre/tray
	Treatment 2: day 15	400ml/plot	2 litre/plot
	Treatment 3: day 28	450ml/plot	2.5 litre/plot
	Assessment: day 35	10 plants/plot	30 plants/plot

RESULTS

Growth conditions impact *in vitro* competition between PGPR and VTEC

The largest impact on competition was the pre-culture temperature used for VTEC Sakai (Table 2), so that almost all PGPR isolates/mixtures out-grew VTEC Sakai that was pre-cultured at 37 °C, except for MB1600 or SBW25 on LB medium. In contrast, VTEC Sakai pre-cultured at 18 °C out-grew all PGPR isolates / mixtures, but only on LB medium. The PGPRs were still able to out-grow VTEC Sakai when a biofilm-conducive medium (for *Bacillus* sp.) MSgg was used. Therefore, growth conditions impacted competition, so that VTEC Sakai was more successful when pre-cultured at plant relevant growth temperature of 18 °C and a rich, undefined growth medium (LB). Only *Ps. putida* JHI-5304, a native tomato endophyte, out-competed VTEC Sakai cultured at both temperatures.

Table 2. Competition assay between VTEC Sakai pUC19 grown at two different temperatures and PGPR isolates/mixtures on two different plating media (LB XGal or MSgg XGal)

VTEC growth temperature	**37°C**		**18°C**	
PGPR growth media	MSgg	LB	MSgg	LB
QST713	x	x	x	✓
GB03	x	x	x	✓
MB1600	x	✓	x	✓
BioNutrients mixture	NA	x	NA	✓
JHI-5304	NA	x	NA	x
SBW25	NA	✓	NA	✓

Key: ✓ VTEC Sakai successful in competition; x PGPR successful in competition; NA not applicable

PGPR do not impact foodborne bacteria colonisation of lettuce or tomato

The impact of PGPRs on the colonisation of fresh produce plants by food-borne bacteria was determined under glasshouse conditions. Lettuce and tomato were selected as representative fresh produce plants associated with food-borne outbreaks, which also support colonisation of food-borne bacteria (Wright et al., 2017). Plants were pre-inoculated with PGPRs and subsequently challenged with VTEC Sakai or Ssf. Challenge of the plants resulted in recovery of ~ 4 $\log_{10}$ cfu VTEC Sakai from lettuce roots after seven days and ~ 5 $\log_{10}$ cfu Ssf from tomato roots (Fig. 1). Treatment with PGPR isolates GB03 or JHI-5304 had no significant impact on the numbers of VTEC Sakai or Ssf recovered from roots compared to the untreated control (Students *t* test). Alternative treatment with the BioNutrients™ mixture or isolate QST713 also had no significant impact on recovery of the food-borne bacteria (not shown). Recovery of PGPRs from treated roots was validated for JHI-5304 on selective medium (not shown). Therefore, application of a range of PGPRs had no impact on colonisation of two food-borne bacterial isolates, on fresh produce plants grown under glass house conditions.

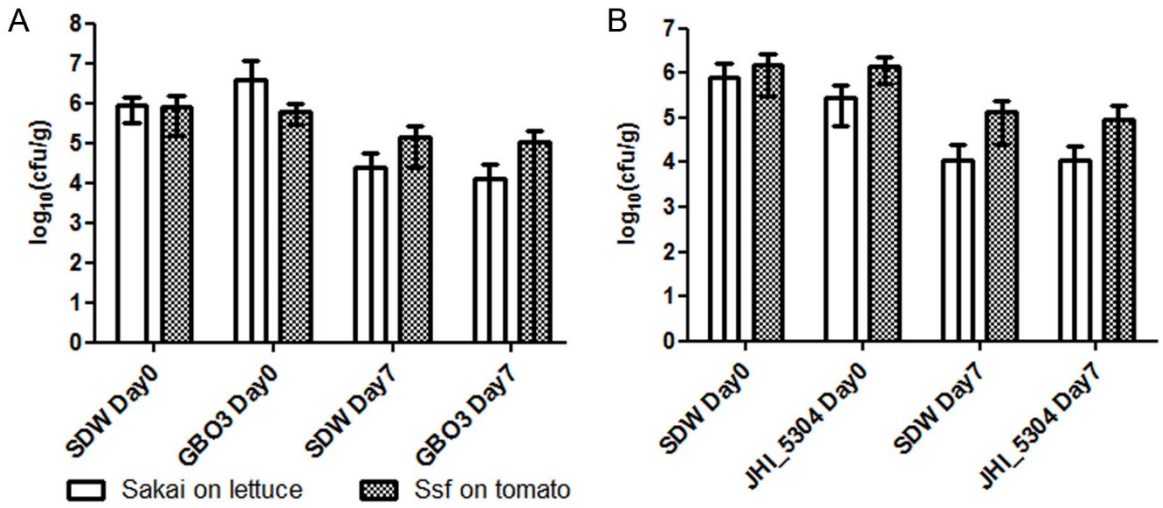

Figure 1. Recovery of *E. coli* Sakai from lettuce roots or Ssf from tomato roots after PGPR treatment. Isolate GB03 (A) or JHI-5304 (B) applied to lettuce (open) and tomato (filled) roots (n=18) and challenged with VTEC Sakai (open) or Ssf (filled). (SDW) sterile distilled water treatment.

Application of PGPR to field grown lettuce and broccoli

The impact of application of PGPRs on yield or disease was assessed on lettuce and broccoli. PGPRs treatments Companion®, BioNutrients™, and *Ps. putida* JHI-5304 were assessed from pre-treatment of transplants prior to planting (non-randomised design), or treatment applied to the base of the plant immediately after planting (randomised design). Application of JHI-5304 and Companion® prior to planting significantly increased lettuce yield, while application of BioNutrients™ had a similar, but not significant effect (Fig. 2). There was no significant difference in yield for the non-treated control plots. Disease severity varied between treatments but was not significantly reduced by any of the treatments. Pre-planting application of JHI-5304 or BioNutrients™ both resulted in an increase from a score of 1 to 2. Application of the same PGPR formulations / isolate to broccoli (var. Parthenon) had no significant impact on yield or visible disease symptoms (not shown).

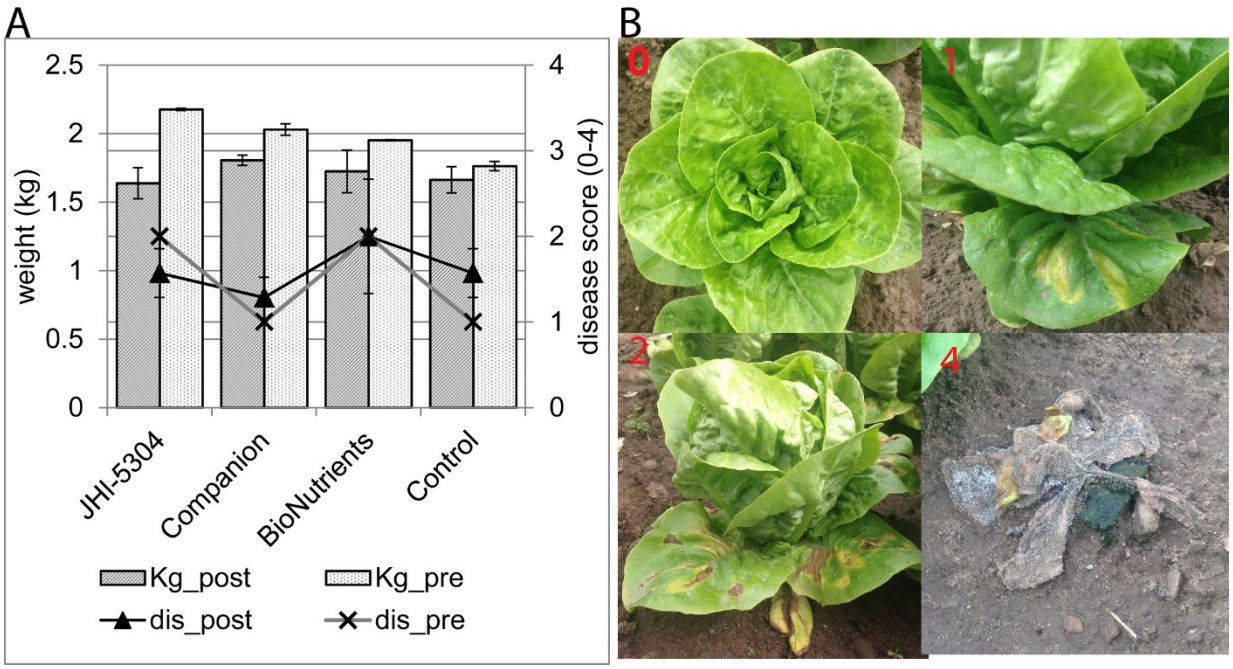

Figure 2. Lettuce (var. Little Gem) treated with PGPR. A: The average weight (kg) of lettuce plants treated pre- (stripped) and post- (stippled) planting and disease assessment, from 0 (no disease) to 4 (maximum disease) for pre- (crosses) and post- (triangles) planting. B: representative images of disease scores (0,1,2,4). Treatments were applied pre- or post- planting of transplants with *Ps. putida* JHI-5304, Companion®, BioNutrients™, or water control. Averages from 10 plants/plots for seven plots treated post-planting or four separate areas per plot treated pre-planting.

DISCUSSION

PGPRs have been widely reported having multiple benefits to plant growth including a variety of disease reduction effects (Eljounaidi *et al*., 2016; Lee *et al.*, 2015; Palaniyandi *et al.*, 2013; Singh *et al.*, 2017). The potential to use them to control bacteria in the food chain has been reported (Jordan *et al.*, 2014), however, under the conditions tested here they did not reduce levels of VTEC Sakai or Ssf on lettuce or tomato roots, respectively. The growth conditions impacted competition between PGPRs and VTEC Sakai, so that pre-culture of VTEC Sakai at a plant-relevant temperature resulted in lack of competition by the PGPRs, and *Bacillus* species were only more competitive under biofilm-conducive conditions at this temperature. Application of PGPRs increased lettuce yield in an application-dependent manner, but had no impact on broccoli, which may be as a result of lack of colonisation or lack of plant growth benefits. Therefore, additional work is required to better understand the mechanisms of microbe-microbe interactions within the complex environment of the rhizosphere (or phyllosphere) before PGPRs can be routinely and effectively applied in an horticultural setting as a means to increase yield and to control food-borne pathogens.

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MOLECULAR ASPECTS OF FUNGICIDE RESISTANCE AND RELEVANCE FOR THE PRACTICE

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Summary: Molecular detection methods for the main resistance mechanisms of QoI, DMI and SDHI fungicides are discussed. Molecular techniques help to rapidly understand and quantify sensitivity changes based on target site mutations in fungal isolates and populations and advantages are discussed, but they mostly cannot replace bioassays, as fitness parameters, virulence, sporulation capacity, or response to changing agronomic conditions of mutants need to be investigated and understood so that effective best fungicide use recommendations can be implemented in practice.

INTRODUCTION

Molecular detection of fungicide resistance is a growing field in agrochemical research due to increasing reports of resistance or insufficient performance of fungicide applications. As it is today quite challenging for agrochemical companies to develop and introduce new fungicides with novel modes of action, the management of fungicide resistance evolution in plant pathogens is an important issue not only to the agrochemical industry, but also to government officials, advisers, and finally to farmers.

The recent progress in molecular technologies elucidating the genetic background of phenotypes made it possible to develop many test systems to quantify resistance in single individuals or field populations of plant pathogens. When fungicides are used to control fungal pathogens, resistance evolves by selection of genotypes already present at very low frequency in field populations that are able to propagate and spread even in the presence of the fungicide. Resistance can be caused by different mechanisms including target site mutations, mutations in alternative pathways, over-expression of genes encoding target enzymes, metabolism/degradation or efflux of fungicide. All these mechanisms are controlled by genes in either the nucleus, mitochondria or extra-chromosomal elements. In order to apply a molecular technique for resistance detection, the mechanisms of resistance in a particular pathogen must be known and sufficiently correlated to the phenotype.

Today, more than 200 fungicides are grouped into different classes based on knowledge generated either from industry or academia (FRAC code list; www.frac.info). For many of the fungicide classes the resistance mechanisms are well described and the target enzymes with corresponding genes known, for example for quinone outside inhibitors (QoIs; gene: *cyt b*), demethylation inhibitors (DMIs; genes: *cyp*51, efflux pumps, others), or succinate dehydrogenase inhibitors (SDHIs; genes: *sdh b, c, d*). Historically, fungicide resistance is measured by bioassays *in vitro* on artificial media or *in vivo* on, e.g. leaf discs or greenhouse grown host plants, either with single individuals or mixed samples. The test design needs to be adapted to the kind of resistance present in the investigated species, such as monogenic resistance (disruptive, qualitative selection) or polygenic resistance (continuous, quantitative selection). In most cases, bioassays provide reliable sensitivity results because they are based on the direct interaction between a given individual and the fungicide without the need

to understand the underlying molecular mechanisms. However, the tests are often labor intensive, time-consuming and may not allow full quantification of resistance. Molecular techniques can complement the bioassay results by delivering quantitative information (frequency of resistance) in bulked samples and allow analysis of a large number of samples in a high through-put process with a short response time and high specificity.

For the detection of alterations in genes leading to fungicide resistance different technologies are available (Sierotzki & Gisi, 2003; Beckerman, 2013; Capote *et al.,* 2012). The techniques include PCR-RFLP, diverse PCR methods (such as real-time PCR, allele-specific Q-PCR, Scorpion PCR, PCR Luminex system), sequencing (Sanger sequencing, pyrosequencing), hybridization (e.g. Taqman, molecular beacon, PCR ASO, d-HPLC), and single-stranded DNA conformation polymorphism (SSCP) analysis (Koenraadt & Jones*,* 1992; Sierotzki *et al.,* 2000; Wille *et al,.* 2002; Bäumler *et al.,* 2003). Sequencing technologies have made tremendous progress in recent years and now whole genome re-sequencing has become fast and relatively cheap. Furthermore, these technologies allow deep sequencing of bulked DNA samples for the detection of changes and mutations in different gene fragments at a high sensitivity. The technologies are of special interest when field samples (mixed individuals) are tested for several genetic changes (mutations), leading to fungicide resistance. Most of the current molecular technologies and test methods focus on one particular genetic change leading to resistance. However, it became obvious over the last decade that more than one mutation (in one gene or several genes) may be responsible for resistant phenotypes, e.g. for SDHI or DMI fungicides.

In the following sections, the mechanisms of resistance to major fungicide classes will be briefly discussed for which validated molecular tests are available and used to support fungicide use recommendations. Most statements and information given in this paper refer to Sierotzki *et al*. (2018), who recently reviewed molecular detection methods for fungicide resistance.

QoI fungicides

The first QoI fungicides were launched in 1996. Isolates of *Blumeria graminis* f sp. *tritici* (Sierotzki *et al*. 2000) and *Plasmopara viticola* (Heaney *et al*. 2000) resistant to QoIs were detected a short time later. They were found to have a mutation in the cytochrome b gene (cyt b) that caused the protein to change from Gly to Ala at position 143 (G143A). Until today, the following three amino acid substitutions in cyt b that govern resistance to Qo inhibitors in plant pathogens have been described: change from a) phenylalanine to leucine at position 129 (F129L), b) glycine to arginine at position 137 (G137R), and c) glycine to alanine at position 143 (G143A), all based on single nucleotide polymorphisms in cyt b. The selection process is qualitative. Based on current knowledge, resistance factors (RFs) associated with G143A, G137R, and F129L are different. RFs attributed to G143A are in most cases greater than 100. Thus, isolates carrying G143A express high (complete) resistance, whereas isolates with F129L or G137R mostly express moderate (partial) resistance (FRAC, www.frac.info).

The mutation G143A is encoded by a nucleotide change in the triplet GGN coding for glycine to GCN coding for alanine. This change, occuring in the majority of plant pathogens, is a simple single nucleotide polymorphism in a relatively conserved area of the cytochrome b gene within a species. This fact made it relatively easy to develop highly sensitive and specific molecular assays based on, e.g. Scorpion PCR or allele-specific PCR. In contrast, the F129L mutation is more complex since it is encoded by a change from TTT/C to C/TTG/A. This means that, at least in some pathogens like *Pyrenophora tritici-repentis*, several SNPs must be considered for the development of a molecular test to detect and quantify QoI resistance. Today, pyrosequencing is mostly used to determine QoI resistance based on the frequency of mutations in the *cyt b* gene of field populations. Q-PCR assays are very sensitive for detecting

even low frequencies of mutations in bulked DNA samples from field populations. However, this approach requires a high specificity for the target species and clear discrimination between mutated and wild-type isolates. Since most efficient molecular tests are based on genomic DNA rather than RNA as template, the intron-exon structure is crucial for the reliability of the molecular test. The primers and probes must be designed such that they work for all DNA configurations within a species. For example in *Alternaria solani*, the gene structure around the exons carrying the F129 and the G143 positions is variable; thus, it is very important to have a sound database of sequence information available to design valuable molecular tests covering the most frequent gene variations in a population.

DMI fungicides

The C14α-demethylase in ergosterol biosynthesis is encoded by the *cyp51* gene and represents the common target of more than 30 agricultural fungicides belonging to diverse chemical classes grouped together as demethylation inhibitors (DMIs). Their intensive worldwide use for the control of various pathogens in a multitude of crops for more than three decades led to intensive research on resistance mechanisms, such as overexpression of the *cyp*51 gene, enhanced azole efflux mediated by the overexpression of membrane-bound efflux pumps (major facilitators and ABC transporters), and, in particular, mutations in the *cyp*51 gene encoding alterations in the target enzyme, which are considered as the most important mechanism of DMI resistance.

The relevance of *cyp*51 target site alterations for the sensitivity towards DMIs has been studied and published predominantly for monocot pathogens such as *Oculimacula* spp. (Albertini *et al*., 2003), *Mycosphaerella fijiensis* (Canas-Gutierrez *et al*., 2009), *Blumeria graminis* f. sp. *hordei* (Délye *et al*., 1998), *B*. *graminis* f. sp. *tritici* (Wyand & Brown, 2005; Yan *et al*., 2009), and *Puccinia triticina* (Stammler *et al*., 2009), but most frequently for *Mycosphaerella graminicola* (Cools *et al*., 2005, 2012; Fraaije *et al*., 2007; Leroux *et al*., 2007; Leroux & Walker, 2010; Mullins *et al*., 2011; Stammler *et al*., 2008). Similar studies were also made and published for dicot pathogens such as *Erysiphe necator* (Délye *et al*., 1997), *Penicillium digitatum* (Hamamoto *et al.,* 2000), *Monilinia fructicola* (Luo & Schnabel, 2008), *Venturia inaequalis* (Schnabel & Jones, 2001), and quite recently also for *Phakopsora pachyrhizi* (Schmitz *et al*., 2013).

DMI sensitivity shifts in European *M*. *graminicola* populations have been frequently discussed during the past 15 years, and a large number of articles published referring to *cyp*51 mutations. Thus, several mutations in the *cyp*51 gene have been described that confer resistance to DMIs, including L50S, D134G, V136A, Y137F, A379G, I381V, N513K, and S524T, which occur mostly in different combinations leading to different phenotypes (Cools *et al.,* 2005, 2012; Fraaije *et al.,* 2007; Leroux *et al.,* 2007; Leroux & Walker, 2010; Mullins *et al.,* 2011; Stammler *et al.,* 2008). These phenotypes often show large variations of *in vitro* sensitivity towards different DMIs (Cools *et al*., 2012; Leroux & Walker, 2010). A detailed update on the number, frequency, and relevance of *cyp*51 mutations detected has been published by Cools & Fraaije (2012).

Due to the broad spectrum of mutations causing DMI sensitivity shifts, pyrosequencing was developed as one of the most reliable methods for the detection of mutations causing DMI resistance in *M*. *graminicola*. Pyrosequencing is especially suitable for quantitative analysis of mutations in infected leaf samples and can provide additional information on the frequency of different mutations in fungal populations (Cools *et al*., 2004). However, molecular detection methods cannot differentiate between fungal DNA from viable and non-viable sources. Consequently, these methods provide no information on potential fitness variations of the individual strains. SNP detection can be performed in single monopycnidial isolates from leaf samples followed by *in vitro* sensitivity tests. The mutations I381V, in combination with amino

acid changes at positions 459 to 461 (deletions or mutations), D134G, V136A, A379G, and S524T have often been described as the most relevant mutations for *in vitro* sensitivity changes towards different DMIs (Cools *et al.,* 2004; Fraaije *et al*., 2007).

In conclusion, molecular techniques can help to understand DMI sensitivity shifts based on mutations in the *cyp*51 gene, but do not replace EC_{50} *in vitro* or *in vivo* sensitivity tests. Over past years, an increasing number of *cyp*51 target site alterations resulting in varying DMI sensitivity have been detected in *M*. *graminicola*. It is conceivable that repeated sexual recombination of the pathogen will generate an even more complex genotype pattern within populations. Under decreased and modified selection pressure (different DMI use patterns) a partial backwards shift in sensitivity to more sensitive pathogen populations may occur as has been observed in some cases (Kuck, 2002). In this context, investigations on fitness parameters, virulence, sporulation capacity and response to changing environmental and agronomic conditions may help to better understand dynamics of DMI resistance evolution on a practical level.

SDHI fungicides

Succinate dehydrogenase inhibitors (SDHIs) block the TCA cycle and consequently also fungal respiration by binding to complex II of the respiratory chain. As investigations of SDHI resistance have advanced, a complex picture of the molecular mechanisms has emerged. Several mutations leading to alterations in the target protein at different positions in three SDH subunits B, C, and D have been detected in field isolates of several plant pathogens such as *Botrytis cinerea* (Stammler *et al.,* 2007; Veloukas *et al.,* 2011)*, Corynespora cassiicola* (Miyamoto *et al.,* 2010a), *Alternaria alternata* (Avenot & Michailides, 2007), *A*. *solani* (Miles *et al.,* 2013), *Didymella bryoniae* (Avenot *et al.,* 2012; Fernandez-Ortuno *et al.,* 2012)*, Podosphaera xanthii* (Miyamoto *et al.,* 2010b), *Sclerotinia sclerotiorum* (Glättli *et al.,* 2009), or in field and laboratory mutants of *M*. *graminicola* (Skinner *et al.,* 1998; Stammler *et al.,* 2010; Fraaije *et al.,* 2012, Scalliet *et al.,* 2012, FRAC). Even within a single species, different mutations were found at one position (e.g. B-P225L/F/T or B-H272Y/R/L/V in *B. cinerea*) and at different positions in different subunits (e.g. B-H277Y, C-H134R, D-H133R in *A. alternata*). Some mutations result in changes to the binding site that affect SDHI binding (e.g. B-H272 exchanges in *B. cinerea*); others result in changes outside of the binding area, which exclude a direct influence on SDHI binding. The impact of mutations on resistance levels is not necessarily correlated with their proximity to the binding site, and exchanges at one position can cause different resistance levels (e.g. H272Y/R/L/V in *B. cinerea*).

Overall, pathogen populations in agricultural crops currently exhibit a variety of the above-mentioned target site mutations (see FRAC, www.frac.info). However, the diversity of mutations complicates the development of molecular assays and interpretation of sensitivity findings in an unprecedented manner. There are mutations with amino acid exchanges leading to a more or less pronounced resistance response to all SDHIs, but some exchanges have been detected affecting sensitivity to specific SDHIs in a somewhat different manner. So, the use of a specific SDHI may influence which mutation will be selected in a certain fungal species. Variability in type and frequency of mutations is therefore not only a result of the fungal species but also of the use pattern of SDHIs. The more that is known about the distribution and frequency of mutations conferring SDHI resistance in field isolates of a single species, the more reliable the molecular monitoring methods will be. This is especially true for *B. cinerea*, of which thousands of less sensitive isolates have been sequenced by different research groups worldwide; nearly all isolates with reduced SDHI sensitivity carried mutations in codons 225 and 272 leading to different amino acid exchanges. Single cases with mutations leading to amino acid exchanges such as B-N230I, D-H132R, C-A85V have also been found. This knowledge was essential for developing reliable monitoring assays based on quantitative mutation analysis. Since different mutations can occur in single codons (e.g. B-

H272Y/R/L/V or B-P225L/F/T), pyrosequencing was the method of choice for establishing an assay for quantitative detection of mutations causing SDHI resistance in populations of *B. cinerea*. Pyrosequencing was developed and described by Ronaghi (2001); meanwhile this method has been used routinely for monitoring target site mutations in different genes of many plant pathogenic fungi. However, the detection limit with pyrosequencing for a SNP in a mixture is about 5% (depending on the assay) and less sensitive than real-time PCR assays, which can detect SNPs down to a frequency of <1%. Different SNPs can occur in a specific codon, and also different codons in different genes (SDH-B, SDH-C, SDH-D) can cause SDHI resistance. It is also expected that combinations of mutations might occur under high selection pressure.

The key question is: which mutation(s) will occur in the field under selection pressure of specific SDHIs? A number of mutations have been described in laboratory mutants of *M. graminicola* (Skinner *et al.,* 1998; Stammler *et al.,* 2010; Fraaije *et al.,* 2012, Scalliet *et al.,* 2012), but the first mutations reported for field isolates of *M. graminicola* were not described in lab mutants: C-T79N, C-W80S, and C-N86S (FRAC). Therefore, it is advisable to first monitor SDHI sensitivity with bioassays and if reduced sensitivity is detected, to identify the responsible mutations with an appropriate molecular assay in the target gene of the isolates. However, SDHI selection pressure in the last several years has been exerted mainly by the SDHI boscalid. Since some target site mutations have different effects on sensitivity to certain SDHIs (including new SDHIs to come), the frequency, type, and combination of mutations in field populations might change as a consequence. Therefore, concomitant "classical" sensitivity bioassays should be run in monitoring programs at least for a number of isolates and from time to time to ensure that molecular assays are still valid, i.e., that the relevant codons are analyzed.

DISCUSSION

The rapidly increasing knowledge in molecular biology and genome sequences of plant pathogens has enabled development of new detection and quantification methods for resistance to fungicides. New sequencing technologies can also be used to detect the frequency and type of mutations in resistant isolates and populations of plant pathogens. Non-quantitative technologies such as PCR-RFLP or oligo-probe hybridization can be used to assess resistance in single-spore isolates. For bulked samples and field populations, application of quantitative technologies such as Q-PCR or pyrosequencing can provide an accurate estimate of resistance frequencies; the limiting factor is the number of samples, rather than the detection method. Molecular detection of resistance can replace bioassays only in very well validated systems, provided there is a good correlation between the resistant phenotype and the molecular marker(s), e.g., for QoI resistance in *M. graminicola*. When more than one mutation in the same gene (e.g. for reduced sensitivity to DMIs based on combinations of mutations in *cyp*51 gene) or mutations in different single genes or combinations of genes (e.g. for SDHI resistance based on mutations in *sdh b*, *c* or *d* genes) are responsible for resistance, molecular tests are more complex. For diploid organisms such as oomycetes, molecular tests provide more accurate results if done with single-spore isolates. If the sensitivity distribution is continuous with no clear differentiation between sensitive and resistant sub-populations, molecular detection of mutations leading to resistant phenotypes can help to better understand evolution and spread of resistance, but bioassays are needed alongside to characterize the significance of resistance for product performance. Molecular methods may help to detect and quantify resistance in an early developmental stage, before performance failures occur, thus supporting decisions on recommendations of product applications. However, this is of benefit only if the response time is short, e.g. a few days from sampling to results, and requires a high level of organization (sampling, shipment, data analysis and communication).

Early detection of resistance depends on a highly sensitive technology that can detect resistance at frequencies close to the natural mutation rate. Molecular quantification of mutations can also be used to study population changes induced by different treatment regimens supporting decision making on which fungicide mixture or alternation partner should be used to delay resistance development as long as possible. Since molecular tests are based on DNA, samples can be shipped very easily from one country to another without the need of isolations. DNA samples can also be used to study the genetic diversity of pathogen populations with non-coding molecular markers (e.g. simple sequence repeats, SSR) supporting investigations on emergence and migration of resistance. It is increasingly evident that the simultaneous detection and quantification of several point mutations will be needed in the future, either in one or several genes causing resistance to one class of fungicides or in different target genes causing resistance to different fungicide classes. New high through-put Q-PCR technologies and new high through-put sequencing technologies need to be further explored for their suitability in resistance research.

In summary, the major advantages of molecular methods in estimating fungicide resistance in plant pathogens are quantification, high sensitivity and specificity combined with the short time needed to generate results, the high through-put of samples, and the reduced need for safety requirements via DNA shipment compared to the movement of viable isolates.

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THE CHALLENGES OF MANAGING MULTIPLE BARLEY PATHOGENS IN WINTER AND SPRING BARLEY

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Summary: Winter and spring barley crops in the north of the UK are at risk of yield and quality losses arising from infection by multiple pathogens. Flexible fungicide programmes which combine a range of actives allow growers to target the range of commonly occurring pathogens and reduce yield losses. In addition there are emerging issues with resistance in several key pathogens so that fungicide programmes utilising a diversity of chemical groups are important to steward against further resistance development. Programmes can be tailored to the disease risk in the crop and this paper considers fungicide programmes in spring and winter barley trials over a number of seasons so that varying disease pressures could be assessed. Results confirm the variability of disease pressure and the yield response to fungicide treatment at high pressure sites. Diverse programmes were more effective in managing disease and reducing yield losses, but these could be tailored to each crop situation and there was scope to reduce rates in low risk situations.

INTRODUCTION

Winter and spring barley represent the major combinable arable crop in the north of Britain (Anon, 2017) but yields are constrained by disease (Walters *et al*., 2012). Pathogens such as *Pyrenophora teres* (net blotch), *Blumeria hordei* (powdery mildew) *Ramularia collo-cygni* (Ramularia leaf spot) and *Rhynchosporium commune* (Rhynchosporium leaf scald) are common in commercial crops and all contribute to losses in yield and quality (Walters *et al*., 2012). Many commonly grown varieties such as Concerto are susceptible to a range of pathogens and uptake of newer varieties with improved resistance can be slow especially for quality markets such as malting where there is a risk to growers that new varieties will not be as acceptable to buyers. As a consequence of disease risk fungicide programmes are applied as standard to crops and are depended on to maintain yield and quality. A range of fungicide active groups are approved for use on barley but there are challenges to fungicide efficacy with a history of resistance developments in several pathogen populations to several fungicide groups (Burnett, 2011). The introduction of succinate dehydrogenase inhibitors (SDHIs) fungicides in barley brought additional control of a broad range of diseases (Flind & Burnett, 2014) and in addition allowed for further diversity in fungicide programmes, thereby allowing improved fungicide resistance stewardship options such a mixing and alternating to be practiced.

Disease levels vary significantly by season and also differ between spring crops and winter crops where the latter allows for a greater disease burden to build up over winter. Ideally fungicide programmes are tailored to suit the disease risk at a site and control decisions reflect previous and current observations of disease pressure at any given site (Hughes & Burnett, 2015). This paper sets out work from trials at SRUC over a range of seasons and

regions and encompasses both spring and winter barley crops so that a range of disease pressures could be encompassed.

MATERIALS AND METHODS

Winter barley trials

Winter barley trials were drilled in five seasons at SRUC trial sites in Midlothian or Lanark (site locations are shown in tables 5 and 7) with harvest dates in 2013, 2014, 2015, 2016 and 2017. Spring barley trials were also drilled for harvest in each of these seasons, with two trials in each of 2016 and 2017. Fungicide treatments were applied at stem extension (GS30-31) and at booting (GS 39-45), termed T1 and T2. Trial treatments varied across years so this paper presents a subset of treatments which were repeated across years and shows untreated disease levels, post-treatment disease levels and yields at 85% moisture content. The winter barley cultivar Saffron was used in 2013 – 2015 inclusive and KWS Cassia in 2016 and 2017. For spring barley trials the cultivar Concerto was used throughout.

Apart from fungicides all other agronomy was as per local standard practice. Plot size was 2.0 x 10.0 m. Each treatment had three-fold replication, laid out in a randomised block design. Details of the fungicides applied are shown in table 1 and details of the fungicide programmes trialled in winter barley table 2 and in spring barley in table 3. Disease assessments (as percentage of leaf layer affected) were made at each spray timing and again during ripening. A sub-set of this disease data is presented in the paper. Ramularia leaf spot scores are not reported in this paper as shifts in sensitivity to key fungicides groups in 2017 (Mehl, pers com) has made historic efficacy data less relevant.

Table 1. Fungicide treatments applied to fungicide trials

Registered product name	Active ingredient/s (a.i.)	g a.i. per litre	Maximum registered application rate
Siltra Xpro	bixafen + prothioconazole	60g+200g	1 l/ha
Fandango	fluoxastrobin + prothioconazole	100g+100g	1.25 l/ha
Bontima	cyprodinil + isopyrazam	187.5g+62.5g	2.0 l/ha
Proline 275	prothioconazole	275g	0.72 l/ha
Zulu	isopyrazam	125g	1.0 l/ha
Bravo	chlorothalonil	500g	2.0 l/ha

Table 2. Fungicide treatments applied to winter barley fungicide trials at T1 and T2 showing varying rates and diversity (dose rate shown as l/ha)

Treat-ment	Programme design	GS 30-31 T1	GS 39-45 T2
1	untreated	nil	nil
2	azole+SDHI	Siltra Xpro 0.6	Siltra Xpro 0.4
3	higher rate T2	Siltra Xpro 0.6	Siltra Xpro 0.6
4	higher rate T1	Siltra Xpro 0.8	Siltra Xpro 0.4
5	higher rate T1&2	Siltra Xpro 0.8	Siltra Xpro 0.6
6	azole+SDHI + multisite	Siltra Xpro 0.6	Siltra Xpro 0.6 + Bravo 500 1.0
7	diverse (+QoI + multisite)	Siltra Xpro 0.6	Fandango 0.75 + Bravo 500 1.0
8	azole + QoI	Fandango 1.0	Fandango 0.75
9	above + multisite	Fandango 1.0	Fandango 0.75 + Bravo 500 1.0

Table 3. Fungicide treatments applied to spring barley fungicide trials at T1 and T2 showing varying rates and diversity (dose rate shown as l/ha)

Treat-ment	Programme design	GS 30-31 T1	GS 39-45 T2
1	untreated	nil	nil
2	lower rate SDHI+azole	Siltra Xpro 0.4	Siltra Xpro 0.4
3	higher rate	Siltra Xpro 0.6	Siltra Xpro 0.6
4	above + multisite	Siltra Xpro 0.6	Siltra Xpro 0.6 + Bravo 500 1.0
5	3 way + QoI	Fandango 0.75	Siltra Xpro 0.6
6	azole + QoI	Fandango 0.75	Fandango 1.0
7	Above + multisite	Fandango 0.75	Fandango 1.0 + Bravo 500 1.0
8	SDHI +cyprodinil	Bontima 0.8	Bontima 1.6
9	alternative SDHI	Adexar 0.8	Adexar 1.0
10	alternative SDHI	Proline 275 0.44 + Zulu 0.3	Proline 275 0.44+ Zulu 0.3

RESULTS

Winter barley fungicide trials

Disease levels ranged from <2% to over 30% on final leaf 3 at the T2 timing, shown in table 4. Averaged over all treatments and seasons, there was a yield advantage of 0.47 t/ha over the untreated controls but the mean response in high pressure disease seasons (2014, 2015 and 2016) was higher at 0.58 t/ha. Yield responses (shown in table 5) were increased with increased SDHI rate at T1 and with the addition of the multisite chlorothalonil. There was a greater yield response to the inclusion of a SDHI at T1 and T2 compared to a QoI at both timings. The addition of chlorothalonil to the QoI plus azole treatment also increased yield.

Table 4. Rhynchosporium % leaf area affected final leaf layer 2 (F-1) or 3 (F-2) assessments taken in untreated plots at T2 application timing and in all plots between GS71 to 83, 20 June – 25th June.

	2013 % Leaf area F-2 GS 76	2014 % Leaf area F-1 GS 75	2015 % Leaf area F-2 GS 71	2016 % Leaf area F-2 GS 80	2017 % Leaf area F-1 GS 75-83
Disease T2	1.75	28.3	33.3	31.8	8.67
1	2.33	27.3	54.3	22.0	15.0
2	6.00	4.00	4.33	-	8.33
3	1.67	0.67	4	-	-
4	0.67	4.33	9	-	-
5	1.33	6.67	-	-	-
6	0.37	2.33	6	-	8.33
7	1.00	4.00	4.33	-	-
8	1.33	11.7	13.33	-	4.00
9	1.00	6.7	8.67	-	-
P value	0.111	<0.001	<0.001	<0.001	<0.001
LSD	4.410	8.430	10.15	3.303	8.422

Table 5. Yield (t/ha) adjusted to 85% moisture content

Year	2013	2014	2015	2016	2017	Mean
Site	Lanark	Perthshire	Kinross	Lanark	Lanark	
1	5.60	6.39	4.58	8.52	4.71	5.96
2	5.72	8.23	5.97	-	4.77	6.17
3	6.19	8.28	5.27	-	5.11	6.21
4	6.47	8.20	5.99	-	-	6.89
5	5.62	8.55	-	-	-	7.09
6	6.08	8.16	5.44	-	-	6.56
7	5.72	8.15	5.07	-	-	6.31
8	5.89	8.20	5.13	-	4.84	6.02
9	5.73	8.16	5.90	-	4.87	6.17
P value	0.51	<0.001	0.175	0.199	0.992	0.055
LSD	0.838	0.684	1.721	0.501	1.218	0.862

Spring barley fungicide trials

Disease levels varied between sites with Rhynchosporium most commonly recorded. Rhynchosporium levels were very low at three of the seven sites, 2013, 2016a and 2017b, with net blotch the only disease recorded at the latter site (table 6).

Table 6. Disease levels 17-22 July as % leaf area affected final leaf layer 2 (F-1) or 3 (F-2). Assessments taken in untreated plots at T1 application timing and in all plots between GS65 to 83, 17th July- 22th July

Year	2013	2014	2015	2016a	2016b	2017a	2017b
	GS75 F-1,-2,-3	F-2 Rhyncho GS 75	F-2 Rhyncho GS 78	F-2 Rhyncho GS 65-71	F-2 Rhyncho GS 79	F-1 Rhyncho GS 77-83	F-1 Net blotch GS 77-83
Disease T1	0.00	0.00	0.00	0.00	0.28	0.00	10.8
1	0.00	10.7	3.67	1.50	8.00	7.33	33.3
2	0.00	0.00	-	1.50	-	0.00	4.70
3	0.00	0,00	-	-	-	-	-
4	0.00	0.00	-	-	-	-	-
5	0.00	0.33	-	-	-	-	-
6	0.00	0.00	-	-	-	-	18.3
7	0.00	0.50	-	0.25	-	7.50	18.3
8	0.00	0.67	-	-	-	-	-
9	0.00	0.67	-	0.50	-	-	-
10	0.00	-	1.00	-	3.25	-	-
P value		<0.001	0.003	0.290	0.002	0.168	0.002
LSD		1.350	1.833	1.462	2.556	6.440	13.91

Table 7. Yield (t/ha) adjusted to 85% moisture content

Treat	2013	2014	2015	2016a	2016b	2017a	2017b	Mean
Site	Mid-lothian	Lanark	Mid-lothian	East Lothian	Lanark	East Lothian	East Lothian	
1	6.76	7.82	7.8	7.66	5.47	6.87	6.11	6.93
2	6.28	8.72	-	8.19	-	7.76	7.04	7.60
3	6.68	8.75	-	-	-	-	-	7.72
4	6.39	9.26	-	-	-	-	-	7.83
5	6.43	8.94	-	-	-	-	-	7.69
6	6.42	8.85	-	-	-	-	6.56	7.28
7	6.48	9.26	-	7.98	-	7.30	6.84	7.57
8	6.73	9.05	-	-	-	-	-	7.89
9	6.30	9.01	-	7.78	-	-	-	7.70
10	6.25	-	8.29	-	6.00	-	-	6.85
P	0.959	0.017	0.061	0.677	<0.001	<0.001	0.461	0.042
LSD	0.815	0.648	1.569	0.427	0.289	0.375	0.712	0.837

Table 7 shows the yield data from the trial series. The mean response to treatment across all sites and treatments compared to untreated controls was 0.57 t/ha but significant yield responses were only noted where disease levels were higher so when 2013, 2016a and 2017b (low disease sites) were taken out of the analysis then responses to fungicides were higher at 1.44 t/ha. Yield response to fungicides increased with increased rate of the SDHI +azole and there was also an increased yield where chlorothalonil was added. Adding a QoI to the SDHI programme also increased yield although substituting a QoI for an SDHI at both T1 and T2 was not as effective in raising yield as the SDHI containing treatments. Substituting the azole for cyprodinil was amongst the highest yielding treatments suggesting that

diversifying from azoles is possible in programmes. Other QoIs were not as effective in raising yield compared to the fluoxastrobin + azole co-formulation. Adding chlorothaonil to this co-formulation also increased yield.

DISCUSSION

The results show the risk that high levels of disease in spring and winter barley present to yield. The fungicide programmes evaluated were effective in reducing disease at high disease pressure sites. Using diverse fungicide programmes can steward products (Hobbelen *et al*., 2014) and reduce the risk of fungicide resistance development but the data in this paper would suggest that there are win-wins in such an approach for individual growers as there was evidence that programmes could be tailored to risk in terms of dose rate. The data presented also indicates yield advantages to diversifying the number of fungicide groups used across the programme. Diverse programmes were more effective in managing disease and reducing yield losses. Flexible fungicide programmes which combine a range of actives will also allow growers to target the range of commonly occurring pathogens and reduce yield losses whilst also allowing better stewardship of fungicides in terms of managing resistance risk.

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MANAGING SEPTORIA AND MAINTAINING YIELD IN WINTER WHEAT THROUGH FLEXIBLE FUNGICIDE PROGRAMMES

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Summary: Control of the septoria leaf blotch in wheat is becoming more challenging and the need to maintain efficacy is a range of actives is a strong driver for forming effective and sustainable fungicide programmes to maintain adequate control and to steward fungicide actives against resistance development. SDHI fungicides have been important in maintaining wheat yields and are widely used in wheat programmes but are at risk of resistance development so this paper summarises three years of trial data to test whether control would be possible without SDHIs in ways which would not overly expose other active groups, should resistance to SDHIs develop. The data presented shows that well-timed programmes of azoles and multi-site fungicides can maintain efficacy, albeit through increased application rates and number. The data also shows that comparable yields can be achieved without over-exposing other at risk groups of chemistry. The paper also considers three seasons of trials evaluating the use of strobilurins in wheat programmes. The data would support the inclusion of strobilurins, where appropriate, in wheat programmes, where they might reduce pressure of other actives and form part of stewardship strategies.

INTRODUCTION

Winter wheat is the highest yielding and most profitable cereal crop in the north of Britain but the high yield potential enabled by long day lengths and moist soils is often limited by high levels of disease. Historically, high-yielding but disease susceptible cultivars such as Consort, Riband and Viscount have been widely grown. *Zymoseptoria tritici*, the causal pathogen of septoria leaf blotch, is the major target of fungicide programmes applied to protect against yield losses from disease. Only a limited number of fungicide groups have efficacy so demethylation inhibitor (DMI) fungicides are widely used on the crop as are succinate dehydrogenase inhibitors (SDHIs) and multisites such as chlorothalonil (Monie *et al*., 2017). Concerns over fungicide resistance developments in *Z. tritici* populations threaten field efficacy and there have been published declines in sensitivity to DMIs (Strobel *et al*., 2014) and a step change loss in the efficacy of the Quinone outside Inhibitor (QoI) or stobilurin fungicide group (Blake *et al*., 2017). In addition, the SDHI group has been classed since launch as at moderate risk of resistance development in *Z. tritici* and in 2016 and 2017 mutants with reduced sensitivity were reported throughout Europe (Dooley *et al*., 2016). It is therefore important to determine whether sufficient disease control could be achieved in wheat crops should efficacy in the SDHI group be lost, and also whether this can be done without over-exposing other chemistry.

In order to steward against resistance and maintain efficacy for both DMI and SDHI fungicides, integrated programmes need to be developed and implemented which, where possible, diversify the number of active groups used. Although resistance to QoI fungicides is widespread in the *Z. tritci* population QoIs may still have a role in programmes for control

against other disease and also to provide some limited efficacy against *Z. tritici* itself so their inclusion could reduce reliance on other chemistry. This paper sets out data from field trials testing the inclusion of SDHIs and strobiluirins and discusses fungicide options and optimum timings.

MATERIALS AND METHODS

Two sets of winter wheat fungicide programme trials on septoria susceptible varieties were established over several seasons at sites in Fife. The first trial series evaluated the contribution of a strobilurin relative to other actives early in the programme and ran in three seasons with harvest years in 2015, 2016 and 2017, termed the 'strobilurin contribution' trials. The seconds series evaluated whether control of septoria would still be possible if efficacy in SDHIs was lost (termed the 'post-SDHI trial'). This ran over four seasons with harvest years in 2013, 2014, 2015 and 2016. The cultivar used throughout was Consort (susceptible to septoria) with the exception of the 2017 harvest strobilurin contribution trial where Trinity, also susceptible to septoria, was used. Plots sizes were 2.0 x 10.0 m and each treatment had three-fold replication, laid out in a randomised block design. Yields were adjusted to 85% dry matter. Apart from fungicide treatments, all other trial inputs and agronomy was as per typical local practice. Fungicides applied to the trial are shown in table 1 and were applied in 200 l water per ha by CP3 knapsack sprayer. The strobilurin contribution trials used treatments targeted at final leaf 4 emerging GS30-31 (T0), GS31-32 (T1), GS39 (T2) with an over-spray to maintain commercially realistic yields at GS50-60 (T3). For the post-SDHI series, treatments were also timed for T0, T1 and T2 but a T1.5 spray was included in some treatments, timed to be equally spaced between the T1 and T2 (GS33-37). Treatments varied slightly between seasons so the treatments shown in this paper present a subset of the data set representing treatments that were duplicated between seasons. For the strobilurin contribution trials, treatments are shown in table 2 and the trial was over-sprayed at T3 with 0.6 l/ha prothioconazole + tebuconazole. For the post-SDHI trial, treatments are shown in table 3 and the over-spray at T3 was also 0.6 l/ha prothioconazole + tebuconazole. There were sometimes small adjustments between comparable treatments between seasons and these are marked.

Table 1. Fungicide active ingredients applied in trials as registered product name, g a.i. per litre and maximum label rate as l/ha.

Registered product name	Active ingredient/s (a.i.)	g a.i. per litre	Maximum registered application rate l/ ha
Adexar	fluxapyroxad + epoxiconazole	62.5 + 62.5	3.0
Bassoon EC	epoxiconazole	83	1.5
Bravo 500	chlorothalonil	500	2.0
Brutus	metconazole+ epoxiconazole	27.5 + 37.5	3.0
Cherokee	propiconazole +cyproconazole + chlorothalonil	62.5 + 50 + 375	2.0
Comet	pyraclostrobin	200	1.25
Ignite	epoxiconazole	83	1.5
Prosaro*	prothioconazole + tebuconazole	125 + 125	1.0
Tracker 1.0**	boscalid + epoxiconazole	233 +67	1.5

*used as a T3 overspray in trials

**contains the SDHI boscalid used for eyespot control but of lower septoria efficacy

*** contains SDHI fluxapyroxad of high septoria efficacy

Table 2. Treatments applied to strobilurin contribution trials in 2015, 2016 and 2017.

	Test	T0 (GS 30-31)	T1 (GS32-33)	T2 (GS39)
1	No fungicide	Untreated	Untreated	Untreated
2	No T0	Untreated at T0	Tracker 1.0l/ha + Bravo 1.0l/ha	Adexar 1.25l/ha + Bravo 1.0l/ha
3	QoI at T0	Comet 200 0.5l/ha	Tracker 1.0l/ha + Bravo 1.0l/ha	Adexar 1.25l/ha + Bravo 1.0l/ha
4	DMI at T0	Ignite 0.75l/ha	Tracker 1.0l/ha + Bravo 1.0l/ha	Adexar 1.25l/ha + Bravo 1.0l/ha
5	CTL at T0	Bravo 1.0l/ha	Tracker 1.0l/ha + Bravo 1.0l/ha	Adexar 1.25l/ha + Bravo 1.0l/ha
6	QoI+ CTL at T0	Bravo 1.0l/ha + Comet 0.5l/ha	Tracker 1.0l/ha + Bravo 1.0l/ha	Adexar 1.25l/ha + Bravo 1.0l/ha
7	DMI + QoI at T0	Bravo 1.0l/ha + Ignite 0.75l/ha	Tracker 1.0l/ha + Bravo 1.0l/ha	Adexar 1.25l/ha + Bravo 1.0l/ha
8	3 way mix at T0	Bravo 1.0l/ha + Comet 0.5l/ha + Ignite 0.75l/ha	Tracker 1.0l/ha + Bravo 1.0l/ha	Adexar 1.25l/ha + Bravo 1.0l/ha
9	QoI at T0, T1 and T2	Bravo 1.0l/ha + Comet 0.5l/ha	Tracker 1.0l/ha + Bravo 1.0l/ha + Comet 200 0.5l/ha	Adexar 1.25l/ha + Bravo 1.0l/ha + Comet 200 0.5l/ha

Table 3. Fungicide treatments in post SDHI winter wheat trial.

	Test	T0 GS30*	T1 GS31-32	T2 GS33-37	T2 GS39
1	No fungicide	Untreated	-	-	-
2	Standard spray SDHI T2	Cherokee 1.0	Tracker 1.0 + Bravo 500 1.0	-	Adexar 1.25
3	SDHI at T1&T2	Cherokee 1.0	Adexar 1.0 +Bravo 500 1.0	-	Adexar 1.25
4	No SDHI, med rate DMI	Cherokee 1.0	Brutus 1.5 +Bravo 500 1.0	-	Brutus 1.5
5	No SDHI, high rate DMI	Cherokee 1.0	Brutus 3.0 +Bravo 500 1.0	-	Brutus 3.0
6	Standard spray + SDHI T2 + T1.5 CTL	Cherokee 1.0	Tracker 1.0 +Bravo 500 1.0	Bravo 500 1.0	Adexar 1.25
7	No SDHI, med rate DMI + T1.5 CTL	Cherokee 1.0	Brutus 1.5 +Bravo 5001.0	Bravo 500 1.0	Brutus 1.5
8	No SDHI, high rate DMI + T1.5 CTL	Cherokee 1.0	Brutus 3.0 +Bravo 500 1.0	Bravo 500 1.0	Brutus 3.0
9	Adds CTL to treatment 4	Cherokee 1.0	Brutus 1.5 +Bravo 500 1.0	-	Brutus 1.5 +Bravo 500 1.0
10	Adds CTL to treatment 5	Cherokee 1.0	Brutus 3.0 +Bravo 500 1.0	-	Brutus 3.0 +Bravo 500 1.0
11	As treatment 10 but DMI at T1.5	Cherokee 1.0	Brutus 3.0 +Bravo 500 1.0	Ignite 0.75*	Brutus 3.0 +Bravo 500 1.0

***** applied as Bassoon EC in 2016 CTL = multisite chlorothalonil

RESULTS

Strobilurin contribution trial

Disease and yield data is presented in table 4. Disease levels varied by season, with more disease pressure in 2016. In all three seasons however, there was less disease where a T0 was added compared to the comparable treatment 2 without a T0. Differences in disease control between T0 options were small but despite the presence of resistance to QoIs, efficacy at T0 was comparable to that achieved with DMI or chlorothalonil treatments. In terms of yield benefit, QoIs were also comparable to DMIs at T0 and both options added 0.2 t/ha meaned over the three seasons and were better than the chlorothalonil yield response. The addition of chlorothalonil to either the QoI or DMI at T1.5 also boosted yield. A three-way mix of QoI + DMI + chlorothalonil was also one of the highest yielding treatments.

Table 4. Septoria severity (% leaf area affected) and yield (t/ha) in strobilurin contribution winter wheat trials.

	Mid-season septoria (leaf 3)			Late-season septoria (flag leaf)			Yield t/ha			
	2015 GS	2016 GS	2017 GS	2015 GS	2016 GS	2017 GS				
Trts	51	39-42	55-59	83	78-83	77-85	2015	2016	2017	Mean
1	12.0	1.90	2.15	24.3	40.0	19.8	9.22	8.25	8.84	8.77
2	3.83	2.50	0.55	12.0	6.90	1.43	10.0	9.40	9.97	9.79
3	5.33	1.30	0.53	5.67	4.90	1.00	10.4	9.53	9.91	9.96
4	4.00	2.50	0.55	5.83	8.80	0.62	10.3	9.51	9.99	9.94
5	2.50	1.25	1.40	6.33	15.2	0.88	10.1	9.29	10.1	9.82
6	4.83	0.18	0.50	1.83	6.80	0.88	10.6	9.47	9.96	10.0
7	1.17	1.40	1.03	9.00	7.10	0.78	10.7	9.46	9.88	10.0
8	2.00	2.12	0.68	4.00	6.20	1.38	11.0	9.32	10.1	10.1
9	3.00	1.88	0.68	5.00	3.20	0.55	10.6	9.45	10.0	10.0
P	0.022	0.002	0.041	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	6.934	1.407	0.956	9.494	12.69	1.700	0.521	0.372	0.331	0.291

Results from the post SDHI trial series are shown in tables 5 and 6. 2013 was a very low disease pressure year but in the other three seasons untreated plots had moderate disease levels by mid to end of season. There were significant levels of disease control in each of these higher pressure seasons and there were also significant yield responses to treatment (table 6).

The results suggest that there is no yield advantage in most seasons to two SDHIs with septoria efficacy as opposed to one SDHI. There was a small yield cost where SDHIs were omitted from the programme where only DMIs were applied and no T1.5 was used but this yield loss was retrieved by the addition of chlorothalonil at conventional T1 and T2 timings. It was also retrieved through the addition of chlorothalonil at T1.5. There was a small yield loss from substituting the chlorothalonil at T1.5 with a DMI.

Table 5. Septoria severity (% leaf area affected) post-SDHI winter wheat trials.

Trt	Mid season septoria (final leaf 3)				Late season septoria (final leaf 2)			
	2013 GS 59	2014 GS 39	2015 GS 43	2016 GS39-43	2013 GS 39	2014 GS39-45	2015 GS 65	2016 GS65-70
1	3.50	5.67	16.67	3.67	0.03	6.33	11.3	11.8
2	1.70	5.00	5.33	2.50	0.00	2.17	2.83	1.65
3	0.27	3.67	6.17	0.45	0.00	0.20	2.67	1.12
4	1.57	2.33	2.50	0.55	0.00	0.67	2.00	2.50
5	0.73	2.00	2.07	0.25	0.00	0.07	1.33	1.00
6	2.67	2.83	4.00	0.03	0.00	0.67	0.73	0.88
7	2.17	0.50	2.23	2.00	0.00	0.50	0.90	2.00
8	1.20	1.67	1.83	0.88	0.03	1.17	2.50	1.27
9	2.67	2.17	1.67	0.50	0.00	0.67	2.83	0.25
10	0.70	1.67	3.33	0.18	0.00	0.40	1.23	0.38
11	0.87	1.33	-	0.12	0.00	0.23	-	1.00
P	0.177	0.02	0.011	0.006	0.594	<0.001	<0.001	<0.001
LSD	2.221	2.553	6.744	1.727	0.038	1.32	2.719	2.017

Table 6. Yield (t/ha at 85% moisture content) in post-SDHI winter wheat trials

Trt	2013	2014	2015	2016	Mean
1	8.87	6.78	9.50	8.53	8.42
2	9.79	9.45	11.4	9.72	10.1
3	9.59	9.76	11.6	9.53	10.1
4	9.34	9.19	11.3	9.80	9.90
5	9.38	9.53	11.3	9.56	9.93
6	9.60	9.81	11.6	10.0	10.3
7	9.44	9.70	11.4	9.81	10.1
8	9.59	9.95	11.6	9.81	10.2
9	9.52	9.94	11.8	9.93	10.3
10	9.50	10.1	11.9	10.2	10.4
11	9.61	10.2	-	9.74	9.84
P	0.549	<0.001	<0.001	0.013	0.023
LSD	0.625	0.731	0.488	0.524	0.589

DISCUSSION

The results show that disease control and commensurate yield benefits can be achieved through a varied range of programmes. This gives scope to factor fungicide stewardship in to programmes without incurring a yield penalty for doing so. The post-SDHI trial series suggests that it is possible to achieve comparable yield responses from programmes that only use one

SDHI as opposed to two SDHIs when disease pressure is low. Removing them completely from the programme did incur a yield penalty but this could be retrieved by inserting a T1.5 spray of chlorothalonil or DMI or by adding chlorothalonil and increasing azole dose at conventional timings. This additional spray incurs an additional application cost which may outweigh the cost difference between two SDHIs and one SDHI. Of these options then using an additional T1.5 DMI timing is likely to increase selection pressure against azoles and so of the two options the addition of chlorothalonil at T1 and T2 would be preferable from a best practice stewardship perspective. The strobilurin contribution trials show that the inclusion of this additional active group can contribute to septoria management and yield despite the presence of fungicide resistance and so should be considered as an additional means of diversifying fungicide actives in programmes, reducing reliance on key actives and maintaining yields. In future, accurate prediction systems which allow growers to react to disease risk and adjust inputs in-season would allow for more tailored approaches; reduce the risk of yield losses, whilst also stewarding products. In the meantime risk management is required which restricts the opportunities to fine tune programmes

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CONTROL OF SEPTORIA TRITICI BLOTCH IN IRISH WINTER WHEAT CROPS

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Summary: Fungicides are routinely applied for the control of septoria tritici blotch in Irish winter wheat crops. The development of resistance to the main fungicide modes of action in Irish *Zymoseptoria tritici* populations, together with increased restrictions on fungicide usage threaten to undermine the viability of winter wheat production in Ireland. As such it is increasingly important to justify the application of such fungicides. To determine the value of fungicides applied from growth stages (GS) 30-65 in terms of yield response, two sets of trials were conducted from 2014 - 2017. In the first instance the value of fungicide applications at GS30 (final leaf 4 fully emerged), GS32 (final leaf 3 fully emerged), GS37 (final leaf 2 fully emerged) and GS39 (final leaf fully emerged), individually and in combination were investigated. In parallel the value of fungicides, +/- the inclusion of chlorothalonil for STB control and yield applied at GS65 (mid-anthesis) were also investigated. A strong site x treatment interaction with application timing (30-39) was observed, whilst in the absence of Fusarium Head Blight (FHB) no additional yield benefits were observed from the application of a range fungicides at GS65. The data suggests increased targeting of fungicides whether for STB or FHB may allow for a reduction in fungicide usage.

INTRODUCTION

Septoria tritici blotch (STB) caused by the fungal pathogen *Zymoseptoria tritici* is the most economically destructive pathogen of Irish winter wheat crops, with potential yield losses of >40% if left unchecked. To prevent such yield reductions disease control programmes are readily utilised by Irish growers. Unfortunately, limitations of agronomic practises (rotation, cultivation practice etc) to restrict STB development and the yield reductions often associated with varietal STB resistance mean these control programmes are heavily reliant on fungicide applications. Currently to achieve STB control fungicides including azoles, SDHIs and the multisite chlorothalonil are in some instances applied 3-4 times from GS30-GS65. This intensive fungicide usage has inevitably led to the development of varying levels of fungicide resistance to both the azoles and SDHIs in the Irish *Z. tritici* population, with impacts on field efficacy (Kildea et al., 2017). These losses in efficacy coupled with increased restrictions on usage pose a serious threat to the sustainability of Irish wheat crops. It is essential therefore to critically evaluate the value of each fungicide application to the overall disease control programme so the two sets of trials presented in this paper dissect yield contribution from a range of fungicide timings.

Previously Creissen et al., (2018) have shown that, where effective fungicides are available, the application of a fungicide at GS30 for STB control and yield protection is unwarranted. However, as weaknesses in efficacy, particularly curativity, become increasingly prevalent in the main modes of action due the spread of fungicide resistances in *Z. tritici* populations

(Blake et al., 2018; Reffus et al., 2018) the value of these earlier applications may become increasingly important. Similarly, if currently available fungicides can no longer be relied upon to provide the necessary curativity, current timings (principally the combination of GS32 and GS39) may leave a major part of the upper canopy unprotected. Conversely, if effective STB control is achieved between GS30-39, application of an azole with or without the multisite fungicide chlorolathonil at GS65 specifically for STB control may be unwarranted and may only serve to increase selection for azole resistance. To address these questions two trial programmes were undertaken between 2014 – 2017. One investigated the role of fungicide applications at GS30 (final leaf 4 fully emerged), GS32 (final leaf 3 fully emerged), GS37 (final leaf 2 fully emerged) and GS39 (final leaf fully emerged), both individually and in various combinations with one another. The second programme investigated the value of the azole fungicides prothioconazole and tebuconazole applied as solos, in combination, and with or without chlorothalonil when applied at GS65 (mid-anthesis) for STB and yield protection.

MATERIALS AND METHODS

Foliar treatments: GS30-39

To determine the role of fungicides applied between GS30-39 five field trials were conducted over two seasons in the main wheat growing regions of Ireland. In 2016 there were three trials located in south-west, north-east and south-east Ireland and 2017 there were two trials (south-west and south-east). All trials were conducted on a STB susceptible or moderately susceptible variety commercially relevant to each location. All trials were laid out as completely randomised block designs with four replications. In 2016 the fungicide chlorothalonil (1.0 l/ha Bravo) was applied at GS30, GS32, GS37 and GS39 individually and in all potential timing combinations, with an additional two treatments remaining untreated at these timings (Table 1 and Table 2).

Table 1. Treatment programmes applied to five winter wheat trials in Ireland in 2016 and 2017. Timing combinations shown in Table 2.

Trial site	GS30	GS32	GS37	GS39	GS 65
2016 SW	CTL	CTL	CTL	CTL	Prosaro
2016 NE	CTL	CTL	CTL	CTL	Prosaro
2016 SE	CTL	CTL	CTL	CTL	Prosaro
2017 SW	Elatus Era + CTL	Elatus Era + CTL	Elatus Era + CTL	Elatus Era + CTL	Prosaro
2017 SE	Elatus Era + CTL	Elatus Era + CTL	Elatus Era + CTL	Elatus Era + CTL	Prosaro

Note that the UK Approval for both Bravo and Elatus Era is for a maximum of two timings.

Except for one of these untreated controls the entire trial received a cover spray of prothioconazole + tebuconazole applied as Prosaro (1.0 l/ha) at GS65 to prevent (FHB). In 2017 the azole/SDHI mixture Elatus Era (0.8 l/ha) (benzovindiflupyr + prothioconazole) was applied with chlorothalonil (1.0 l/ha Bravo) at each of the timings between GS30-39. All plots were harvested, and yield determined as t/ha at 15% moisture.

Ear treatment: GS65

Field trials were conducted at three locations in Ireland (south-west, south-east and north-east) in both 2014 and 2017. All trials were conducted on a single variety commonly grown in the local area (two varieties were included in the south-east in 2017) and laid out as completely randomised block designs with four replications. In each trial six fungicide treatments representing those most commonly used were tested at GS65 in addition to an untreated control (Table 3). Prior to the test application all plots including the untreated control received a final leaf 3 and final leaf 2 fungicide application typical of commercial programmes (mixtures of azole, SDHIs and chlorothalonil) in each year. Levels of STB were assessed on 10 main tillers selected from throughout each plot at GS75 and yields determined and present as t/ha at 15% moisture.

Statistical analysis

Preliminary analysis of the foliar treatments identified a strong site x treatment interaction and hence the impact of fungicides applied between GS30-39 were determined per individual site. Across site (excluding the 2016-NE site) comparisons to determine the overall impact of each leaf application was determined by ANOVA with Contrasts. The impact of ear treatments was determined using ANOVA, with the specific value of the addition of CTL determined by ANOVA with Contrasts. All statistical analysis was determined using GenSat Version 14.1.

RESULTS

In all trials STB was the major disease present (data not shown). With the exception of the individual fungicide application at GS65 all fungicide treatments in the foliar treatment trials provided significant yield benefits at the different sites. Significant differences were however observed between the different treatments (Table 2). As single applications, treatments applied at either GS37 or GS39 provided the greatest yield response, whilst in four of the five sites the single application at GS30 provided the lowest response. Two or more application did not necessarily always result in significant yield responses compared to single applications. However, at all sites the greatest yield response over the untreated was achieved by those treatments that received a fungicide at all timings. In most sites this was not significantly different to treatments that received three treatments, specifically at GS32, GS37 and GS39. When compared across sites all timings had a significant impact on yield when analysed by Contrasts ($P<0.001$) (Figure 1).

In the ear application trials low levels of disease were observed (<5%) on the untreated control at GS75 and as such no further analysis on disease data was conducted. Significant differences in yields ($P<0.001$) were observed between the different sites, however no differences were observed between the different fungicide treatments (Table 3). The addition of chlorothalonil had no impact on final yield ($P<0.001$).

Table 2. Effect of fungicide treatment at GS30-39 as t/ha individually and in combinations at five sites in Ireland in 2016 and 2017

Treatment	2016-SW	2016-SE	2016-NE	2017-SW	2017-SE
Untreated	7.9	6.8	8.6	7.1	7.5
GS30	8.4	8.0	9.6	8.6	8.4
GS32	9.0	9.1	9.2	9.4	8.8
GS37	8.7	9.0	10.5	9.6	9.4
GS39	8.6	8.5	10.5	9.6	9.3
GS65*	7.9	7.9	nd	8.4	7.6
GS30, GS32	9.1	9.3	10.2	9.4	9.7
GS30, GS37	8.7	8.9	10.8	10.2	10.0
GS30, GS39	8.7	8.8	10.2	9.9	10.2
GS32, GS37	9.2	9.9	10.4	8.8	10.3
GS32, GS39	8.9	10.1	10.1	10.0	10.3
GS37, GS39	8.8	9.5	11.0	10.3	10.6
GS30, GS32, GS37	9.2	10.1	10.6	10.6	10.7
GS30, GS32, GS39	9.4	10.1	10.6	10.7	11.0
GS30, GS37, GS39	8.8	9.5	11.1	10.1	11.3
GS32, GS37, GS39	9.4	10.3	11.2	10.8	11.0
GS30, GS32, GS37, GS39	9.4	10.5	11.3	10.8	11.6
P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.001*
L.S.D	0.51	0.39	0.63	0.60	0.59

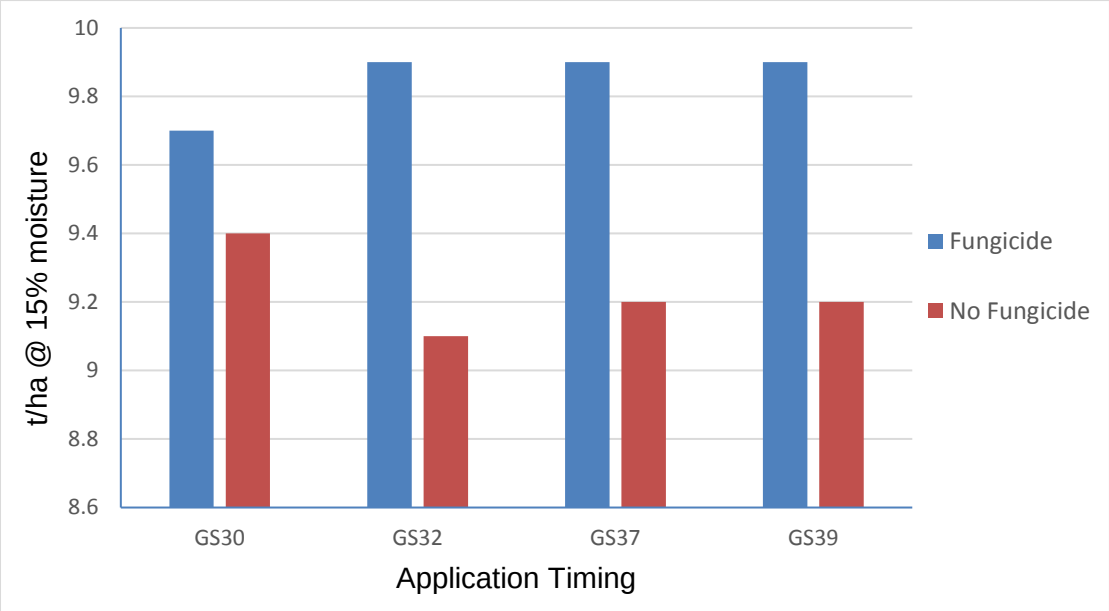

Figure 1. Differences in yield (t/ha) from all fungicide combinations including/excluding an application at the different growth stages.

Table 3. Effect of fungicides on winter wheat when applied at mid-flowering

Fungicide[1]	Active Ingredient(s)	Rate (l/ha)	Yield (t/ha)[2]
Untreated	-	-	10.3 *a*
Proline	prothioconazole (PTZ)	0.8	10.3 *a*
Prosaro	PTZ & tebuconazole (TBZ)	1.0	10.5 *a*
Folicur	TBZ	1.0	10.3 *a*
Proline & Bravo	PTZ & chlorothalonil (CTL)	0.8 & 1.0	10.4 *a*
Prosaro & Bravo	PTZ & TBZ & CTL	1.0 & 1.0	10.4 *a*
Folicur & Bravo	TBZ & CTL	1.0 & 1.0	10.5 *a*

[1]All treatments included a fungicide application at GS32 & GS39
[2]Yields with the same letter do not differ significantly: Tukey's HSD test ($P<0.05$)

DISCUSSION

Unsurprisingly a large amount of variation was observed in yield responses within the foliar treatment trials, with specific treatments performing significantly differently between sites. This is most likely a reflection of both local disease pressures and weather conditions that occurred at each site prior to or post application. In each trial septoria tritici blotch was the dominant pathogen with high levels of disease present (data not shown), reflected in yield differences between the untreated and fully protected treatment observed (1.5 - 4.1 t/ha). Although the greatest yield response was consistently achieved when a fungicide was applied at each timing, corresponding to the emergence of each of the final four leaves, it was not always significantly different to that achieved with either three applications or two, dependent on timing. This highlights the potential that, even though fungicide efficacy is declining targeted applications can be as effective as a prophylactic approach. However, the inconsistency between the sites as to the effectiveness of the different treatments does emphasis the difficulties often facing growers in making decision on when to apply fungicides and why the prophylactic approach maybe favoured. Further analysis of the data from these trials, including various parameters involved in STB epidemic progression is required.

Although low levels of disease and no yield responses were recorded in the ear treatment trials, both 2014 and 2017 were regarded in commercial practice as moderate disease pressure seasons. At each trial site high levels of STB and significant yield losses were recorded within untreated plots throughout the site. The lack of disease development and yield responses observed in these trials is likely due to control achieved by the earlier fungicide applications. This suggests that under Irish conditions where STB is controlled adequately earlier in the season fungicide programmes targeting STB at GS65 are unwarranted. Further analysis is ongoing to determine levels of *Fusarium* spp. present in the harvested grain, however the above suggest that if improvements in the prediction and precision of FHB risk can be achieved more targeted applications at GS65 can be achieved. Any such reduction in usage will undoubtedly reduce the selection for fungicide resistance amongst the Irish *Z. tritici* population.

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CONTROLLING RAMULARIA LEAF SPOT IN BARLEY CROPS

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Summary: Ramularia leaf spot is major pathogen of barley crops across the temperate regions of the world. The disease is caused by the fungus *Ramularia collo-cygni.* Symptoms appear late in crop development leading to a reduction in yield and quality. Recent changes in the sensitivity of the fungus to two of the major fungicide groups, used extensively in barley disease control, has exacerbated the problem of controlling disease levels. Cultivar choice offers some option to growers but the optimal control may be achieved using IPM programmes containing the multi-site fungicide, chlorothalonil.

INTRODUCTION

Barley is the second most important cereal crop grown in the UK with nearly 300,000 ha were planted in 2017. Ramularia leaf spot (RLS) caused by the fungus *Ramularia collo-cygni* is now a major disease of barley crops in the UK (Havis *et al.,* 2015). Yield losses due to RLS have been estimated at anything from 20% to 70% worldwide, and in the UK losses are estimated to be around 0.5 tha^{-1}. Control depends largely on chemical methods as there are no fully resistant varieties available. The vast majority of symptoms appear in the crop post-flowering. However, at this point in crop development, no fungicides applications are permitted so sprays have to used prophylactically. Recently a decline in the field efficacy of some fungicides has been noted in European countries (M. Hess, pers comm). One alternative to chemical control is Integrated Pest Management (IPM) which involves a number of approaches such as determining acceptable disease levels, preventative cultural practices, monitoring, biological control, and lastly, responsible use of fungicides. In previous work biological control used on its own has not given consistent control (AHDB*,* 2014). Non-chemical seed treatments have been shown to have some effect on RLS levels in Scottish trials (Havis *et al.,* 2012) and, the use of elicitors to control barley diseases has also been demonstrated in previous work (Walters *et al.,* 2012).

MATERIALS AND METHODS

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Efficacy trials

A replicated field trial was undertaken at Drumalbin farm, Lanarkshire in 2017. Spring barley (cv. Concerto) was sown in 10m x 2m plots in a randomised block design. The trial received an overspray of 1 lha^{-1} chlorothalonil and 1.25 lha^{-1} pyraclostrobin at GS30. The following fungicides treatments were applied to the crop: prothioconazole + bixafen (pro+bix), prothioconazole (pro), chlorothalonil (chlor), fluxapyroxad (flux), chlorothalonil + pethiopyrad (chlor+pent), isoprazam (iso) and pethiopyrad (pent). The fungicides were applied at full recommended rate, one half full rate and one quarter full rate. Fungicide treatments were applied at GS45-49. Ramularia leaf spot levels were assessed at GS80 in the top two leaf layers. The trial was taken to yield and yields were converted to tonnes per hectare at 85% dry matter.

Variety trials

Ramularia leaf spot levels were assessed visually in AHDB Recommended List trials in northern Britain and Northern Ireland in 2017. Disease levels were assessed between GS75 and GS85 in untreated and treated plots for both winter and spring barley crops. Treated plots received a full fungicide programme. Disease was assessed by one person to avoid variation due to individual assessors.

Sensitivity assays

R. collo-cygni isolates were produced from infected leaf samples from SRUC trial sites in 2012, 2015 and 2016. Conidiophores were picked from infected leaves using a dissecting needle. Isolates were maintained on PDA agar. They were tested for sensitivity to the fungicides pro, flux and chlor using the multi-well plate assay developed by Piotrowska *et al.* (2016). EC_{50} values were log-transformed and plotted in a box plot to show the range of figures for each fungicide/year.

Integrated Pest Management trials

Two spring barley trials were carried out in 2017 to investigate the effect of IPM schemes on disease control and yield. The trials were undertaken at Cauldshiel, East Lothian and Drumalbin farm, Lanarkshire. Seed (cv. Propino) was treated with an elicitor (Regalia ®) or biological (Companion ®) seed treatment and sown in 10m x 2 m plots. The plots were then managed as a conventional crop with a fungicide programme (bix + pro at 0.5 lha^{-1} plus pyraclostrobin (pyr) at 0.5 lha^{-1} at GSZ 31 followed by pro (0.4 lha^{-1}) plus chlor (1.0 lha^{-1}) at GS53 or using an IPM scheme Regalia (2.5 lha^{-1}) at GS24, followed by pro+bix+pyr at 0.25 lha^{-1}, then pro (0.25 lha^{-1}) + chlor (0.5 lha^{-1}) at GS53. Disease levels were assessed during the trial and area under disease progress curve (AUDPC) values calculated. Plots were taken to yield and figures converted to tha^{-1} at 85% DM.

RESULTS

Ramularia leaf spot levels were high at the Drumalbin site in 2017. Untreated disease levels were over 14% in the upper leaf layers (Figure 1). The only spray which gave significant control of RLS was the chlor treatment. The chlor + pent treatment had reduced disease levels but greater variation between plots. Yields were increased by a number of treatments but in this trial none were significantly different from the untreated (LSD [P=0.05], 0.54 tha^{-1}).

RLS levels were variable across the all of the UK in 2017 with reports of disease in the drier parts of England as well the wetter northern parts of the UK. Mean disease levels range from between 1% and 4 % for the northern trial sites (Figure 2). Lowest disease levels were recorded in cvs. KWS Cassia and Volume and highest levels in cv. California.

Levels of RLS were higher in spring barley than winter barley in 2017. Mean figures ranged from 7% to 10% (Figure 3). The highest levels were seen in cvs. Concerto and Olympus while cvs. RGT Asteroid and KWS Sassy had the lowest disease levels (Figure 3).

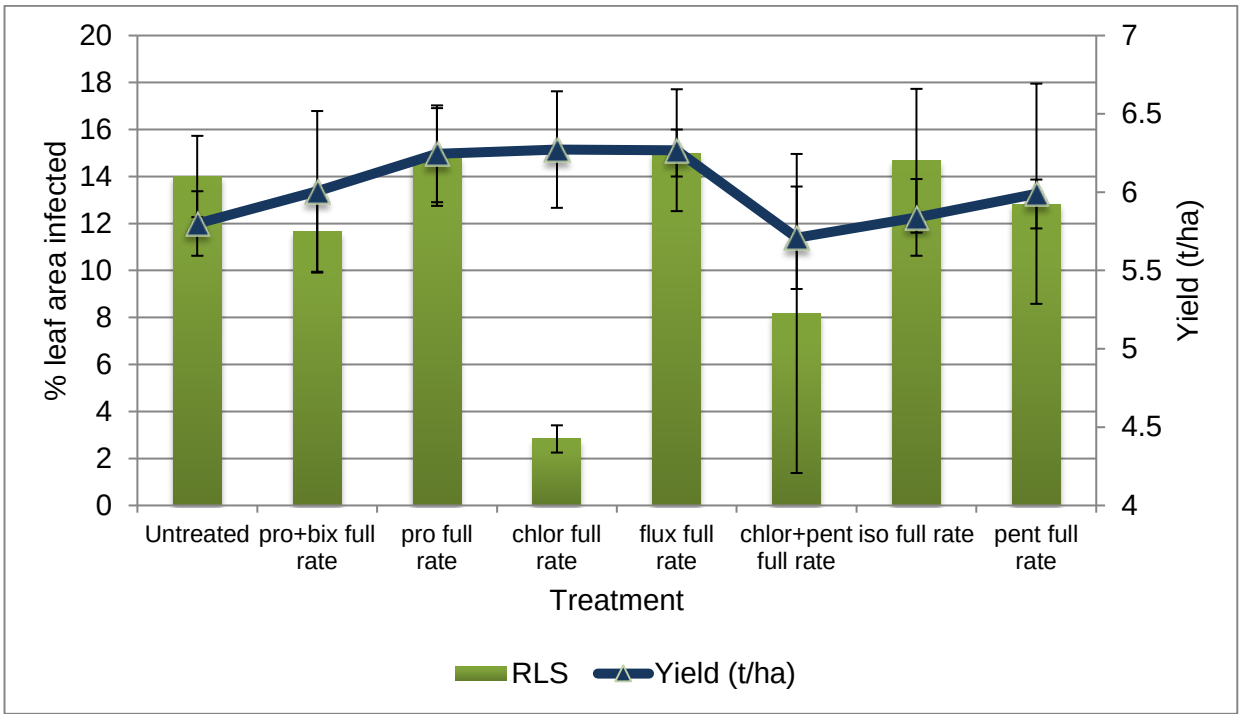

Figure 1. Control of Ramularia leaf spot in Spring barley (cv. Concerto) and yield response at Lanark trial site in 2017.

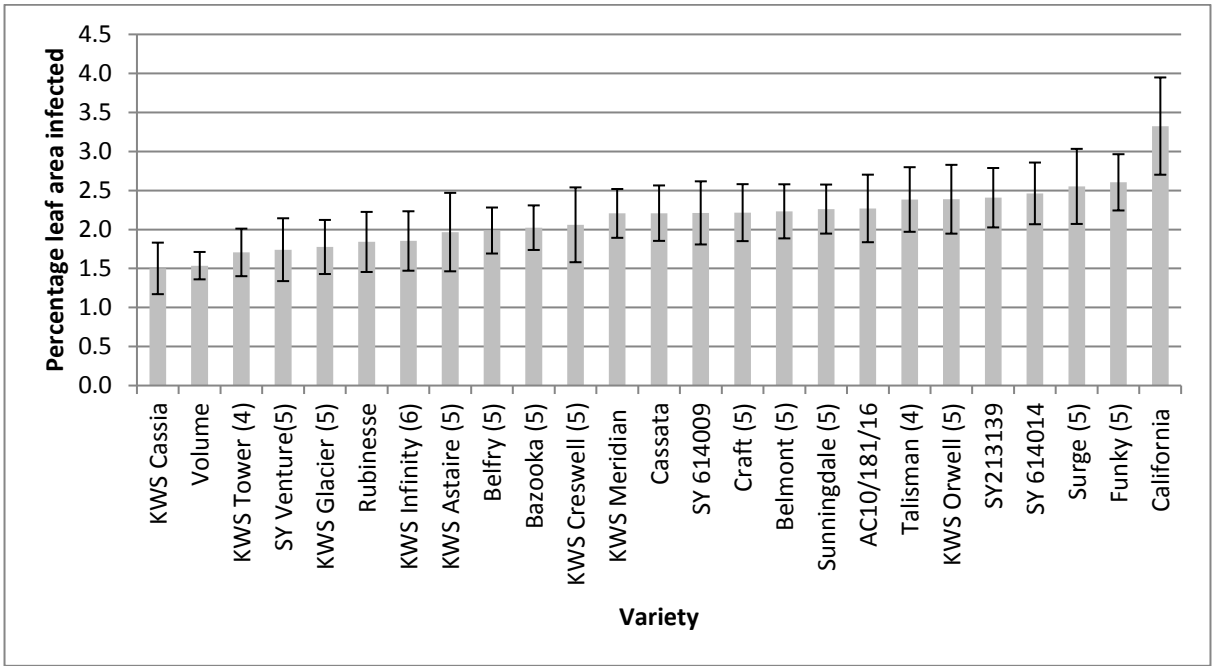

Figure 2. Levels of Ramularia leaf spot (RLS) in winter barley trials (northern Britain) in 2017. Figures in brackets are official AHDB ratings

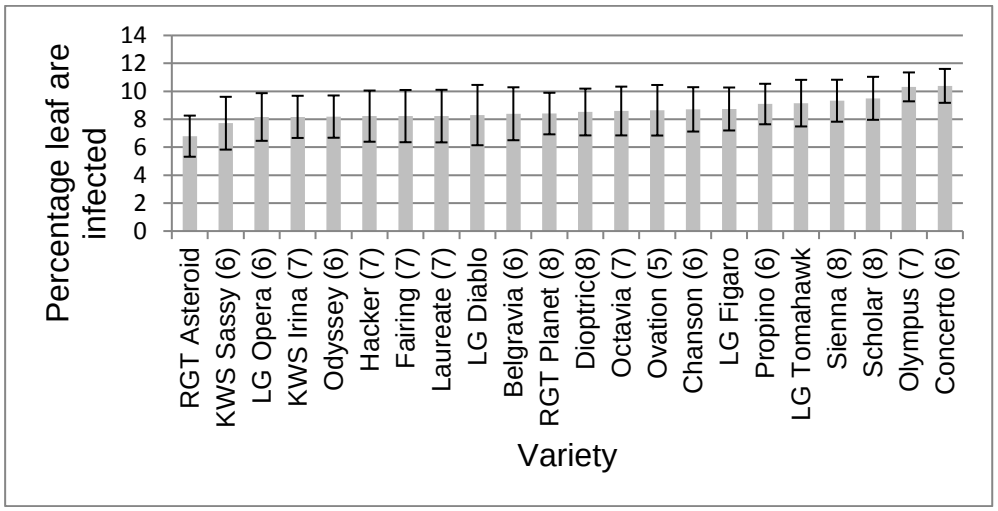

Figure 3. Levels of Ramularia leaf spot (RLS) in spring barley trials (northern Britain) in 2017.

Figures for the sensitivity of *R. collo-cygni* to the three fungicides showed a significant shift between 2012 and 2016 (Figure 4). In 2012 the mean values and ranges were very similar for the pro, chlor and flux. However, by 2016 the fungus was much less sensitive to the flux and the mean values for pro were closer to the flux than the chlor. The values for chlor had only showed a small shift in the mean value over the years (Figure 4).

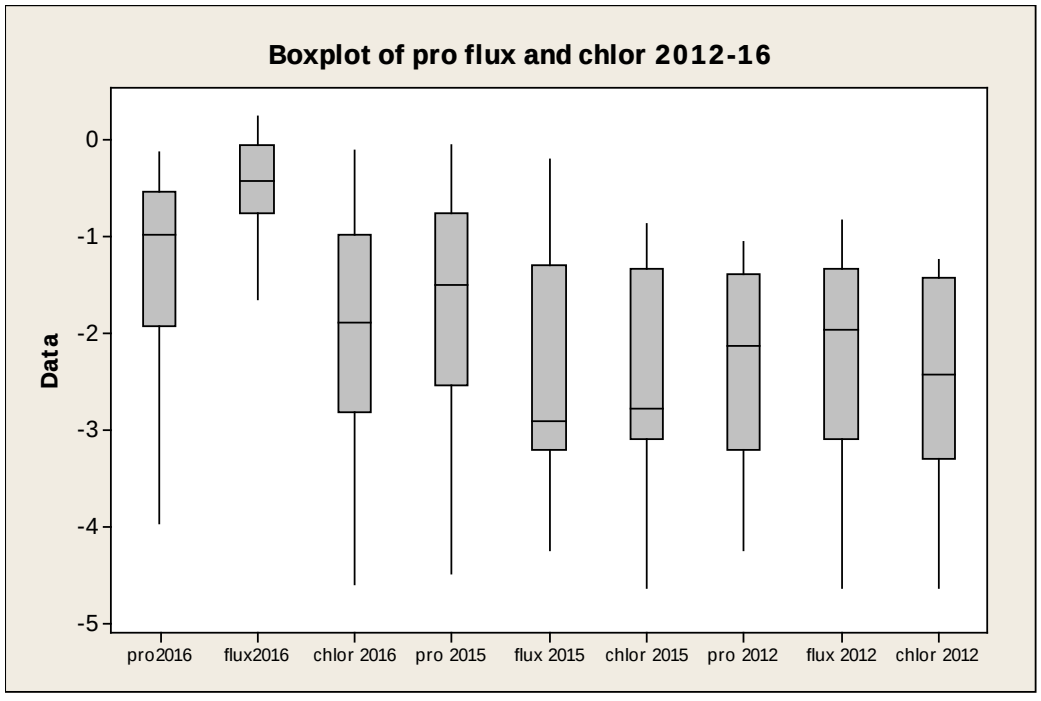

Figure 4. Sensitivity of *Ramularia collo-cygni* isolates to three widely used fungicides (2012-16)

The results from the IPM trial showed that RLS could be managed effectively using an IPM programme or conventional programme, providing both contained chlorothalonil. The seed treatments alone had little effect on RLS in the crop.

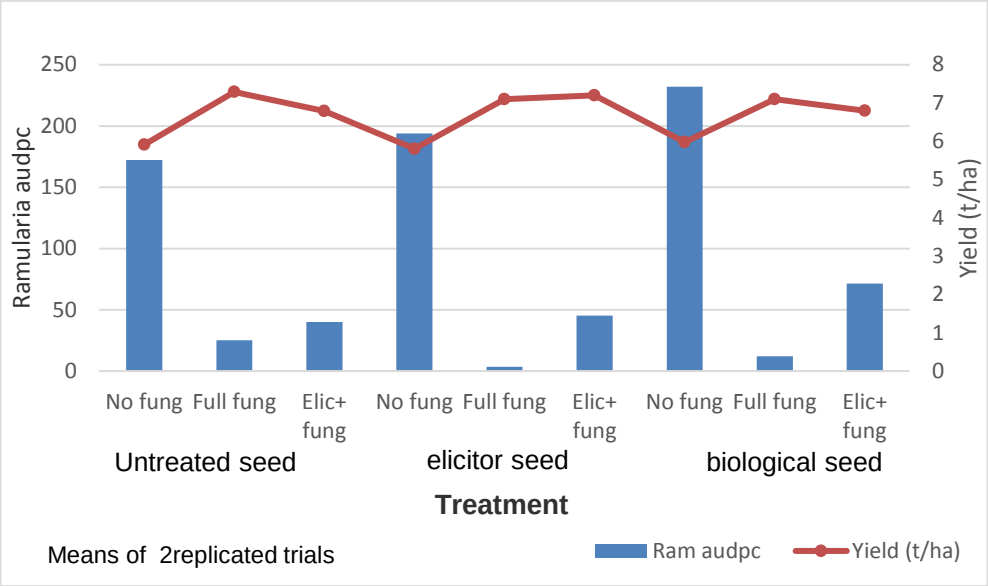

Figure 5. Control of Ramularia leaf spot in spring barley (cv. Propino) in 2017

DISCUSSION

Ramularia leaf spot emerged as a major pathogen of barley in the last 15 years (Havis *et al.,* 2015). In recent years there have been reports of the appearance of mutations in the fungus conferring reduced sensitivity to the succinate dehydrogenase inhibitors (SDHI) (FRAG, 2017). The fungus was described as medium to high risk of developing resistance to this group of fungicides (Piotrowska, 2014). The results from the field trial (Table 1) indicate that field efficacy of the straight SDHI products has reduced to practically nothing. Similar results were obtained in the AHDB Fungicide Performance trial in 2017. The straight triazole product (pro) also had no effect on disease levels. The products containing a mix of traizole +SDHI (pro+bix) gave marginally better control in this trial but only gave a 3% reduction in RLS levels. The only significant control of RLS was achieved by the chlorothalonil. These findings were not a complete surprise and the data on fungicide sensitivity (Figure 4) combined with reports from Germany (Bayer, 2017) indicated a major shift in fungicide efficacy. It was believed *R. collo-cygni* would follow a similar resistance pattern to *Zymoseptoria tritici* as the two fungi are genetically similar and developed resistance to the quinone outside inhibitor (QoI) fungicides at the same time (Fountaine & Fraaije, 2012). A slow decline has been reported in triazole efficacy against *Z. tritici* over time but this decline has been gradual and SDHI efficacy is still high (Cools *et al.,* 2011). The data in Figure 4 indicated shifts in sensitivity of the fungus to a triazole and SDHI fungicide but the shift was far greater for flux than for pro. The data presented from the field trial in combination with the sensitivity data shows that the only effective fungicide against *R. collo-cygni* is the multi-site chlorothalonil. Cultivar resistance figures to RLS for winter and spring barley are now available in the AHDB Recommended List and online (AHDB, 2018). The data presented in Figures 2 and 3 show that there are some differences between varieties in terms of resistance to symptom expression but no variety has a rating above 7 in the current list. Given the pressure on fungicides to control the disease more varietal resistance would be a welcome help to farmers. Unfortunately, new varieties produced by new crosses may take up to 8 years to make it to commercial practice (JHI, 2018). Controlling plant disease using integrated systems is now the aim of European governrnents. The UK and Scottish governments have both signed up to this policy initiative. (Scottish government, 2016). This approach utilises a number of alternatives to remove reliance on chemical control. The data presented in Figure 5 indicates that elicitor seed treatments in combination with an elicitor and reduced rate fungicide programme can give

significant disease control and a yield response similar if not superior to the conventional fungicide programme. The programmes all contained chlorothalonil at the latest spray timing and this will have been a major influence on the control achieved. More trials are required to show that this response holds across seasons and cultivars and also to optimise the combination of elicitors and fungicides. Previous work on elicitors in barley has shown that disease responses are influenced by cultivar (Walters *et al,* 2012). Given the results presented here it is apparent that in the short-term control of RLS will depend on judicious and appropriate use of chlorothalonil either in a conventional or IPM system until greater cultivar control is available.

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DETERMINING THE ROLE OF THE PHYTOTOXIC SECONDARY METABOLITE RUBELLIN D IN THE PATHOLOGY OF *RAMULARIA COLLO-CYGNI*, THE FUNGUS RESPONSIBLE FOR RAMULARIA LEAF SPOT DISEASE OF BARLEY

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Summary: The non-host specific phytotoxin rubellin D is produced by the pathogenic fungus *Ramularia collo-cygni*, the agent responsible for Ramularia leaf spot (RLS), an important disease of barley (*Hordeum vulgare*) in northern Europe. Development of RLS symptoms in crops has been associated with the release and action of phytotoxins such as rubellin D in the host plant despite no supporting evidence of this interaction. Here, we infiltrated leaves of barley landraces with rubellin D to investigate the link between RLS and this phytotoxin. Results suggest that rubellin D sensitivity and RLS susceptibility may not be directly linked but other factors might determine the outcome of the interaction between barley and *R. collo-cygni*. Research using the model plant *Arabidopsis thaliana* to determine the mode of action of rubellin D *in planta* is on-going.

INTRODUCTION

The disease Ramularia leaf spot (RLS), caused by the Dothideomycete fungus *Ramularia collo-cygni* (Rcc), has now been recognised as a major disease of barley in temperate regions worldwide. RLS develops late in the growing season, usually after flowering has occurred leading to typical yield losses of about 20-40% (Havis *et al*., 2015). Furthermore, RLS reduces grain quality which results in increased screenings and subsequent devaluation of the crop (McGrann & Havis, 2017). Early diagnosis of RLS is difficult due to the endophytic nature of Rcc for most of the growing season until the pathogen becomes necrotrophic as a response to unknown stresses, and typical disease symptoms appear (Walters *et al*., 2008).

Many plant pathogenic fungi deploy toxic secondary metabolites that can influence the success of infection and severity of disease development. Victoria blight of oats, caused by another Dothideomycete fungus, *Bipolaris victoriae* (syn. *Cochliobolus victoriae*), is dependant of the action of the host specific toxin victorin with the disease developing only on cultivars sensitive to victorin (Lorang *et al*., 2004). Similarly, cercosporin, a non-host specific toxin produced by a wide range of *Cercospora* species influences disease severity caused by *Cercospora nicotianae* (Choquer *et al*., 2005).

The infection biology of Rcc is now fairly well understood (Kaczmarek *et al*., 2017); however, the critical determinants of the interaction between Rcc and its host at the molecular and cellular level remain largely elusive. Despite the observation of plant cell death away from Rcc hyphae suggesting the action of a phytotoxin (Kaczmarek *et al*., 2017); the role of the secondary metabolism and in particular that of the rubellin toxins have not been investigated *in planta*. Rubellins are light-activated, anthraquinone toxins produced by Rcc (Miethbauer *et al*., 2003). Light-dependent phyto-toxicity of rubellins (Heiser *et al*., 2004) combined with the observations that light is a major determinant of RLS severity (McGrann & Brown, 2018) led to

the hypothesis that rubellin toxicity was involved in symptom development. The recent discovery that reactive oxygen species (ROS), primarily hydrogen peroxide (H_2O_2) mediate symptoms appearance (McGrann & Brown, 2018) further support the possible link between rubellin and RLS development as previous *in vitro* studies reported rubellin-induced ROS-mediated fatty acid peroxidation (Heiser *et al*., 2003 2004). Currently, no studies have investigated the nature of the link between RLS and rubellin. Considering the important role phytotoxins play in mediating the interaction between a pathogen and its host, understanding their mode of action may provide insight into disease development. Therefore, the effect of rubellin D, the most stable rubellin produced by Rcc, was investigated in barley and in the model plant *Arabidopsis thaliana*.

MATERIALS AND METHODS

Plant material used in this study

Barley landraces were selected for infiltration with rubellin D based on disease assessment in field conditions in 2012 at the SRUC trial site in Lanark, Scotland. Two moderately resistant spring barley landraces, coded 5.18.13 and 8.161.13, showing less than 5% RLS leaf coverage, two landraces with an intermediate RLS response, 9.189.13 and 9.221.13, exhibiting between 5 and 10% RLS leaf coverage, and the susceptible line 7.91.13 exhibiting more than 10% RLS leaf coverage were used. *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) plants grown at 18°C, 16h light photoperiod, 80% relative humidity and 250 mol m^{-2} s^{-1} light intensity, were also used in this study to compare plant cell death response between a non-host and a host plant.

Infiltration solutions

A stock solution (10 mM) of rubellin D (Enzo Life Sciences, Farmingdale, NY, USA) was obtained by dissolving the toxin in dimethyl sulfoxide (DMSO; Sigma, Dorset, UK) and stored at -20°C. Working concentrations (50 µM) were obtained by dissolving the stock solution in 10 mM magnesium chloride ($MgCl_2$; Sigma, Dorset, UK) on the day of use. A 10 mM $MgCl_2$ solution supplemented with DMSO equivalent to that of the rubellin D treatment was used as a control.

Chemical infiltrations of barley and *A. thaliana* leaves

Leaf infiltrations were performed on the prophyll leaf of two week old barley plants at GS 11-12 (Zadoks *et al*., 1974) using a 1 mL needleless syringe and pressure infiltrating 100 µL of rubellin D on the abaxial side of the leaf on either side of the central vein at mid-length of the leaf. Infiltrations of the full area of *A. thaliana* leaves were performed on the abaxial side of the third or fourth leaf of 4-week-old *A. thaliana* plants. Lesions were allowed to develop on barley and *A. thaliana* for 72 and 24 hours respectively, at 18°C, 80% relative humidity, 250 mol m^{-2} s^{-1} and a 16h/8h light/dark photoperiod prior to being photographed and measured using the ImageJ software (Abràmoff *et al*., 2004).

Data analysis

Data were analysed in GenStat 16th edition (VSN International, Rothamsted, UK). Three independent experiments consisting of ten leaves each were carried out for the rubellin D infiltration of barley landraces. Raw data were analysed by general linear model, using the model Landraces*Experiment.

RESULTS

To investigate the role of rubellins in disease symptom development, rubellin D was infiltrated into the leaves of five barley landraces, which are lines adapted to local environmental and agricultural conditions, previous identified as expressing differential RLS susceptibility in field trials. Rubellin D infiltrations in barley leaves failed to reproduce typical disease symptoms as the lesions observed showed a necrotic area consisting of dehydrated tissue surrounded by a chlorotic brown area instead of rectangular, reddish brown necrotic tissue ringed by a yellow halo typical of RLS symptoms (Figure 1).

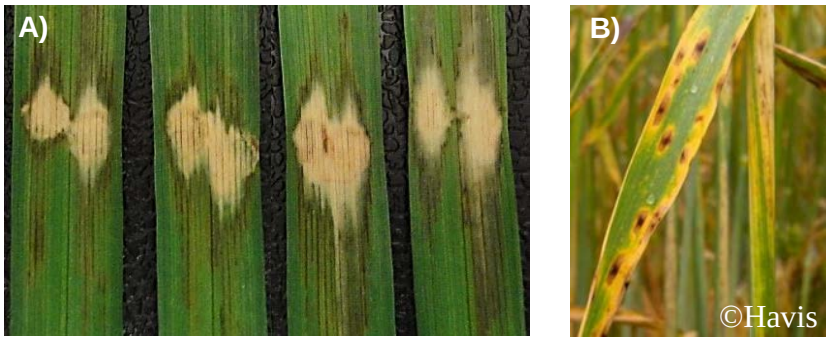

Figure 1. Comparison of Rubellin D-induced lesions on barley cv. Concerto (A) and typical Ramularia leaf spot symptoms (B).

The two RLS resistant landraces, 5.18.13 and 8.161.13, exhibited similar sensitivity to rubellin D based on the size of the lesion formed 24 hours post-infiltration to that observed for the susceptible line, 7.91.13, whereas the two landraces showing intermediate resistance to RLS, 9.189.13 and 9.221.13, showed significantly lower sensitivity to the toxin ($p<0.05$) than the resistant ones (Figure 2).

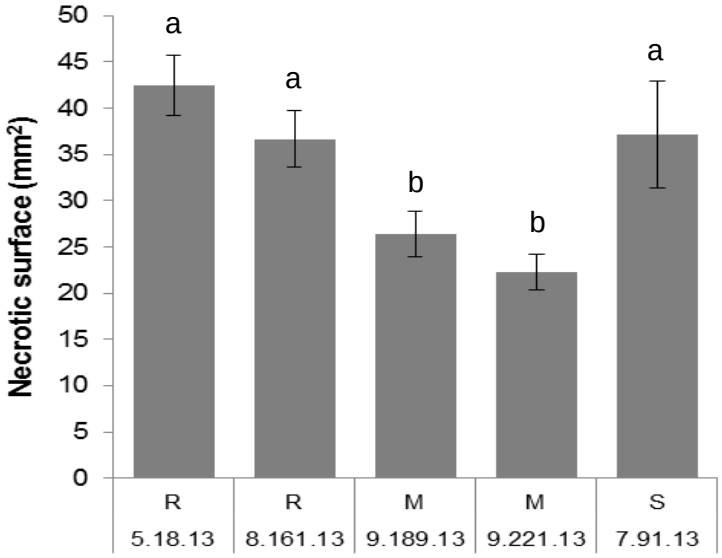

Figure 2. Rubellin D-induced lesion size observed in barley landraces. Resistance (R), susceptibility (S) or intermediate resistance (M) to RLS is indicated above the line's name. Bars sharing the same letters were not significantly different at $p<0.05$. Data represent predicted mean ± SE of three independent experiments.

Rubellin-induced cell death appears to be more complex than previously thought as sensitivity to the toxin, highlighted by lesions development, does not necessarily relates to RLS susceptibility. Investigating the mode of action of rubellin D *in planta* may therefore provide insights into understanding the nature of the interaction between Rcc and its host. To assess the suitability of the model plant *A. thaliana* to study the mode of action of rubellin *in planta*, rubellin D-induced cell death phenotype was compared between barley and *A. thaliana* leaves (Figure 3).

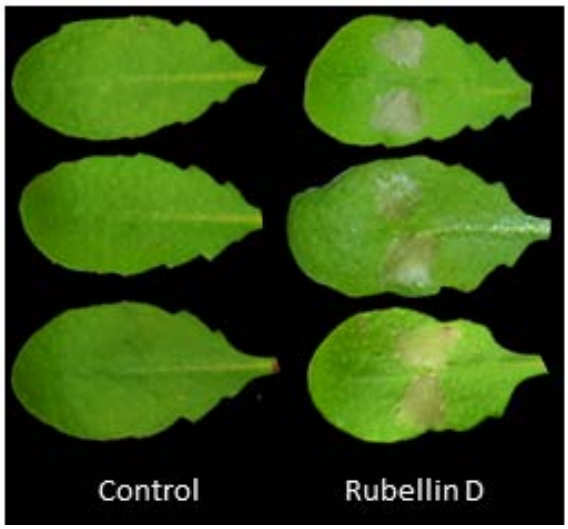

Figure 3. Rubellin D–induced necrosis observed after infiltrations on *A. thaliana* Col-0

The phenotype observed following rubellin D infiltration in *A. thaliana* exhibited well contained lesions consisting of dehydrated, collapsed tissue. This finding suggests that *A. thaliana* should be a suitable model to interrogate the interaction between rubellin D and plants to determine which pathways are targeted by this toxin.

DISCUSSION

Lesions formed by rubellin D infiltration into barley leaves did not resemble characteristic RLS lesions (Figure 1). This result contrasts with previous observations that toxins produced by plant pathogenic fungi were able to reproduce disease symptoms. The non-host specific anthraquinone dothistromin produced by the pine pathogen *Dothistroma septosporum* is structurally related to rubellins and is able to reproduce typical red band needle blight symptoms when infiltrated into pine needles (Kabir *et al*., 2015). Mutants of *D. septosporum* impaired in dothistromin production where still able to induce disease symptoms although the severity of the symptoms was reduced (Kabir *et al*., 2015). Similarly, cerato-ulmin, a toxin involved in the development of the Dutch elm disease, caused by *Ophiostoma ulmi* (syn. *Graphium ulmi*), induces similar symptoms to that observed on diseased plants (Bowden *et al*., 1996). These results highlight that close relationships between phytotoxins and their involvement in disease development exist. However, considering that rubellin D failed to reproduce typical RLS symptoms the link between rubellin and RLS appear to be more complex than previously thought.

Although the *in vitro* mode of action of rubellin D, which involves ROS-mediated lipid peroxidation, is similar to that previously reported for the light-activated toxin cercosporin

(Daub & Ehrenshaft, 2000), their role in disease development may differ greatly. Rubellin D infiltrations into barley landraces exhibiting differential RLS susceptibility showed that high sensitivity to the toxin did not necessarily imply high disease susceptibility (Figure 2), suggesting no clear link between RLS and rubellin. This finding contrasts with that of previous studies showing that a direct correlation existed between disease susceptibility and cercosporin sensitivity in *Cercospora oryzae*, the causative agent of narrow brown spot disease (Batchvarova *et al*., 1992). Similarly, sugar beet cultivars susceptible to Cercospora leaf spot disease exhibited higher cercosporin sensitivity than resistant varieties (Balis & Payne, 1971). Recently, cercosporin sensitivity in oilseed rape (*Brassica napus*), brown mustard (*B. juncea*) and wild radish (*Raphanus raphanistrum*) was linked with susceptibility to white leaf spot disease caused by the Dothideomycete *Pseudocercosporella capsellae* (Gunasinghe *et al*., 2016). The observation that rubellin failed to reproduce disease symptoms suggests that rubellin may not be the only determinant in the interaction between Rcc and its host. However, the plant response to rubellin D was only investigated in a small sample of landraces; therefore a more extensive study using a range of barley genotypes and commercial varieties with differing susceptibility to RLS is required to confirm whether barley sensitivity to rubellin D is indeed not correlated with RLS susceptibility. It should be noted that the amount of rubellin D infiltrated to observe a phenotype in barley leaves was approximately 50 times higher than the level previously reported in naturally infected barley leaves (Miethbauer *et al*., 2003). Therefore, some aspect of rubellin biology may have been amplified in this study.

Infiltrations of *A. thaliana* with rubellin D were performed to assess the suitability of the model plant to study rubellin-induced cell death mechanism. The phenotype induced by rubellin D infiltration in *A. thaliana* was similar to that observed on barley leaves (Figure 3) therefore; *A. thaliana* appears to be a suitable model to investigate the mode of action of the non-host specific rubellins *in planta*. Rubellin D-induced phenotype is similar to that observed in mutants exhibiting spontaneous programmed cell death. In these mutants, spontaneous lesion formation is dependent on salicylic acid, one of the key players in the plant immune response (Devadas *et al*., 2002). Further investigating the mode of action of rubellin D at the cellular level, particularly the role of the salicylic acid pathway in rubellin-induced lesion formation may increase our understanding of the interaction between Rcc and its host, and potentially help identify breeding targets for RLS resistance.

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UNDERSTANDING THE CONTROL OF YIELD FORMATION IN A TWO- AND A SIX-ROW WINTER BARLEY VARIETY TO TARGET DISEASE MANAGEMENT

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Summary*:* A field experiment was carried out to investigate if fungicide yield response differed between a conventional two-row and a hybrid six-row winter barley variety. The experiment was sown at sites in , Ireland and Scotland in three seasons in a split-split plot design with two seed and N rates in the main plots, a hybrid six-row (Volume) and conventional two-row (KWS Tower) variety in the sub-plots and fungicide treatment as the sub-sub plots. The results indicated there was no need to alter fungicide programme based on row type as there was no variety interaction with fungicide treatment. Additionally, the average yield of the two varieties was not significantly different. In a further experiment the source-sink balance during grain filling in the two varieties mentioned was investigated using manipulations of assimilate supply and sink size The results showed that the six- and two-row variety had a similar source-sink balance during grain filling, with evidence of a co-limitation of yield by source and sink as each of the manipulations had significant effects on final grain weight but not to the same degree as the manipulation of assimilate supply during grain filling.

INTRODUCTION

Yield formation can be analysed in terms of sink or source limitation of grain filling. 'Sink-limitation' refers to a limitation imposed by the number of grains the crop can set and their storage capacity. 'Source-limitation' refers to situations where the supply of assimilates for grain filling is insufficient to meet the grain storage capacity. Alternatively grain filling can be co-limited by both source and sink (Borrás, Slafer, & Otegui, 2004). Understanding how yield is formed in cereals is central to the design of effective disease management programmes. In the cool temperate climates of the UK and Ireland, the control of wet weather diseases is fundamental in achieving the maximum yield potential of cereal crops. Disease epidemics can have varying impact on yield depending on when in the crop's life cycle the epidemic occurs. Therefore, efficient and targeted disease management strategies are fundamental parts of the management practices in these regions. Evidence that yield in two-row winter barley is sink limited (Bingham, Blake, Foulkes, & Spink, 2007) has led to formation of a disease management strategy focusing on protecting the canopy during the period when the sink capacity is being determined. In wheat, a study conducted in the cool temperate climate of the UK suggested that that the source-sink relationship operated in close balance (Beed, Paveley, & Sylvester-Bradley, 2007), while a recent study carried out in Ireland suggested that in certain variable seasons, there is the potential for winter wheat crops to be source limited (Lynch et al., 2017). These findings are reflected in the disease control strategy for wheat, in which the focus is on protecting the upper leaves of the canopy to maximise production of assimilates during grain filling rather than the development of a large sink capacity.

In recent times, there has been increased utilisation by growers of hybrid six-row barley varieties for a number of reasons, such as their excellent performance in national recommended list trials in Ireland and the UK. Six- and two-row winter barley differ in their yield components. Six-row types produce fewer ears m^{-2} and more grains ear^{-1} (leading to an overall greater number of grains m^{-2}, but a lower average grain weight when compared with two-row varieties (Garcia del Moral, Garcia del Moral, Molina-Cano, & Slafer, 2003). As these varieties are relatively new to the market there has been very little independent research conducted to support management practice, with growers currently implementing the same fungicide timing strategy for both row types. It can be hypothesised that because of the larger number of grains produced that grain filling in six-row types will be less sink-limited than in two-row types. Moreover, this may necessitate a different approach to the management of six-row varieties in which the emphasis should be placed on maximising canopy lifespan and assimilate production during grain filling rather than on the development of sink capacity. Currently there is insufficient understanding of the source-sink balance of six- compared to two-row varieties and the response of these row-types to fungicide timing.

In order to investigate if fungicide timing needs to be altered based on ear type a field trial was carried out in Ireland and Scotland in 2014/15, 2015/16 and 2016/17 to investigate the yield response to a range of fungicide timings in both a conventional two-row and hybrid six-row winter barley variety.

Further to this a field experiment was conducted in 2015/16 and 2016/17 in Ireland, to determine the source-sink balance of a hybrid six-row and conventional two-row variety through manipulation of their source:sink ratio during grain filling

MATERIALS AND METHODS

Fungicide response experiments

The trials were carried out in 2014/15, 2015/16 and 2016/17 across two sites Teagasc, Oak Park, Carlow, Ireland and SRUC, Edinburgh, Scotland. The trials were laid out in a split-split plot design with four replications, with the main plot being seed and N rate. Here two programmes were used (Table 1), the first was the standard recommended rates of both seed rate and N, the second was the standard rates plus 25% of both inputs in order to test the yield response to fungicide at higher grain numbers. The N fertiliser was applied in two applications, 30% of total at growth stage (GS) 25-29 and 70% of total at GS 31 (Zadoks, Chang, & Konzak, 1974).

Table 1. Seed rate & Nitrogen programme

Variety Type	Variety	Seed Rate seeds/m2		N kg/ha	
		Standard	+25%	Standard	+25%
Two-row	KWS Tower	360	450	190	230
Six-row	Volume	270	360	190	230

The sub plot was variety, two varieties were used, KWS Tower (conventional two-row) and Volume (hybrid six-row). The sub-sub plot was fungicide timing, with programmes listed below

1. Untreated
2. GS 31/2 (1 spray)
3. GS 31/2, GS 49 (2 spray)
4. GS 25, GS 31/2, GS 49 (3 spray)
5. GS 25, GS 31/2, GS 49 plus GS 69 (4 spray)

The GS 25 timing used 0.4 L/ha of prothioconazole 250g/litre (Proline®, Bayer Crop Science, Monhem am Rhein, Germany) and 0.4 L/ha of fenpropimorph 750 g/litre (Corbel®, BASF, Ludwigshafen, Germany). The GS31/32 and GS39/45 used 1.8 L/ha of epoxiconazole 41.6 g/litre, fluxapyroxad 41.6 g/litre and pyraclostrobin 66.6 g/litre (Ceriax®, BASF, Ludwigshafen, Germany). The GS69 timing consisted of 0.4 L/ha prothioconazole 250g/litre and 1 L/ha of chlorothalonil 500 g/litre (Bravo®, Syngenta, Basel, Switzerland). All other crop management inputs were according to standard farm practice.

Foliar disease assessments were conducted at various growth stages during each season on 10 shoots per plot. The top 3-4 fully expanded leaves were assessed on each shoot, with both individual foliar diseases and percentage green leaf area assessed. Before the plots were harvested grab sampling for assessment of harvest index was conducted. Grabs of about 10-15 shoots at five locations within each plot were conducted. Samples were divided into straw (leaf and stem) and ears, both shoot and ear counts were conducted. Samples were weighed then dried in an oven at 70°C for 48 hours. Samples were then re-weighed and ears were threshed with all grain collected and weighed. Grab sample data allowed ears m^{-2} and grains per ear to be calculated. Plots were harvest using a Sampo 2010 (Sampo Rosenlew Ltd, Finland) plot combine with yield expressed at 85% dry matter. A grain sample was taken from each plot for assessment of screenings through a 2.5mm sieve and thousand grain weight (TGW) using a grain counter.

Source-Sink experiments

A field trial to investigate the source-sink balance in Tower and Volume was carried out in 2015/16 and 2016/17 at Teagasc, Oak Park, Carlow, Ireland on plots within the experiment investigating the fungicide response. In this experiment plots of both a six- and two-row winter barley variety were sown at the standard seed rate for each variety and received the standard N rate (Table 1). The fungicide treatments used were untreated and the 4 spray programme. The treatment period for the source-sink manipulations was 14 days post anthesis until GS87. Three different source-sink manipulations were used;

I. Shading the crop canopy, 2 x 3m shades were placed 0.5 m above the crop canopy.
II. De-graining the upper half of the ear. All ears along a 0.5m length of row were de-grained at 2 locations within each plot. The de-graining method involved removing the top half of the ear without damaging the lower grains or the awns attached to these grains.
III. Opening up a single row by pushing back adjacent rows along a 3m row length to allow greater light interception by the isolated row.

Shading was imposed only on fungicide treated plots of both varieties, while de-graining and row-opening was imposed on both fungicide treated and untreated plots of each variety. At the end of the treatment period, treated and marked control areas were sampled and brought to the lab where ears were removed, threshed and mean grain weight (MGW) measured using a grain counter.

All statistical analysis was carried out using GenStat 14th Edition statistical software.

RESULTS

Fungicide timing

An initial ANOVA was conducted on each site and season combination individually. These results showed (Table 2) that there was no significant interaction between seed & N rate x variety and fungicide in any of the six site-seasons, thus site-season was treated as a random effect in the ANOVA model for analysis of the whole data set presented in Table 2. The results show that there was no significant effect of increasing seed & N rate on yield (p=0.993) nor were the varieties affected differently by increasing the seed & N rate (p=0.214). Both Volume and KWS Tower produced an average yield across all treatments of 9.2t/ha. While fungicide treatment had a significant effect on yield (p=<0.001) with all programmes having a positive effect on yield, varying the seed & N rate didn't alter the crop's response to fungicide (p=0.175). Interestingly the response to fungicide treatment did not differ between each of the varieties as shown in Figure 1.

Table 2. ANOVA table of fungicide timing effects on yield.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site x block stratum	23	1215.069	52.8291	40.34	
Site x block x Seed & N rate stratum					
Seed & N rate	1	0.0001	0.0001	0	0.993
Residual	23	30.1236	1.3097	1.01	
Site x block x Seed & N rate x Variety stratum					
Variety	1	0.0003	0.0003	0	0.989
Seed & N rate x Variety	1	2.0566	2.0566	1.59	0.214
Residual	46	59.6218	1.2961	2.66	
Site x block x Seed & N rate x Variety x Fungicide timing stratum					
Fungicide	4	386.1547	96.5387	197.78	<.001
Seed & N rate x Fungicide	4	3.1158	0.7789	1.6	0.175
Variety x Fungicide	4	2.8015	0.7004	1.43	0.222
Seed & N rate x Variety x Fungicide	4	0.1394	0.0348	0.07	0.991
Residual	355	173.2788	0.4881		
Total	466	1825.801			

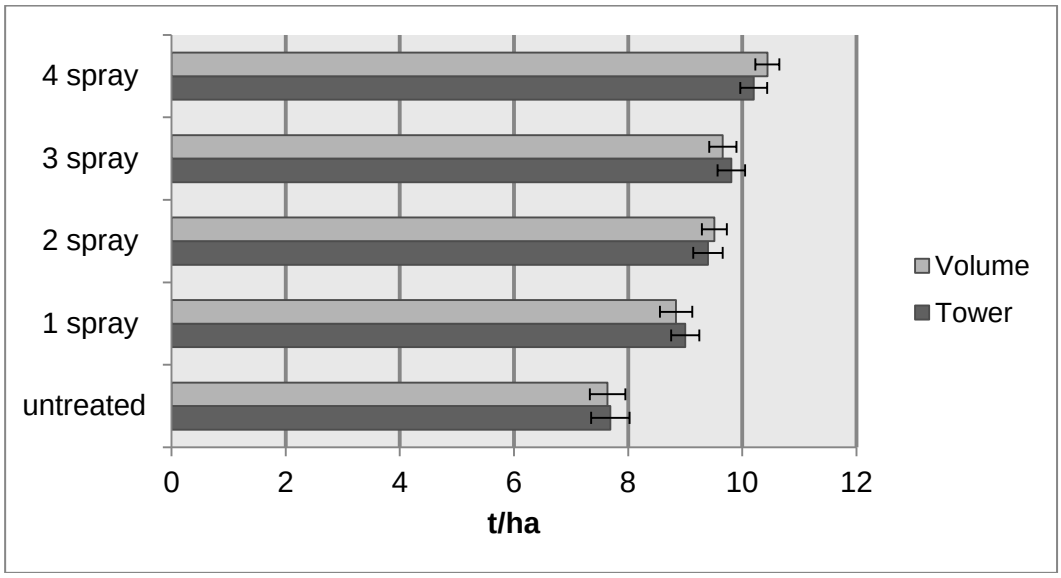

Figure 1. Fungicide programme effects on yield of Tower and Volume averaged across seed rate & N regimes

Table 3. Source-sink manipulation effects on MGW (mg)

	Manipulation			
	Shading	Row-open top	Row-open bottom	De-grain
Control	42.33	38.97	42.06	42.06
Treated	37.87	41.64	44.81	45.51
LSD	1.582	1.044	1.185	1.274
Significance (P)				
Variety	***	***	***	***
Fungicide		***	***	***
Manipulation	***	***	***	***
Variety x Fungicide		***	***	***
Variety x Manipulation	ns	ns	ns	ns
Fungicide x Manipulation		ns	ns	ns
Variety x Fungicide x Manipulation		ns	ns	ns

For interaction terms: *** show significance at <0.001, ns is not significant.

Source-sink

The data presented in Table 3 shows that there was no significant interaction between variety and each type of manipulation, suggesting that the source-sink balance during grain filling was comparable in each variety. Interestingly each manipulation had a significant effect on MGW. Increasing assimilate supply by 50% through de-graining increased MWG by on average 7.4%, while increasing assimilate through row-opening increased final MGW by 9.2% in the

top half of the ear and by 7.9% in the bottom half. A 68% reduction in assimilate supply (shading) caused a 10.6% reduction in grain weight. The lack of a significant interaction between fungicide treatment and manipulation would suggest that disease had no effect on the source-sink balance in either variety. There were significant interactions between fungicide and variety on MGW, caused by a significant increase in MGW from fungicide treatment in KWS Tower only (data not shown).

DISCUSSION

The results presented would indicate that despite their differing yield components the six- and two-row winter barley varieties in this study do not differ in their response to fungicide timing, thus there is no need to alter timing based on row-type. Interestingly these experiments contradicted the national recommended list trials in both the UK and Ireland as the conventional two-rowed variety produced a similar yield compared to the hybrid six-row variety. The finding that both varieties had a similar response to fungicide timing may be explained by their similar source–sink balance seen in the source-sink manipulations. The hypothesis that a six-row type may be less sink-limited than a two-row type was found to be incorrect in this study, in fact each variety had a degree of co-limitation as the relative response in grain dry weight did not match the reduction/increase in assimilate supply during the grain filling period.

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DIFFERENTIAL ADAPTATION OF SPRING BARLEY CULTIVARS TO INVERSION AND NON-INVERSION TILLAGE

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Summary: Spring barley cultivars were grown under different tillage conditions for three or four years and assessed for disease and yield. Overall the higher yielding cultivars under inversion tillage conditions gave reduced yields when grown under non-inversion tillage, whereas low yielding older cultivars showed relatively lower reductions in yield under non-inversion tillage. However, a few cultivars showed preferential yield performance for either inversion or non-inversion tillage irrespective of their overall yield performance. The traits that are attributable to these differences are yet to be identified but are likely to be root-associated. No consistent cultivar or tillage interaction with rhynchosporium symptoms was observed overall.

INTRODUCTION

Elite cultivars of cereals have been bred generally under inversion tillage and high input agronomy resulting in high yield and quality when grown at field scale under similar conditions. Such yield potential gains often translate to other agronomic regimes such as low input, non-inversion tillage or organic. However, particular cultivars gain a reputation as being adapted to such agronomic conditions but these are seldom validated in controlled trials. Comparing wheat cultivars under organic and conventional low and high nitrogen conditions, elite cultivars generally yielded highly under all conditions but a few cultivars were adapted preferentially to either organic or high nitrogen conventional conditions (Newton *et al*., 2017). Here a range of spring barley cultivars was compared, some mixtures or blends and some root hair mutants, across three or four seasons under different soil tillage conditions but primarily comparing inversion and non-inversion tillage for yield and rhynchosporium symptoms.

The soil cultivation platform used was Mid Pilmore at the James Hutton Institute Mylnefield Farm, set up in 2003 (Newton *et al*., 2012) and previously used to show differential response of winter barley cultivars to tillage treatments, primarily differentiating inversion and non-inversion treatments. The site was sown with barley continuously so some soil microbial populations will be skewed but also any effects of previous cropping differences will be minimised. However, It is known that cultivation can affect microbial diversity and distribution (Sun *et al*., 2014; Sun *et al*., 2011) so any cultivar interactions with cultivation treatment will always compound both physical and microbial factors and there is no evidence that this will be further compounded by the microbial community selected by continuous barley.

MATERIALS AND METHODS

Cultivars

Spring barley trials were sown in each year from 2013-2016. Each trial had 35 entries and the same 35 were sown in 2013, 2014 and 2015 trials. These were: Optic (referred to as Optic1), Optic2 (a repeat from a different seed batch), Westminster, Waggon, and Concerto as widely-grown standard comparison cultivars; all six 2-component equal proportion mixtures of Optic (Op), Westminster (We), Waggon (Wa) and Concerto (Co) together with the 4-component equal proportion mixture (Op/Wa, Op/We, Op/Co, Wa/We, Wa/Co, We/Co and Op/We/Wa/Co); three root hair mutants of Optic (T-short root hairs-R, Q-no root hairs-S and V-short root hairs-R); and 20 other cultivars representing a diversity of origins and attributes (Propino, Appaloosa, Riviera, Prestige, Carafe, Scarlett, Tocada, Kennia, Morex, Derkado, Aramir, Bowman, Troon, Vada, Decanter, B83-12/21/5, Golden Promise, Carlsberg, NFC Tipple and Melius). In 2016 11 new cultivars were added (RGT Planet, KWS Sassy, Olympus, Octavia, Sienna, Odyssey, Origin, Fairing, Belgravia, Ovation and Scholar). To make room for these within the trial platform, 11 cultivars had to be removed and these were selected either because they were the 2-component mixtures (six entries) or around the middle of the distribution of responses to cultivation treatment in the 2013-15 trials (Prestige, Carafe, Scarlett, Derkado and B83-12/21/5).

Trial design

Five tillage treatments originally established in autumn 2003 represented different levels of soil disturbance: (T1) zero tillage and (T2) minimum tillage to 7 cm depth where the non-inversion treatments and the inversion or ploughed treatments were followed by power harrowing consisting of (T3) conventional plough to 20 cm depth, (T4) plough to 20 cm followed by compaction by wheeling the entire plot with a Massey Ferguson 6270 tractor fitted with 16.9R-38 rear tyres (8.8 Mg total load, 2.9 Mg wheel load and 110 kPa contact pressure) and (T5) deep plough to 40 cm depth (Newton *et al*., 2012). These treatments were selected to provide different physical constraints to root growth and water availability. Fifteen blocks each 33m × 33m were marked out in an even grid with five blocks in each of three north-south columns representing the three treatment replicates. Blocks were separated from each other by strips at least 3 m wide that were sown with grass seed after the first trial year was sown. Within each of the 15 blocks, half of the trial was winter sown (not reported here) and half spring sown with the 35 entries described above. Plots measured 1.55 m wide× 6.0 m long, reduced to 4.8 m harvested length by plot definition, and were sown at a sowing rate of 360 seed/m2 with an eight-row Hege plot drill with five plots per bed. Nitrogen fertiliser was 350 kg/ha of 22-4-14(7.5SO3) applied as top dressings. Standard pre- and post-emergence herbicide treatments were applied but no fungicide treatments were used. Straw was removed from all of the plots following harvest. The T1 zero tillage treatment was discontinued due to weed problems that compromised comparisons and is not reported here.

Assessments

Diseases were scored on a 1-9 whole plant severity scale (Newton & Hackett, 1994) when above trace levels, and scored again at approximately two-weekly intervals. Scores were both analysed directly and converted to percentage infection and the Area Under the Disease Progress Curve (AUDPC) was calculated. Plots were harvested when ripe using a Wintersteiger plot combine and the grain was dried to constant moisture and weighed.

RESULTS

Disease

Rhynchosporium occurred as the main disease every year but levels were never high and unlikely to have much direct impact on yield. The frequency of scoring varied from year to year so the most appropriate comparison was made using the mean of the raw scores (Fig.1). Conversion of the scores to percentages required transformation to restore a normal distribution of residuals. Differences in the mean rhynchosporium score were significant for year, treatment, cultivar, year*treatment and year*cultivar but neither treatment*cultivar nor treatment*year were significant at any level. Figure 1 illustrates the similarity of responses of the three inversion tillage treatments in all years except 2013 and the trend towards increased infection with minimum tillage in some years (2014 and 2016).

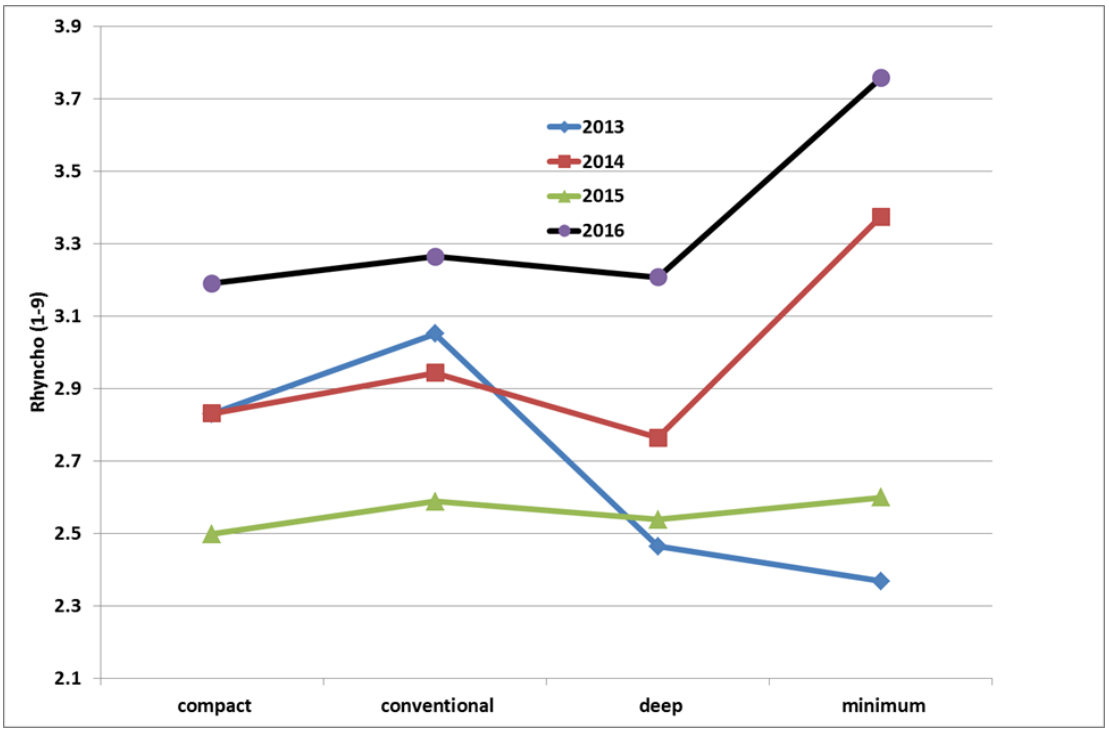

Figure 1. Significant interaction between cultivation treatment and year for rhynchosporium score.

Yield

The 2013, 2014 and 2015 trials were identical in terms of cultivars and agronomy and therefore these were analysed together for yield. As would be expected there were highly significant interactions ($p<0.001$) for year, cultivation treatment and cultivar. Year*treatment, year*cultivar and treatment*cultivar interactions were also all highly significant ($p<0.001$) though not year*treatment*cultivar. Year*treatment clearly shows the lower yield effect of the minimum tillage treatment and the overall difference between years (Figure 2).

The yield*cultivation treatment differences ordered by the mean of the inversion tillage treatments show the marked similarity in performance between the three inversion tillage treatments, the contrasting yield performance of the non-inversion minimum tillage treatment and the variation in non-inversion tillage yield with respect to the inversion tillage yields. The gap between the inversion and non-inversion tillage treatments tends to increase with overall or inversion tillage yield. The lowest yield cultivars tend to differ little in yield response between tillage treatments whereas high-yielding cultivars such as Appaloosa, Waggon and

Concerto, show relatively poor non-inversion tillage yield. The highest yielding four commercial cultivars under minimum tillage (Figure 3) were Westminster, Melius, Concerto and Waggon whilst Bowman, Golden Promise Derkado and Vada were the lowest four for yield.

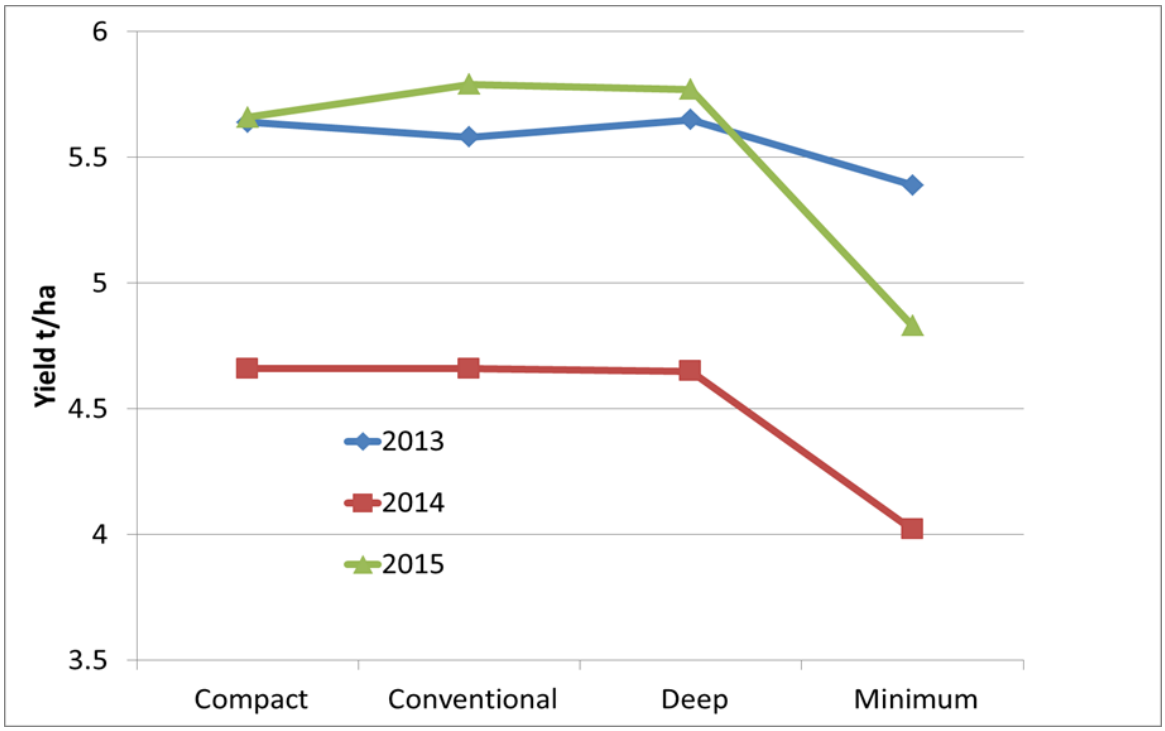

Figure 2. Mean yield response to soil cultivation treatment in each year.

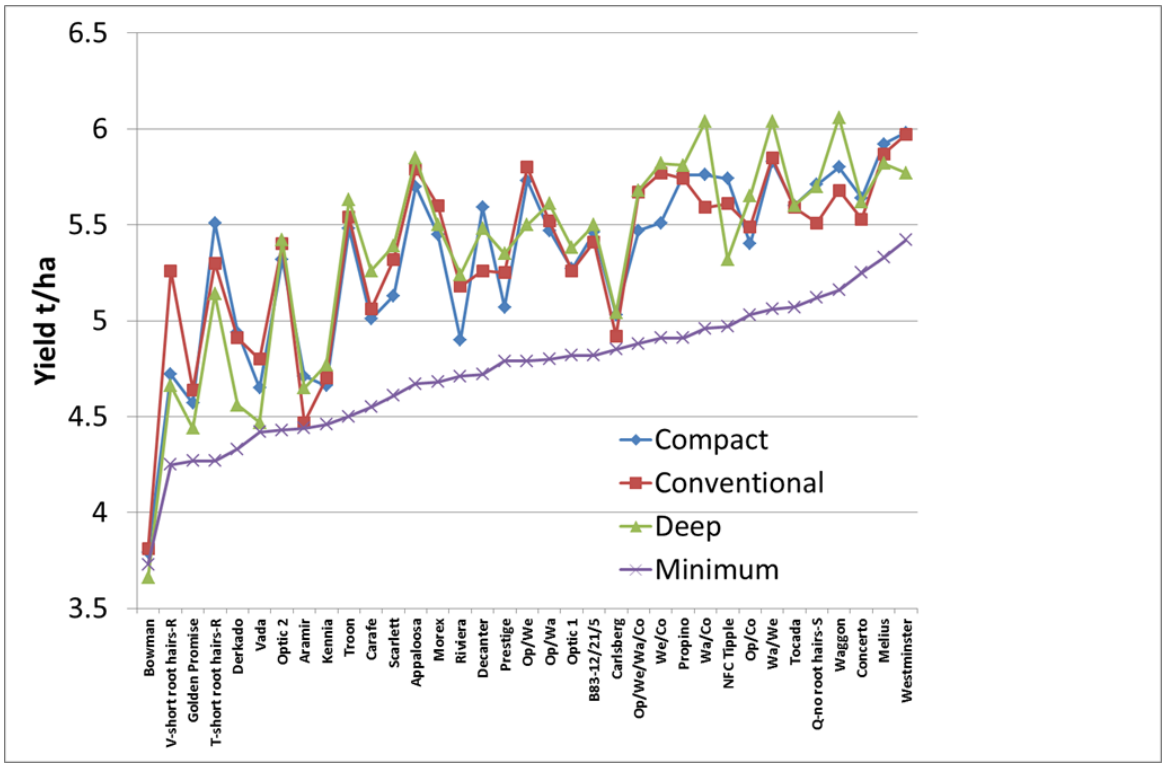

Figure 3. Mean cultivar yields ranked by non-inversion tillage treatment yields.

Selecting the top third non-inversion and inversion tillage yields, Westminster is ranked 1 and 2 respectively, Melius 2 and 3, Waggon 4 and 4 respectively, so higher inversion tillage yields are often reflected in higher non-inversion tillage yields (Table 1). However, cultivars such as Appaloosa ranks 6 in inversion but 23 in minimum (19% yield reduction) and Troon drops from rank 15 to 26 (19% yield reduction). Conversely, Concerto goes from rank 13 in inversion to 3 in non-inversion (6% yield reduction) and Carlsberg from 14 to 28 (3% yield reduction).

Table 1. Relative (rank) yield performance of cultivars under inversion and non-inversion tillage treatments in 2013-2015.

Non-inversion tillage			Inversion tillage			Differ	% yield
Rank	Cultivar	Yield	Rank	Cultivar	Yield	-ence	reduction
35	Bowman	3.73	28	Bowman	3.75	0.02	0.6
33	Golden Prom.	4.27	34	Golden Prom.	4.55	0.28	6.2
31	Derkado	4.33	32	Vada	4.64	0.22	4.7
30	Vada	4.42	30	Derkado	4.80	0.47	9.9
26	Troon	4.50	28	Carlsberg	5.00	0.15	2.9
23	Appaloosa	4.67	15	Troon	5.55	1.05	**18.9**
14	Carlsberg	4.85	13	Concerto	5.60	0.35	6.2
4	Waggon	5.16	6	Appaloosa	5.78	1.11	**19.2**
3	Concerto	5.25	4	Waggon	5.85	0.69	11.7
2	Melius	5.33	3	Melius	5.87	0.54	9.2
1	Westminster	5.42	2	Westminster	5.91	0.49	8.2

In 2016, several of the eleven new cultivars included showed good non-inversion tillage yield, notably KWS Sassy, RGT Planet, Fairing, Sienna, Origin and Olympus. Scholar is notable as having good inversion tillage yield but its non-inversion tillage yield is 36% lower, dropping from 3 to 16 in the cultivar rankings. In contrast, Fairing and KWS Sassy only lose 17% of their yield in non-inversion tillage, changing from 14 to 4 and 7 to 1 in the rankings respectively (data not shown). However, these data are from a single site in just 1 year and showed particularly poor non-inversion tillage yields overall and therefore need further validation.

DISCUSSION

Whilst most cultivars of spring barley used in these trials performed similarly, relative to trial means under all tillage conditions, a few did not. The variance from their expected yield was correlated with whether tillage was inversion or non-inversion. In these experiments and previous work there was no significant difference between the three inversion tillage treatments with respect to cultivar yield. Although only one non-inversion tillage treatment was used in this work, previously zero and minimum tillage treatments had performed similarly and both had been very different from the inversion tillage treatments.

For disease, namely rhynchosporium, there was a trend towards increased disease in non-inversion tillage treatments previously and this was found here too, though levels were low. However, no tillage by cultivar interactions were found.

In the 2013, 2014 and 2015 trials the lowest yield cultivars also tended to have a smaller difference between inversion and non-inversion tillage yield, shown most clearly in the lowest yielding cultivar, Bowman. The highest yield cultivars under non-inversion tillage tended to have the highest yield under inversion tillage too but the yield difference between inversion and non-inversion was not correlated with cultivar yield overall. Amongst the middle-ranking cultivars there were some contrasting yield trends with respect to tillage treatment interactions. Both Appaloosa and Troon showed large yield reductions comparing inversion tillage with

non-inversion tillage (~19%) but Concerto and Carlsberg had only small yield reductions (3-6%) under non-inversion tillage. In 2016, the non-inversion tillage treatment showed greatly reduced yield overall but amongst the 11 new cultivars trialled in 2016 both KWS Sassy and Fairing had relatively small reductions. KWS Sassy was also the top yield cultivar under non-inversion tillage whereas Fairing was fourth and lower ranking still under inversion tillage (14th). However, the two highest-yielding cultivars under inversion tillage, RGT Planet and Sienna, both showed a much larger yield reduction under non-inversion tillage. Furthermore, the third highest-yielding cultivar under inversion tillage, Scholar, gave a 36% reduction in yield under non-inversion tillage, but these data are from a single trial and therefore preliminary.

Clearly the yield gains in some recent cultivars shown in RL trials may not be realised under non-inversion tillage. It could be argued that our non-inversion tillage treatment may equate to, or serve as a proxy for, sub-optimal agronomy or some on-farm conditions. If the inversion tillage equates to RL trial high input, optimum conditions, then these data provide evidence that choice of cultivars should consider the level of inputs and agronomic treatments, at least for cultivations.

It was observed previously that soil tillage treatment differences have most impact on yield in years of environmental stress, particularly drought and it is under such conditions that cultivar differences are most likely to be expressed. None of the trials reported here were subject to strong stress conditions but it was still possible to identify cultivars with differential responses to tillage treatments. Although cultivars more suitable to non-inversion tillage were identified, the transient commercial life of cultivars means that by the time these trials have been completed these data may be of limited on-farm application. More valuable will be to use these differential response cultivars to identify the traits responsible and previous work suggested that rooting structure physical traits are some of the most likely candidates (Adrian Newton & Glyn Bengough, unpublished data).

ACKNOWLEDGEMENTS

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IMPROMALT: IMPROVING THE MALTING QUALITY OF WINTER BARLEY FOR SUSTAINABILITY OF DISTILLING INDUSTRY

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Summary. The distilling industry now uses the majority of UK malting barley purchases. Spring barley is the prefered crop as it offers a higher spirit yield per tonne of malt used than winter barley but the Scottish barley crop no longer provides enough malting barley to meet the amount that distillers require. The higher yield of winter barley would theoretically provide a greater yield of spirit per hectare at current winter barley quality levels. But, breeding of winter malting barley has not advanced at the same rate as for the spring crop. This paper describes progress in a consortium project with industry called IMPROMALT project that has identified key genetic characters improving malting quality in spring barley that are not found in winter barley. Working with the breeding companies in the project consortium, IMPROMALT will transfer these spring genetic characters into several winter barley cultivars in an attempt to bridge the quality gap between the two crops.

INTRODUCTION

Climate change predictions suggest warmer and drier summers in the UK that could limit the amount of available soil moisture and hence grain fill during the summer. This is likely to result in increased grain nitrogen contents that may well render much of the spring barley crop over the current 1.65% threshold that maltsters have set for distilling malting barley. Whilst the average grain nitrogen content from Scottish barley samples has been below the threshold for the last 5 years, it is a mean value and many individual samples exceed the threshold and are rejected for malting to avoid processing problems. The average value has exceeded the threshold in 6 out of the last 20 years (https://cereals.ahdb.org.uk/markets/survey-results.aspx). Increased average temperature and atmospheric carbon dioxide content has been predicted to increase crude protein and hence nitrogen content of spring barley in Denmark and increase the uncertainty of malting barley cultivation (Niero *et al*, 2015).

The earlier maturity of winter barley offers a possible escape from potential restrictions of the growing season due to late season drought, which would spread not only the harvest load but also the risk to the UK malting crop.The average yield of winter barley in the UK has also been 1.2t/ha greater than that of spring barley over the last five years. If the quality of the winter crop was the same, the improvement in spirit yield per hectare would mean a reduction of 17% in the land area required to produce sufficient malting barley to meet the distilling industry requirement. Unfortunately, the average malt extract of winter malting barley is approximately 2% less than the spring crop (Figure 1), which is equivalent to a significant loss of approximately 9l of predicted alcohol yield per tonne of malt in the distillery.

Data gathered during the BBSRC LINK project called 'Association Genetics of UK Elite Barley' (AGOUEB) showed that the hot water extract levels of older spring and winter malting barley cultivars such as Golden Promise and Maris Otter (respectively) was similar and that whilst breeding progress had improved the character since then, improvement was much greater in the spring crop. The introduction cv.Triumph to the UK in 1980 was a significant step forward in malt extract of the spring crop, which has been progressively enhanced since then through varieties such as Chariot, Optic, and Concerto. Whilst the malt extract of winter barley has also improved over the same time-scale, there hasn't been the equivalent injection of quality that Triumph provided to the spring crop. Varieties like Pipkin, Puffin, Pearl and SY Venture have all been purchased in significant quantities by maltsters but none of them have come close to the malt extract potential of their spring counterparts.

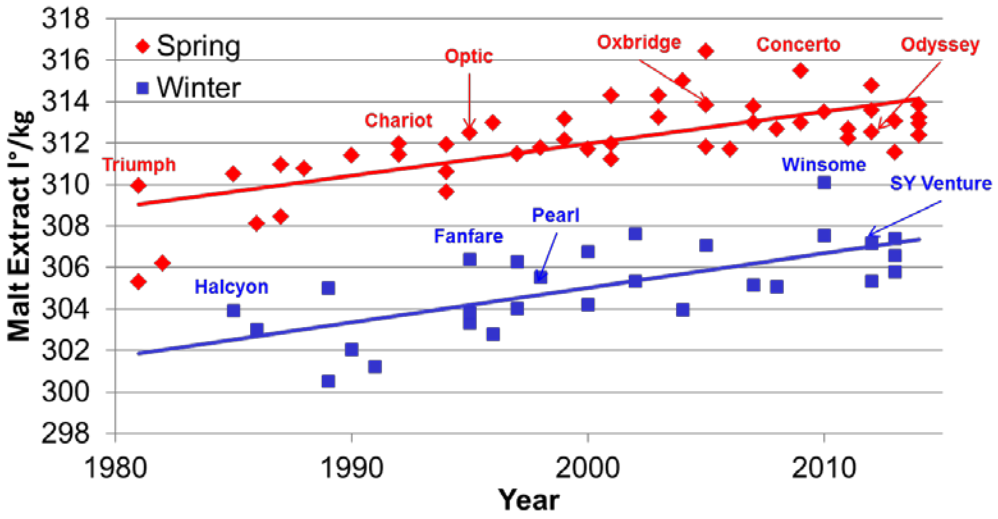

Figure 2. Malt extract mean of UK recommended varieties that had advanced to at least the testing stage in malting evaluation compared to the year of their first being placed on the list.

The development of high throughput DNA marker platforms in the 2000s has facilitated detailed analyses of the genetic similarities between different barley varieties. The AGOUEB project (Thomas *et al.,* 2014) used the data obtained from an assay for some 3000 Single Nucleotide Polymorphisms (SNPs) in known barley genes to compare all the UK malting barleys that the member companies of the Maltsters Association of Great Britain (MAGB) have bought at least 50,000t of in any one year since 1991 (Figure 2) with Golden Promise and Maris Otter included as reference points for older spring and winter malting barley cultivars respectively. The data that obtained was used in a hierarchical clustering analysis to reveal the genetic similarities between the 34 different varieties to show that all the winter varieties including Maris Otter were on a completely different branch of the resulting dendrogram to the more recent spring varieties. Interestingly, Golden Promise was located on the same main branch as the winter varieties, reinforcing a conclusion from pedigree analysis that it shares a common background with Maris Otter and its derivatives. Triumph and all the spring derivatives were on a separate branch, which is a strong indication that the malting quality advances brought about in the spring crop by the introduction of Triumph have yet to be brought into the winter crop. The analysis also revealed sub-divisions in both crop types that distinguished between the older and more recent varieties within each crop type. In the case of the spring crop, this appears to coincide with the introduction of Optic, indicating that its introduction brought about another change in the gene-pool of the spring crop. Whilst many performance factors will contribute to these genetic differences between current winter and spring UK malting barley varieties, it does provide strong evidence that the relative quality gap in the winter crop may be due to the lack of introduction of good malting quality characteristics from Triumph and, more especially, Optic.

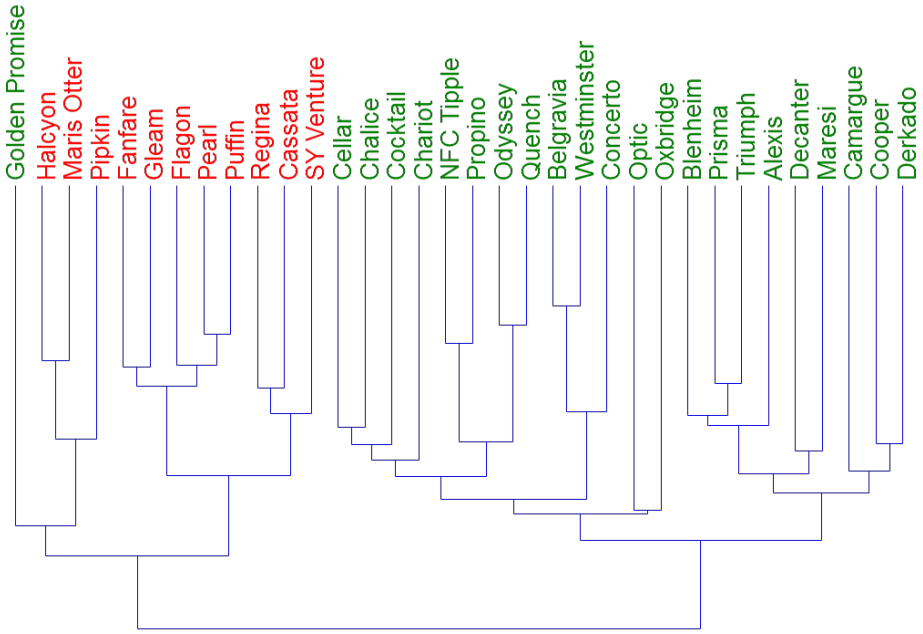

Figure 2. Dendrogram produced from hierarchical clustering analysis of the DNA fingerprints from all UK malting barleys that MAGB member companies had purchased at least 50,000t of in any one year since 1991. The shorter the line connecting any two varieties the closer the genetic relationship. Varieties in green and red are spring and winter types respectively.

Further analysis of the genetic fingerprints of the then elite UK barley germplasm in the AGOUEB project showed that all the winter malting cultivars had the Maris Otter haplotype at the beta-amylase genetic locus at the end of chromosome 4H whereas most of the more recent springs had an alternative haplotype. The Maris Otter haplotype was the same as the Proctor haplotype, which had been shown to be associated with low apparent attenuation based on EBC analysis (Eglinton, Langridge and Evans, 1998)) and hence was likely to have a lower fermentability (IBD) analysis. This was most likely due to genetic linkage of the locus to one of the two vernalisation loci that determine winter habit, although the genetic distance of approximately 4-5 centi-Morgans suggests that producing a recombination in the interval should be relatively easy as they would be expected to occur at a frequency of 1 in every 20-25 progeny. Breeders tend, however, to cross within germplasm groupings so that crossing within the winter barley gene-pool would not produce a recombinant as the low fermentability beta-amylase allele was fixed in the germplasm.

The AGOUEB project also combined the genetic information with the phenotypic information collected as part of the official trialling process and then used Genome Wide Association Scanning (GWAS) to identify SNP markers that were significantly associated with charcters such as malt extract production. This analysis revealed a region on chromosome 1H that was highly significantly associated with malt extract and a number of other malting characters in winter barley. Alleles at the most significant marker locus in the region were in equilibrium in the winter crop, indicating that it would segregate in many crosses made between elite winter barley varieties. The AGOUEB results also revealed that the allele at the locus on 1H that was associated with increased malt extract had virtually been fixed in spring barley, reflecting the selection for increased malting quality in the crop. More importantly, the marker haplotype (the allelic pattern revealed by a series of linked markers) in the region carried by Triumph and its derivatives was not found in any of the winter varieties, even the malting ones. Comparison

with the springs shows that the better winter malting barleys carry the same haplotype as Golden Promise, the Proctor haplotype, suggesting that replacing the Proctor haplotype in the region with the Triumph one might provide an increase in winter barley malting quality.

Further investigation revealed a region on chromosome 3H that was highly significantly associated with malt extract in spring barley but there was no significant association in the winter crop. The allele associated with increased malt extract at the most significant locus was in slight excess (64%) in the spring varieties but was a minor allele (9%) in the winter varieties, especially the accepted malting varieties. Looking at the marker haplotype in the region, it was clear that once again the Proctor haplotype in the winters had been replaced by first the Triumph haplotype in the springs with that in turn being replaced by the Optic haplotype more recently. Whilst there was some variation amongst the winter haplotypes, none carried anything closely related to the Optic haplotype. This again suggested that introducing the Optic haplotype in the region may provide an increase in malt extract.

IMPROMALT is a Consortium of breeding companies, academic research institutes, and representatives of malsters and distillers that was formed to test the hypothesis that introgression of three key regions associated with increased malting quality in spring barley into a winter barley background will result in a significant improvement in winter barley quality to reduce the quality gap to the spring malting crop. One key objective was to update the AGOUEB data set with all barley material that had been added to the National List since 2006 and not only keep updating it during the project but also take advantage of developments in genotyping and use a 9000 SNP marker array for the new genotypes and also re-analyse the AGOUEB set to improve the marker density. Combining all the genotypic and phenotypic data and re-analysing it with Genome Wide Association Scans (GWAS) meant that there was greater power to identify the significant associations that the project hypothesised would improve winter malting quality.

MATERIALS AND METHODS

A crossing scheme was designed to introgress the three target regions listed above in the most efficient manner and the breeding company members produced winter barley lines from these crosses that carried different combinations of these targets. The lines would then be grown in trials and harvested samples sent for micro-malting analyses by member companies of the MAGB to test the success of the strategy.

RESULTS

The project has added some 170 additional lines to the AGOUEB data set and genotyped all 713 with the 9k SNP assay to form the IMPROMALT data set. The addition of the recent data has notably reduced the genomic regions (Figure 3) that were forecast contain the improved malting quality alleles from a spring background, which reduces the chances of introgressing some characters from the spring background that may adversely affect winter habit. In addition, it also reduces the numbers of potential candidate genes contained in the intervals thus increasing the chances of identifying the causal genes, which then opens up the possibility of mining germplasm collections for novel alleles, some of which may lead to even better malt extract potential.

The objective was to maximise the winter background whilst introgressing the spring targets as efficiently as possible. A backcrossing scheme was chosen as the best way of fulfilling the objective and that using two backcrosses to the recipient parent significantly reduced the population sizes that would need to be produced. For instance, if a spring segment with a

recombination event within one centi-Morgan of its border was targeted then 1% of the progeny from the cross will have the desired recombination. But, a similar event on the other side of the introgressed segment would also be required, resulting in a population size of at least 10,000 to have a chance of finding one individual that fulfilled the criteria. By selecting for one border in the first backcross and the second in the second, the required numbers are reduced to 200, which was much more manageable.

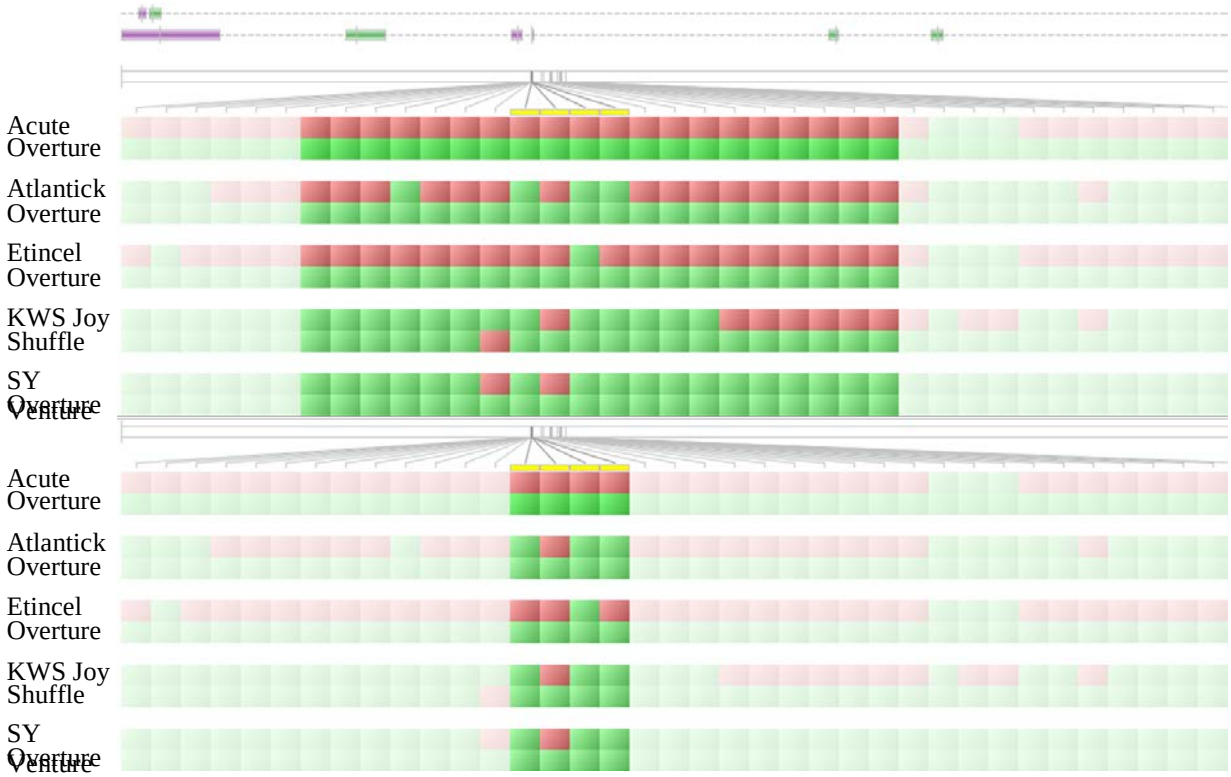

Figure 3. Predicted size of the Intogressed segment and graphical genotypes at 5 winter x spring crosses for one QTL target introgression identified from the AGOUEB analysis (top) and the IMPROMALT analysis (bottom). Overture is the reference genotype so that each cell coloured green has the same allele as Overture and those coloured red have the alternative allele. Opaque regions are those predicted not to contain the region affecting malt extract. Letters in each cell refer to the base calls at each SNP and the parental varieties are listed on the LHS.

Two spring varieties, Overture and Shuffle, were selected as they carried increasing alleles at all three target loci and the participating breeders used them in crosses to the two row winter varieties Acute, KWS Joy and SY Venture and the six row varieties Atlantick and Etincel. Each breeder was responsible for the development of introgression lines from one of the crosses and the markers identified from the refined IMPROMALT analysis were used to select the lines at each backcrossing stage. One breeder then utilised a selfing series and the others used doubled haploid production to produce inbred or near inbred lines from the crosses. Markers were then used to make a final selection of introgression and control lines that have or are being multiplied for field trialling.

The most advanced lines were grown in trials for harvest 2017 under a winter malting barley management regime. Samples of cleaned and graded grain from the trials have been prepared for dispatch to MAGB member companies and the Scotch Whisky Research Institute

for micro-malting and the perfomance of the introgression lines compared to controls to determine if a significant improvement in malt extract has been made. Some of the crosses were not as advanced as the others and were at the multiplication stage for harvest 2017 and have been entered into trial for harvest 2018 together with the best agronomic lines from the 2017 trials for micro-malting post harvest.

DISCUSSION

Whilst it is possible that one of the lines could be submitted for National List trials, it is more likely that the best lines will form valuable parental stocks for utilisation in the participating breeders own programmes and that selections from that material are more likely to make a market impact. The use of natural variation to improve the malt extract potential and hence the spirit yield of winter barley could make winter barley a viable alternative to the spring crop that would reduce the footprint of barley production for the distilling industry. This would also require the introduction of the low glycosidic nitrile (GN) characteristic that is now mandatory for all new varieties that are approved for use in distilling. Both the spring parents carried this characteristic and so do some of the selections. The low GN character is located on the same chromosome as one of the target introgressions but some distance apart so introgressions that carry segments that include the two may also have some deleterious characters for adaptation to winter growing so a separate round of crossing and selection may also be required to introgress low GN into winter barley. For example, a frost hardiness locus is located between the two (Fisk *et al*, 2013) and it may be that the winter allele is required in UK environments to prevent excessive frost damage.

ACKNOWLEDGEMENTS

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ACCESSING NEW PRODUCTS AND INNOVATIONS: THE IMPACT OF POLICY AND REGULATION ON UK AGRICULTURE

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Summary: Agricultural policy used to be easy: produce enough food at an affordable price to keep your population nutritiously fed. For today's society in Europe, the complexities of policy in this area are almost inexhaustible; do we need productive agriculture at all or can we rely on imports? If we are going to produce food, how do we do so in a sustainable way that maximises biodiversity, minimises the use of water and chemicals and reduces waste? Whatever the rights and wrongs of exiting of the European Union, it is an excellent opportunity to totally re-assemble and re-wire the UK's agricultural policy. Attempting to parallel the current EU model long-term, whilst reducing subsidies, would reduce farmer competitiveness, reduce investment and risk condemning the UK to museum agriculture. Balancing productive farming with excellent environmental stewardship would result in a vibrant agricultural system attracting inward investment and requiring low subsidies.

INTRODUCTION

The pressure is on, and the challenges are large: on one side there are medium to long-term issues of growing enough food to feed an ever-increasing population in the face of ever more compromising climate change. Professor Chris Elliott from the Institute for Global Food Security at Queen's University in Belfast pointed out at the 2018 Oxford Farming Conference that one third of the global population are currently living in areas of water debt; by 2025, two-thirds of an increased population will be in the same situation.

On the other side is the short-termism of policy making at the European Commission, the European Parliament and the parliaments of the Member States of the European Union. Increasingly there is a third side to what is now a triangle of policy determinants which threaten to erode even further our ability to promote evidence-based policy making – that of post-truth populism, enabled and emboldened by social media, and the rise of the pseudo-polling organisations such as SumofUs and 38 Degrees.

Agricultural Policy as a function of food security

The whole landscape where policy has to impinge has changed: in the 1960's and 1970's, the focus was on a three-way pull of productive agriculture and keeping food affordable with an increasingly important aspect, as the former improved the latter, of promoting biodiversity by reducing the environmental impact of agriculture.

Today, and quite rightly, we have a model that has added at least three more pieces to the agricultural policy jigsaw (Figure 1); that of delivering sustainably, reducing waste, and minimising agricultural residues in food and the environment irrespective of any risks associated with their presence. The latter is epitomised by the European Union's regulatory obsession with hazard cut-off criteria which can result in the loss of useful tools for farmers to control destructive weeds, pests and diseases, irrespective of any stewardship activities to minimise risks.

US President Woodrow Wilson pointed out that “In the Lord's Prayer, the first petition is for daily bread. No one can worship God or love his neighbor on an empty stomach”; perhaps the Chinese proverb "The man with the full stomach has many problems, but the man with an empty stomach only has one” is just as relevant. Either way, once you essentially eliminate the issue of supplying food to a population, the issues of what food is produced, how it is produced, and the environmental consequences of its production become much more important.

Nevertheless, food security issues do remain and any drop in agricultural productivity in Europe will have an impact globally; a study by the Humboldt Forum (Noleppa *et al.*, 2013), for example, suggested that for every 1% increase in overall EU agricultural productivity, an extra 10 million people a year could be fed. The reverse, however, was also true: for every 1% reduction in productivity, food for many millions of people would have to be imported into Europe, reducing the self-sufficiency of the latter and presumably having impacts on those countries outside of Europe exporting to bridge the gap. Policy where it impacts on agricultural productivity must be viewed through that prism, and the EU did respond during the last sustained upward pressure on food prices between 2008 and 2010, by relaxing the rules on set-aside.

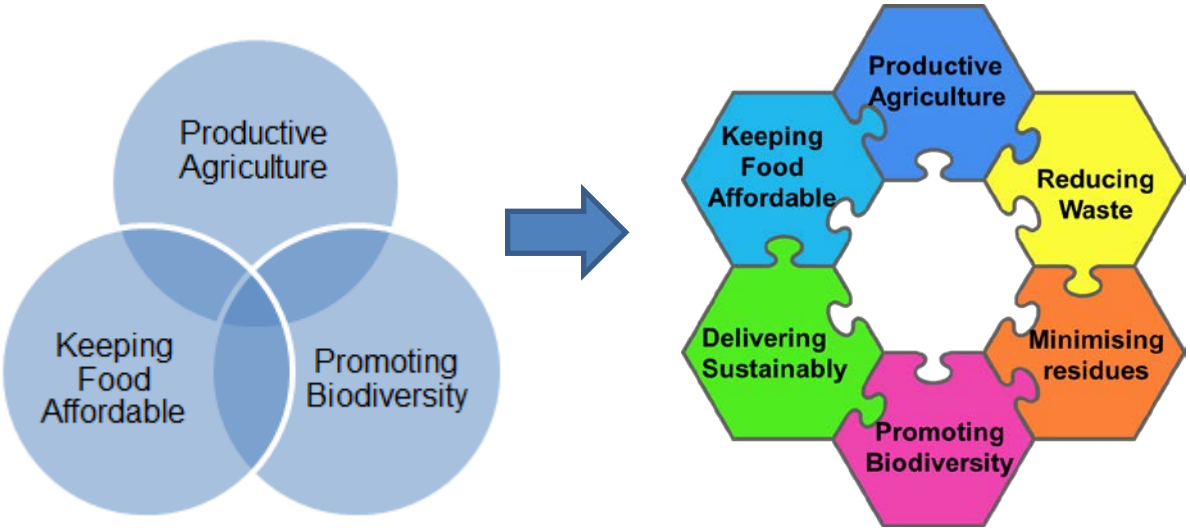

Figure 1. Increasing complexity of agricultural policy as food security issues diminish.

The Impact of the UK exiting the European Union

The missing piece of the jigsaw in Figure 1 is the impact of exiting the European Union. This is not just a United Kingdom-centric view; you only have to look at the concerns of the impact on the Republic of Ireland to realise that Brexit will have ramifications across Europe. Traditionally, the UK has been seen as a moderating influence on European agricultural policy, arguing for science-led, evidence-based decision making, promoting the freeing-up of market controls with a concomitant reduction in subsidies within the Common Agricultural Policy framework. The loss of the UK’s moderating influence in the European Council may yet be one of the most profound impacts of Brexit, not ignoring, of course, the UK’s large monetary contribution to the process.

Whether the so-called Brexit is seen as a threat, a challenge, or an opportunity for the UK, a change in agricultural policy is highly likely, especially in light of the UK Government’s repeated assertion that single farm payments in England (agriculture being an area devolved

to the Welsh and Northern Ireland Assemblies, and the Scottish Government) would not continue in the medium-term.

We know that the once-called “Great Repeal Bill” ending 40 years of EU legislation will actually be closer to a “copy-everything-that-is-in-EU-law-into-UK-law” bill; the question is when, and to what extent, will agricultural policy in the UK start to deviate from EU thinking?

Prior to the General Election in 2017, there was a feeling that the UK would start to move closer to a global (outside of the EU) model of risk-based regulation when it came to pesticide availability (if you can demonstrate that it can be used safely, then the innovation principle trumps the precautionary principle), and, once the latter is determined to be as safe as the “conventional” alternative, a market forces approach to innovative plant breeding (the customer rather than the politician decides whether food made from plants or animals using novel technologies is acceptable). This methodology potentially gives farmers early access, or at least earlier access than their EU competitors, to new products, helping them to be more competitive and perhaps more productive just at the time when subsidies in the UK are reduced or eliminated.

There is no expectation that the regulations with respect to the environment would be eroded in the process. Indeed, a more productive agricultural system must and will be compatible with measures to not just conserve but promote biodiversity in the countryside. Such a model would attract investment from across the supply chain, including those involved in crop protection and innovative seed breeding, and could be a blueprint for a low subsidy-based agricultural system that balances productive food production with targeted biodiversity outcomes (Figure 2).

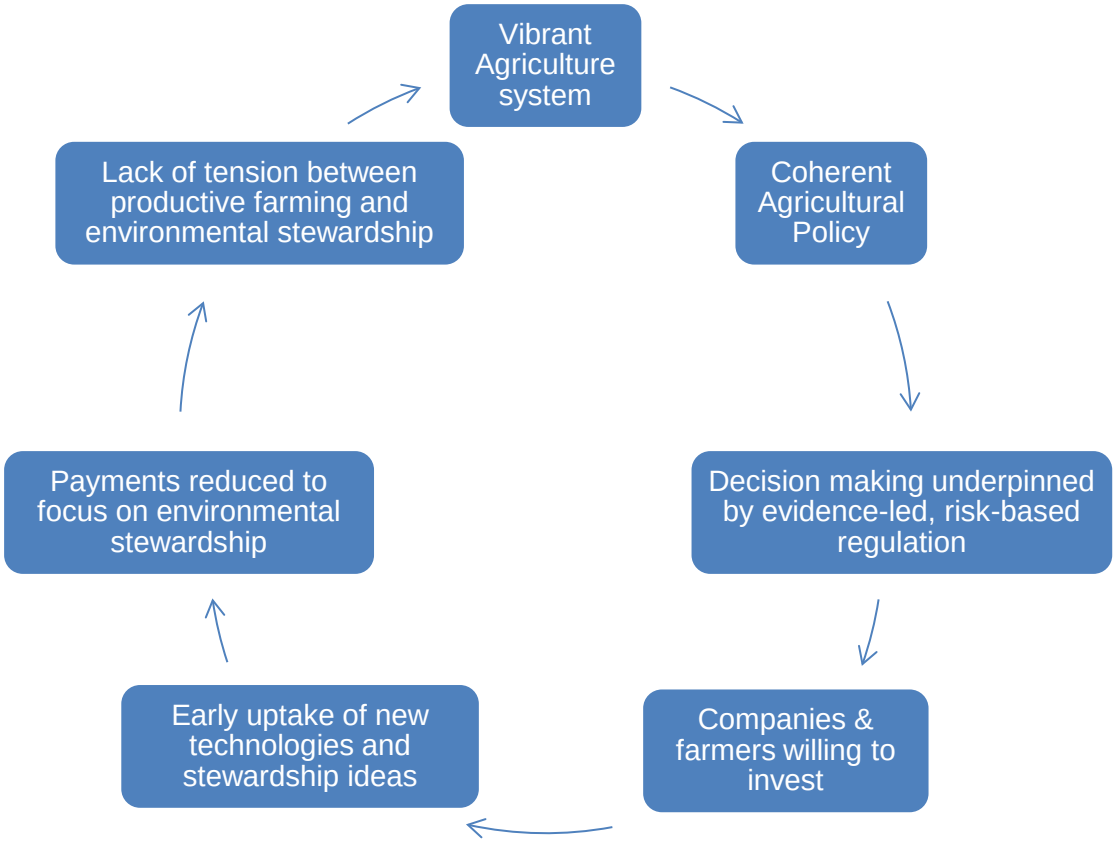

Figure 2. A vibrant agricultural system requiring much less support payments.

The policy drift since the General Election, however, has thrown this model into doubt and is of concern. Announcements to date have been vague and appear to be more aligned to protecting the Government's green aspirations that dealing with the challenges that Brexit will produce. Rather than putting into place a policy that promotes productive agriculture, increases UK farming competitiveness and reduces the legislative burden, pronouncements to date have very much aligned with the EU's obsession with a "museum agriculture" approach. The latter reduces productivity, politicises the regulatory process such that only those products and technologies that pass the "qualified majority" vote come to the market, and uses a scatter-gun approach to improving biodiversity. The difference is that European farmers can "afford" to be less competitive because of CAP payments.

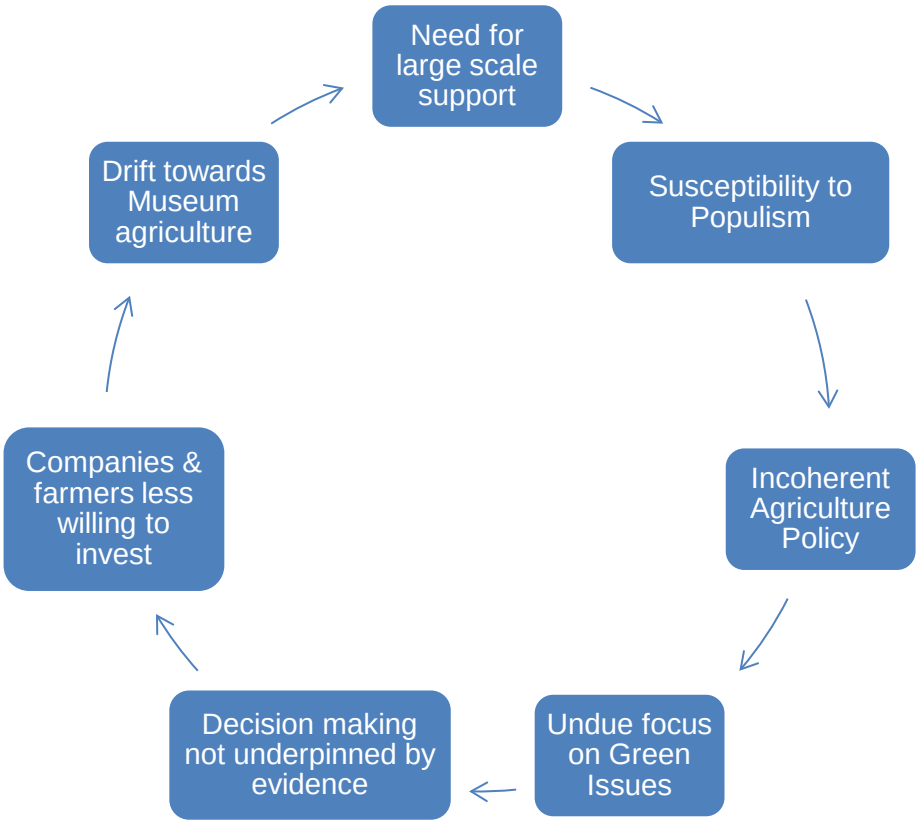

Figure 3. A lack of coherent policy leads to museum agriculture.

Policy matters when it comes to investment decisions

The museum agriculture approach also deters investment. A consultancy study undertaken by PhillipsMacDougall (Annonymous, 2013) recognised that the agrochemical industry as a whole spends around £5 billion every year on researching and developing new products. The proportion that is spent specifically for EU markets has collapsed in recent years from 33% of total in the 1980's to 25% in the 1990's and to less than 8% today. It takes around £300 million and nearly 12 years to bring a product to market. The increasing politicisation of the regulatory system in Europe, which allows the European Commission to propose the withdrawal of products, which have previously passed all of the regulatory hurdles put in front of it, whenever single issue groups campaign loud enough, does not give the industry an

incentive to invest. Clearly, with the current policy flow in Europe, the focus of investment will tend be where regulation is predictable and science and evidence led. As a result, European farmers find themselves rarely seeing products designed for their use, but accessing those products that were designed for use in North or South America, South Africa, South East Asia or Australia, but that also will work in Europe.

Bayer is currently commercialising a new low-dose nematicide based on the succinate dehydrogenase inhibitor (SDHI) fluopyram for use in potatoes in the UK. Would that product have been developed solely for the UK or even European markets unless it worked first elsewhere in the world? It seems unlikely.

Likewise on-farm investment is equally impacted; a survey conducted of its members by the Nation Farmers Union in December 2017 showed very low confidence in prospects for the industry with twice as many respondents intending to reduce investment than increase investment as a result of uncertainty associated with Brexit. As NFU President Meurig Raymond (2017) said at the time: “These results are a clear signal that the lack of certainty is impacting business decisions right now and that, in turn, has the potential to make farm businesses less resilient for when the UK leaves the EU”.

Innovation Model for the UK

So what about innovations coming from the UK? At any one time Bayer is involved with around 40 crop production and protection projects and 20 PhD students at 25 universities, research institutes and SMEs in the UK. This commitment is a reflection of the quality of the science in this area in the UK. Some of this activity will be near market but much of it is not.

Of course much academic research is not carried out with a company associated with it. If it involves novel plant breeding, its application to agriculture in the EU and thereby, at least for the moment, is highly problematic. Professor Jonathan Jones’ work at the Sainsbury Laboratory in Norwich is a case in point. Bringing to the market a potato that dramatically reduces the need to spray potatoes against late blight would make absolute sense, especially if you could add consumer benefits such as the reduction of acrylamide in the potato when cooked, or reducing waste by slowing the browning process once a potato is cut. The reality is that although there is a process in the EU via the European Food Safety Authority (EFSA) to demonstrate that such potatoes are safe to eat, the fact that a potato tuber is a living organism and can be planted to produce a genetically modified (GM) plant means that getting political approval for such an innovation is highly problematic. It is beyond irony that EFSA’s strapline is Trusted Science for Safe Food since despite 4 billion meals containing GM ingredients having been consumed around the world without one substantiated health issue, EFSA’s pronouncements are not trusted by governments who collectively must give political approval for a product to be commercialised in Europe.

Currently the blight resistant potato has been licenced to the American company Simplot; could we see a situation post-Brexit where the UK is prepared to allow commercialisation of its own research (Figure 4)?

CONCLUSION

Agricultural policy matters: it provides a clear framework for those working throughout the food supply chain and a basis for commitments and investments in the industry. If you have confidence in the predictability of how rules and regulations are made, implemented and policed, you are more likely to see different parts of the supply chain respond positively. A lack of, or a poorly thought-through, policy will have the reverse effect, causing inertia in decision making, reducing investment and invoking a cycle towards museum agriculture.

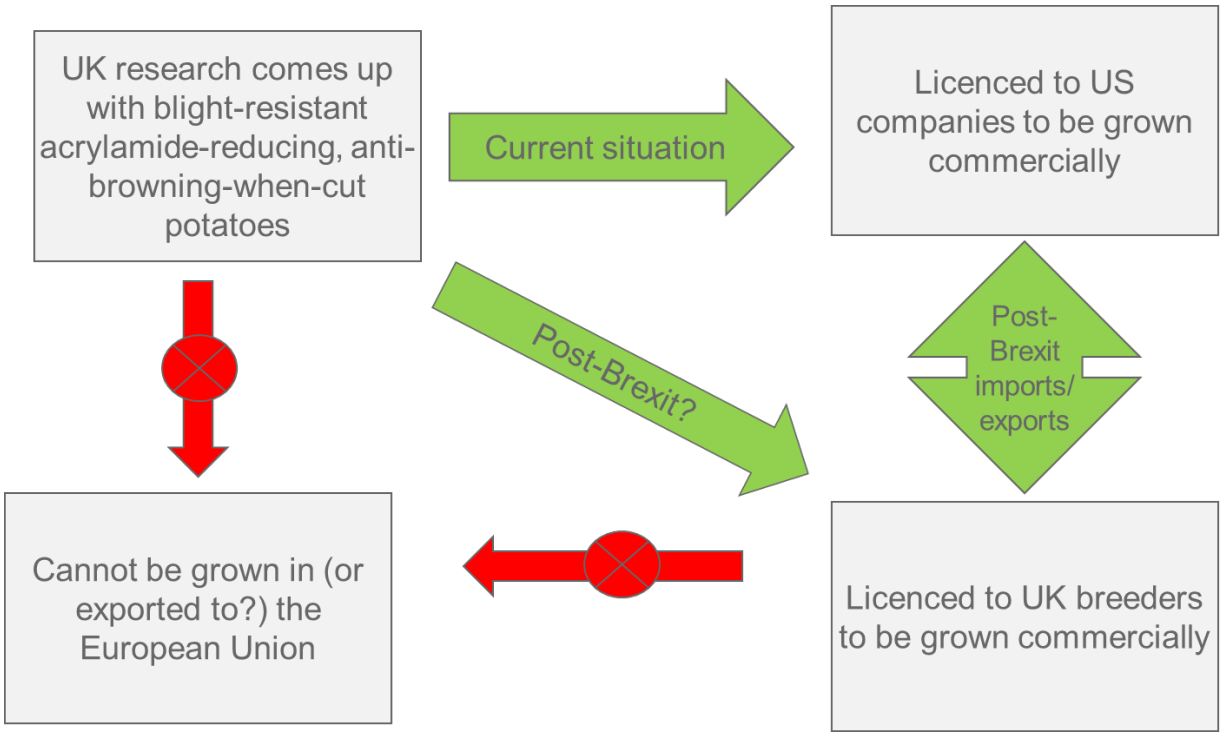

Figure 4. Translation of UK research: opportunities for GM post-Brext?

Allowing farmers access to new technology earlier than their European counterparts may pose problems in terms of accessing the EU markets in some cases, but reducing support payments without improving agricultural productivity could be catastrophic for the UK post Brexit. A shift from the EU hazard cut-off criteria based regulatory system to the more globally accepted risk-based, less politicised system would allow farmers to access new products and perhaps technologies earlier without necessarily reducing the export potential for UK agriculture. A balance therefore is needed; getting the right policy in place as soon as possible will allow the food supply chain to commence the evolution that will undoubtedly be required such that UK agriculture can not only survive Brexit, but perhaps be the blueprint for other countries to follow.

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FENPICOXAMID, A NEW NATURAL PRODUCT DERIVED FUNGICIDE FOR CONTROL OF *ZYMOSEPTORIA TRITICI* (SEPTORIA TRITICI BLOTCH) AND OTHER DISEASES IN CEREALS

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Summary: Fenpicoxamid (Inatreq™[1] active) is the first molecule from a new class of fungicides (picolinamides). It is derived from the natural product UK-2A which is produced by fermentation of an actinomycete (*Streptomyces sp. 517-02*) and then undergoes a minor synthetic modification post-fermentation for stabilisation. Fenpicoxamid is converted back to UK-2A in the presence of fungi and in plants which inhibits fungal respiration at the Q_i site of the respiratory cyt bc1 complex and represents a new target site within the cereal fungicide segment. Significantly, fenpicoxamid shows no target site cross-resistance to cereal fungicide chemistries and is effective against *Zymoseptoria tritici* (Septoria tritici blotch) isolates with decreased sensitivity or resistance to triazoles and strobilurin fungicides. Fenpicoxamid will offer growers effective control of *Z.tritici* with related yield benefits, as well as being an innovative resistance management tool that must be used in combination with other fungicides to control a range of cereal diseases.

[1] Trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

INTRODUCTION

Zymoseptoria tritici (Septoria tritici blotch) is one of the most important foliar diseases of wheat in temperate regions worldwide. This tenacious pathogen accounts for approximately 70% of annual fungicide useage on wheat in the EU (Fones et al, 2015). In the UK, average annual yield losses of around 20% have been observed on untreated susceptible varieties from the AHDB list (2012-2013) (HGCA topic sheet, 2012). Yield losses of around 5 – 10% (ADHB, 2016) are typically seen in wheat varieties with a higher disease rating when treated with fungicides. *Z. tritici* is challenging to control in wheat growing regions where there is widespread resistance to the strobilurin class of fungicides coupled with substantial sensitivity losses to the triazole molecules and increased pressure on succinate dehydrogenase inhibitors (SDHI's). An additional challenge is the possibility in the near future that a number of important fungicides will be restricted or removed from the EU wheat grower's toolbox due to increased regulatory scrutiny. Compounding this is the impact of an ever-more complex and overburdened regulatory environment in the EU which now means that it takes on average 11.3 years (Lorsback and Sparks, 2016) for a new active ingredient to reach the market from time of initial discovery.

Fenpicoxamid is the result of more than a decade of research and is the first molecule from a new class of fungicides called picolinamides. It offers novel chemistry with a new target site for cereal fungicides by inhibition of cell respiration in the mitochondria at Q_i site of the respiratory

cyt bc1 complex. It will be the first new target site to be introduced to the cereal fungicide market in over a decade and comes at a time when new fungicides solutions will be critical to manage *Z. tritici* resistance issues with current chemistries. Fenpicoxamid provides strong curative and residual protectant efficacy in wheat against *Z. tritici* with good protectant activity on yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia recondita*). Outside Europe, it will also be developed for use in banana against black sigatoka (*Mycosphaerella fijiensis*). This paper sets out data to support the claim that fenpicoxamid will deliver class leading biological performance on *Z. tritici* and is effective on isolates resistant to current chemistries including strobilurins and triazoles.

Discovery, development and production

Fenpicoxamid's discovery began with a soil sample collected and analysed at Osaka City University in Japan in 1996. An actinomycete bacteria (*Streptomyces* sp. 517-02) isolated from the soil was found to produce a compound with natural fungicidal activity and subsequently referred to as UK-2A (Ueki et al, 1996; Hanafi, 2016). At Dow AgroSciences *in vitro* bioassay studies and high volume glass house efficacy screening of UK-2A showed promising activity against a range of Ascomycete plant pathogens including *Z. tritici* and Basidiomycetes including wheat rusts but minimal activity on Oomycetes (Owen et al, 2017). Efficacy of UK-2A was seen to translate poorly to the field environment and this was linked to a number of inherent properties of the molecule including photo-instability. UK-2A, nevertheless, was considered an attractive candidate for semi-synthetic modification and optimization of antifungal activity and other key attributes. Over 300 analogs were prepared and the one-step post-fermentation modification of the picolinamide OH group of UK-2A was eventually identified as the lead development candidate. This minor modification of UK-2A to form fenpicoxamid brings UV stability, increases efficacy at a lower dose and allows delivery in a novel formulation which improves retention and uptake of fenpicoxamid from the leaf surface (Owen et al, 2017).

The manufacture of fenpicoxamid follows a two-step process. UK-2A is first produced by conventional fermentation within a contained bioreactor. The growth medium and fermentation conditions are carefully optimised to maximise the titre of UK-2A produced by the Streptomyces species. Following fermentation, UK-2A is recovered from the broth, purified and then converted to fenpicoxamid post fermentation through a single step modification which adds the stabilising group (Figure 1). The final product is then formulated to optimize storage, application delivery and product performance

Biological conversion of fenpicoxamid back to the original natural substance UK-2A

Metabolism studies in *Z. tritici* and wheat protoplast cell suspensions have shown that fenpicoxamid is converted back to UK-2A by removal of the isopropylcarboxymethyl ether masking group that was added post fermentation for stability (Figure 1). Moreover, biochemical studies indicate that UK-2A is the active moiety responsible for the fungicidal activity. (Young et al, 2017; Owen et al, 2017). Preliminary laboratory studies conducted at Dow AgroSciences suggest that the removal of the isopropylcarboxymethyl ether masking group is mediated by carboxylesterase (CE) enzymes (unpublished). Typically, multiple CEs are present in both fungi and plants. Substrate specificity is poorly understood for most CEs but is known to differ greatly between enzymes (Satoh & Hosokawa, 2006). At present it is unclear if specific CEs in *Z. tritici* and/or cereals are responsible for the conversion and further research is ongoing in this area.

Fenpicoxamid sprayed onto the wheat leaf surface becomes bound to the cuticular waxes where it remains in parent form. Fenpicoxamid spray deposits are essentially a reservoir of material which is only activated to UK-2A once taken up into germinating fungal spores

(protectant activity). Small but biologically relevant amounts of fenpicoxamid penetrate into the leaf tissue where they are converted to UK-2A by the plant and/or when taken up by fungal tissue within the leaf (curative activity).

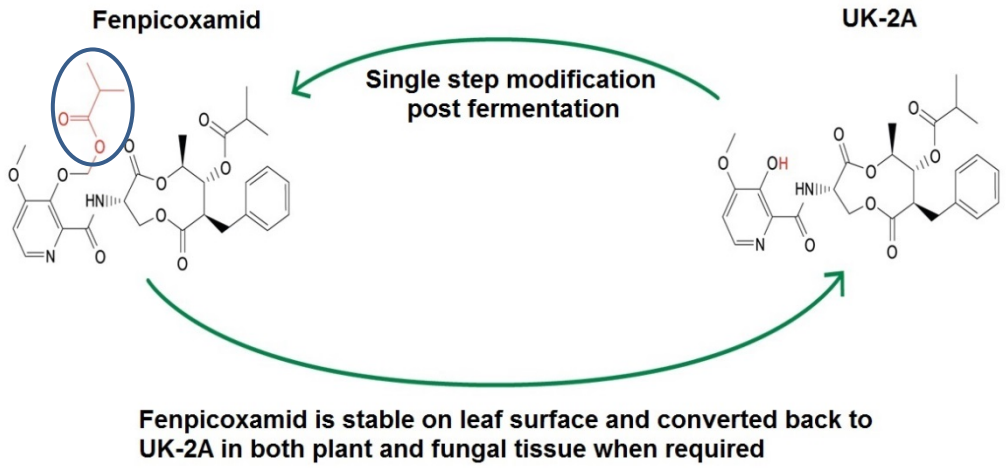

Figure 1. Modification of UK-2A to fenpicoxamid post fermentation, and conversion back to UK-2A in plants and fungi

METHODS AND MATERIALS

Efficacy Trials

The following experiments show the efficacy of fenpicoxamid at 100 g a.i. /ha when tested in field trials across Maritime EPPO zone in 2014 which was a very heavy pressure year for *Z. tritici* and 2015 where there was generally moderate disease pressure. However, in all trial situations susceptible varieties were used and trials were placed in locations where *Z. tritici* is typically an issue to growers. Twenty two trials were conducted to GEP standards in Belgium (2), Czech Republic (2), Denmark (3), Germany (4), Republic of Ireland (5) and UK (6) over a two year period from 2014 to 2015. All trials were designed as randomised complete blocks with 4 replicated plots per treatment and plot sizes of 20 to 36 m^2. The reference standards were prothioconazole applied at 198 g a.i. /ha and bixafen + prothioconazole at 281 g a.i. /ha, Two applications were made in each trial, the first being between growth stages Zadoks 31-33 and the second application being made at Zadoks 39-57. All applications were made using a precision small plot sprayer calibrated to deliver 150-250 litres/ha. Assessments were made at 28 to 44 days after the second application timing with a mean assessment interval of 34 days after the second application. Visually, percentage infection on a 0-100 scale was assessed on the upper three leaves (flag, flag -1 and flag -2) and the mean percentage control was then calculated across all leaf levels. Yield was also assessed per plot and tonnes per hectare calculated and corrected to 14% moisture and the relative percentage increase over the untreated were calculated. Yield and % Control data were analyzed across trials with a linear mixed model where treatment was modeled as fixed effect and trial was random effect. Models where estimated with the method of Restricted Maximum likelihood (REML) and means were compared with Tukey test (α=0.05). The results from the studies are shown in Table 1.

Sensitivity test

UK-2A is a potent inhibitor of mitochondrial electron transport and binds at the Q_i site in the cytochrome *bc*1 complex (Machida et al, 1999; Young et al, 2017). This results in inhibition of respiration and subsequent fungal growth. The Q_i ubiquinone binding site is distinct from the Q_o site targeted by the strobilurin class of fungicides (Young et al, 2017). There is no target site-based cross resistance between fenpicoxamid and strobilurin, triazole and SDHI fungicides. This claim is supported from studies reported by Owen et al, 2017 (Figure 2) which investigated cross resistance. These studies were carried out on three isolates (UK-4, UK-7 & UK-12) of *Z.tritici* from the United Kingdom with confirmed target site mutations conveying resistance to both strobilurin and triazole classes of fungicides. All isolates contained the G143A site mutation conferring strobilurin resistance and the CYP51 gene encoding the sterol C-14 demethylase target site for azole resistance. Additionally, isolate UK-12 carries CYP51 over- expression genes, as well as the multi-drug resistance gene MgMfs1 (Omrane et al, 2015). A 'wild type' *Z. tritici* isolate ATCC 26518, highly sensitive to all four chemistries was also included. Dose response greenhouse tests were conducted on wheat plants which were inoculated with the *Z.tritici* isolates. Plants were inoculated either three days prior or one day post fungicide application. Test products were applied at 200 litres/ha using an automated track sprayer and each fungicide was evaluated at doses of 100, 25, 6.25, 1.56 and 0.39 g a.i /ha. Inoculated plants were then removed to the greenhouse and assessed when symptoms first developed on untreated plants. Percentage disease control was calculated using the ratio of disease on treated plants relative to untreated. The effective concentration values to give 80 percent control (EC_{80}) were calculated from the dose response curves. The results from these studies are shown in Figure 2.

RESULTS

The results presented in Table 1 summarise the performance of fenpicoxamid against *Z. tritici* in 22 trials when tested over a two year period in Maritime countries. Untreated mean levels of infection on the flag leaf, flag -1 land flag -2 leaves were 57.52% in 2014 and 49.17% in 2015. Fenpicoxamid provided high levels of *Z. tritici* control and related yield benefits that were superior to prothioconazole and equivalent to a leading market standard mixture product containing bixafen + prothioconazole.

Table 1. Percentage Control of *Z. tritici* and yield (t/ha) from 22 trials established in Maritime EPPO zone in 2014 (12) and 2015 (10)

Treatment	Mean % Control *Z. tritici* (years)	Yield t/ha (Rel % increase)
fenpicoxamid	87.9 a (2014/2015)	11.0 a (125%)
100 g a.i. /ha	87.4 a (2014)	10.6 a (130%)
	85.8 a (2015)	11.6 a (116%)
prothioconazole	69.3 b (2014/2015)	10.5 b (119%)
198 g a.i. /ha	76.5 b (2014)	10.2 a (125%)
	54.4 b 2015)	11.2 b (110%)
Bixafen +	87 0 a (2014/2015)	11.1 a (125%)
prothioconazole	85.3 a (2014)	10.5 a (129%)
281 g a.i. /ha	82.3 a (2015)	11.8 a (118%)

Means followed by the same letter (comparisons within same year period) are not significantly different (Tukey test α=0.05)

There were significant differences between treatments when compared across trials and years (2014-2015) for % Control ($F_{2,42}$= 22.46; *P*<0.0001) and Yield ($F_{3,63}$= 65.42; *P*<0.0001) (Table 1). By year, treatment differences were also significant for % Control (2014, $F_{2,22}$= 4.59; *P*=0.0215; 2015 $F_{2,18}$= 33.05; *P*<0.0001) and Yield (2014, $F_{3,33}$= 35.86; *P*<0.0001; 2015, $F_{3,27}$= 44.88; *P*<0.0001) (Table 1). In 2014 trials, a significant yield increase of 30% was observed with fenpicoxamid when compared to the untreated which yielded 8.2 t/ha. When this is compared to 2015 results where disease pressure was less aggressive, the increase in yield from fenpicoxamid was 15% over that of the untreated which yielded 10.0 t/ha, but still significant.

Control of fungicide resistant isolates of *Z. tritici* by fenpicoxamid and current cereal fungicides was investigated in low volume greenhouse tests as 1 day protectant (1DP) and 3 day curative (3DC) tests. The results presented in Figure 2 clearly show the only isolate that was effectively controlled by the strobilurin and azole fungicides is ATCC 26518 whereas fenpicoxamid and also fluxapyroxad provide strong control of all four of the tested isolates including those resistant to strobilurin and azole fungicides. These results indicate that fenpicoxamid can provide cereal growers with an important new tool for management of *Z. tritici* isolates with target site resistance to strobilurin and triazole fungicides.

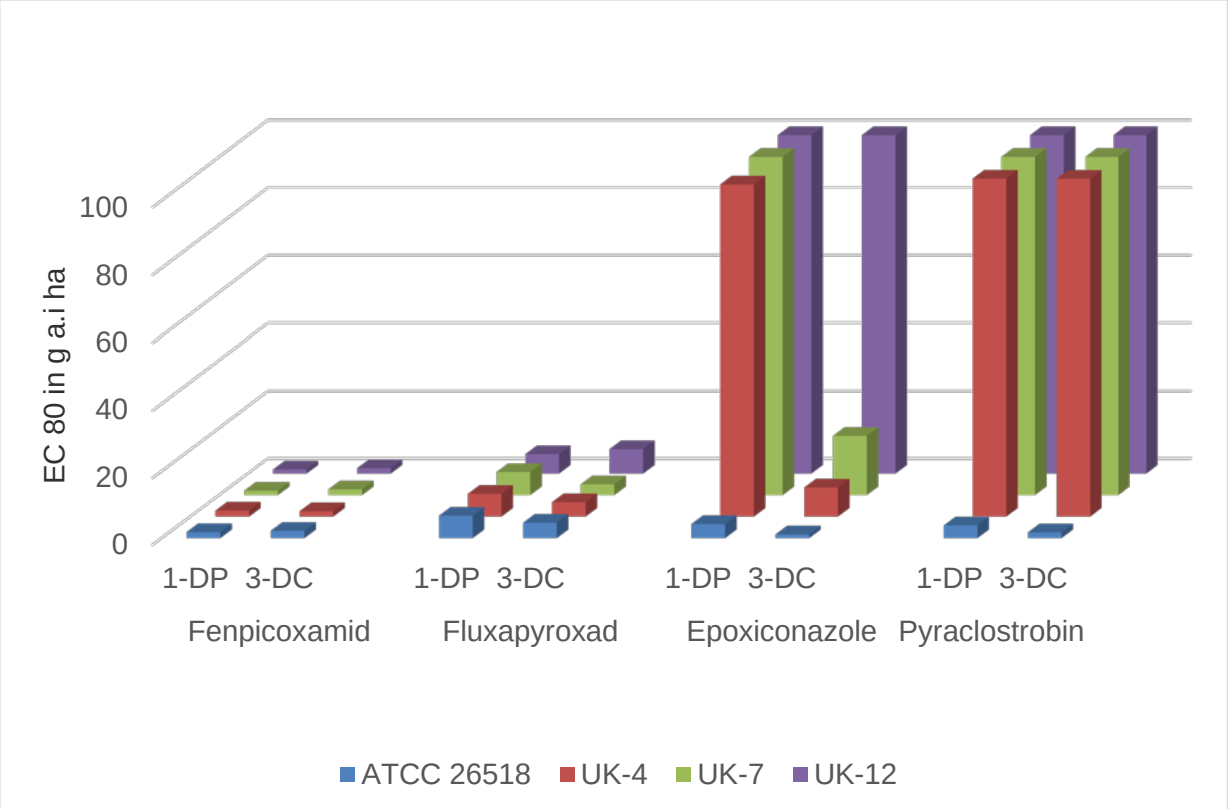

Figure 2. Comparative control of fungicide resistant isolates of *Z. tritici* by fenpicoxamid and current cereal fungicides in low volume greenhouse tests as reported by Owen et al, 2017.

DISCUSSION

Fenpicoxamid is a new tool for controlling plant pathogenic fungi in cereals. Fenpicoxamid has demonstrated during its development outstanding biological performance on *Z. tritici* with complementary yield benefits and crop safety in multiple field trials across successive years.
The novel chemistry and new target site of fenpicoxamid offers an additional fungicide solution for cereal growers. Its strong efficacy against *Z. tritic*i isolates with reduced sensitivity to strobilurins and triazole fungicides, and lack of target site cross-resistance to fungicide chemistries used in cereals highlights its importance as a future resistance management tool. *Z .tritic*i is classified by the Fungicide Resistance Action group (FRAC) as a pathogen with a medium risk of resistance development. It is proposed that fenpicoxamid will be classified in FRAC group C4#21. Fenpicoxamid is a single site inhibitor and as such it will need to be used within the framework of an effective resistance risk management strategy. To ensure that fenpicoxamid will remain effective and that resistance risk is minimized, it should never be applied alone and only recommended for use in mixture with other effective modes of action against *Z. tritic*i.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Meiji Seika Pharma Co. Ltd. for discovery of UK-2A and the assistance of Dow AgroSciences colleagues and external researchers.

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EFFICACY OF FENPICOXAMID FOR THE CONTROL OF ZYMOSEPTORIA TRITICI (WHEAT LEAF BLOTCH)

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Summary: *Zymoseptoria tritici* represents a major threat to wheat growers across all temperate climates, with reported yield losses of up to 50% in severe epidemics (Eyal *et al*., 1973: Eyal *et al*., 1987).

Fenpicoxamid is the first cereal fungicide to be developed from a new picolinamide class of chemistry which will be assigned to, FRAC group C4-21, (QiI, quinone inside inhibitor). It controls of a range of cereal pathogens including *Zymoseptoria tritici, Puccinia striiformis* and *Puccinia triticina.*

A series of field trials were established to evaluate the efficacy of fenpicoxamid (Inatreq^{TM1} Active) with prothioconazole for the control of *Z. tritici*. Fenpicoxamid with prothioconazole provides robust control of *Z. tritici* across curative and protectant situations, superior to that provided by the market standard bixafen + prothioconazole. Fenpicoxamid represents a new site of activity in the cereal fungicide segment with no cross resistance to current chemistry used in cereals.

[1] Trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

INTRODUCTION

Wheat yield loss from *Zymoseptoria tritici* in the UK varies from year to year, but susceptible varieties on the AHDB recommended list can average a 20% yield loss in untreated trials (HGCA Topic Sheet 113, 2012).

Over recent years the intensity of the fungicide programme within the winter wheat crop has increased with many crops now receiving four sprays targeted against *Z. tritici* (Crop Monitor 2015). At the same time *Z. tritici* has developed widespread resistance to the strobilurins (Fraaije *et al*., 2003), reduced sensitivity to the azoles (Cools *et al*., 2012) and more recently shown development of resistant strains to SDHi's. (Dooley *et al*., 2016).

Future changes in pesticide legislation within the European Union is likely to further restrict the number of registered active ingredients available to growers and is slowing the development of new active ingredients which are required to advance the control of *Z. tritici.*

Fenpicoxamid is a new active substance developed by Dow AgroSciences for the cereal market being the first molecule from a new class of fungicides called picolinamides. It offers novel chemistry with a new target site for the cereal fungicide segment. Fenpicoxamid inhibits cell respiration by binding at the Qi site of the respiratory cyt bc1 complex (Young *et al* 2017). The molecule will be assigned to FRAC group C4-21, (QiI quinone inside inhibitors). It will be the first new target site to be introduced to the cereal fungicide market in over a decade and comes at a time when new fungicides solutions will be critical to manage *Z. tritici* resistance issues with current chemistries. Fenpicoxamid provides strong curative and residual protectant

efficacy in wheat against *Z. tritici* with good protectant activity on yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia triticina*). Extensive studies show that there is no cross resistance from fenpicoxamid to other groups of chemistry currently used in the cereal fungicide market (Owen *et al*., 2017).

Fenpicoxamid is being developed in co-formulation with prothioconazole and contains 50g a.s./litre fenpicoxamid and 100g a.s./litre prothioconazole, as an EC formulation.

This paper summarises trials undertaken between 2014 and 2017 in Belgium, Czech Republic, Denmark, France, Germany, Republic of Ireland and the UK to evaluate the efficacy of fenpicoxamid + prothioconazole for the control of *Z. tritici*.

MATERIALS AND METHODS

Trial design

Eighteen replicated field trials were conducted in winter wheat to GEP standards in Belgium (1), Czech Republic (2), Denmark (1), France (5), Germany (2), Republic of Ireland (2) and UK (5) in 2014. An additional three trials were conducted in 2017 in Germany (1), France (1) and the UK (1) to evaluate the efficacy of fenpicoxamid + prothioconazole against new and existing references for the control of *Z. tritici*. All field trials were designed as randomised complete blocks with 4 replicated plots per treatment and a minimum plot size of 2.5 x 12 m. All applications were applied using a precision small plot sprayer calibrated to deliver 150 to 250 litres/ha. Each treatment was applied twice to the same plot at T1 (Zadoks 31-32 of the crop, leaf 3 emerged) and again at T2 (Zadoks 37-39 of the crop, leaf 1 just visible to leaf 1 unrolled) in the trials in 2014 and applied only once at T2 (Zadoks 37-39 of the crop, flag leaf visible to flag leaf unrolled) in the trials in 2017. In all trial situations susceptible varieties were used and trials were placed in locations where *Z. tritici* is typically an issue to growers.

Crop safety assessment

Trials were assessed for any visual symptoms of crop injury. Parameters examined were % visual chlorosis, growth inhibition (stunting) and vigour reduction. Assessments were typically made at 1, 2, 4 and 6 weeks after application.

Efficacy assessment

Visually, percentage infection on a 0-100 scale was assessed on the upper three leaves (leaf 1, leaf 2 and leaf 3) and the mean percentage control was then calculated across all leaf levels. Assessments were made immediately prior to application and typically at 4 and 6 weeks after application. Final assessment data are presented in Figures 1 and 3.

Crop Yield

Harvesting was carried out using a small plot combine. Yield is expressed as t/ha at 14% moisture content. Figures 2 and 4.

Trials Details

Table 1. Details of Field Trials

	Application timing	Crop growth stage at application
Zymoseptoria tritici	T1	BBCH 31 to BBCH 32
	T2	BBCH 37 to BBCH 39

Formulation Details

Details of all formulations tested are in Table 2.

Table 2. Product and Formulation Details

Treatment	Active substance concentration	Formulation type	Dose rate (litres/ha)
fenpicoxamid + prothioconazole	50 + 100g a.s./litre	EC	2.0
prothioconazole	275g a.s./litre	EC	0.72
bixafen + prothioconazole	75 + 160g a.s./litre	EC	1.25
fluxapyroxad + metconazole	62.5 + 45g a.s./litre	EC	2.0
benzovindiflupyr + prothioconazole	75 + 150g a.s./litre	EC	1.0
bixafen + fluopyram + prothioconazole	65 + 65 + 130g a.s./litre	EC	1.5

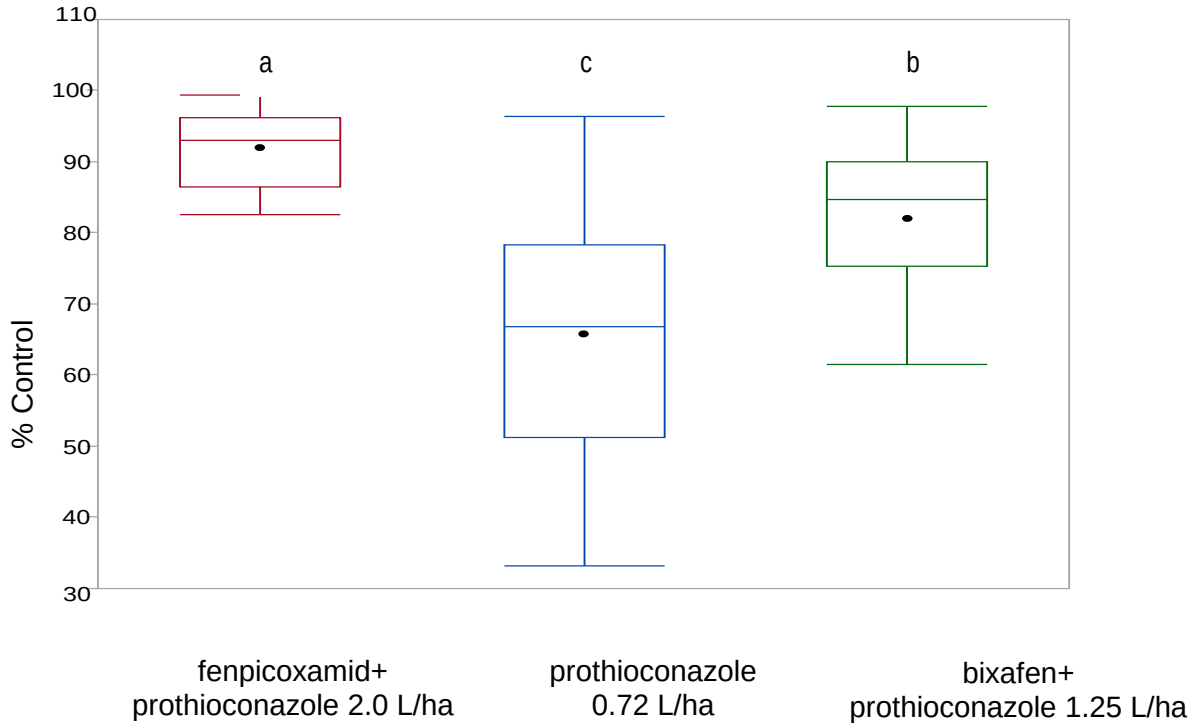
RESULTS

Visual percent control of *Zymoseptoria tritici* from treatments applied at T1 and again at T2 (2014 trials) is shown in Figure 1.

Crop yield in t/ha from treatments applied at T1 and again at T2 is shown in Figure 2.
Visual percent control of *Zymoseptoria tritici* from a single treatment applied at T2 (2017 trials) is shown in Figure 3.

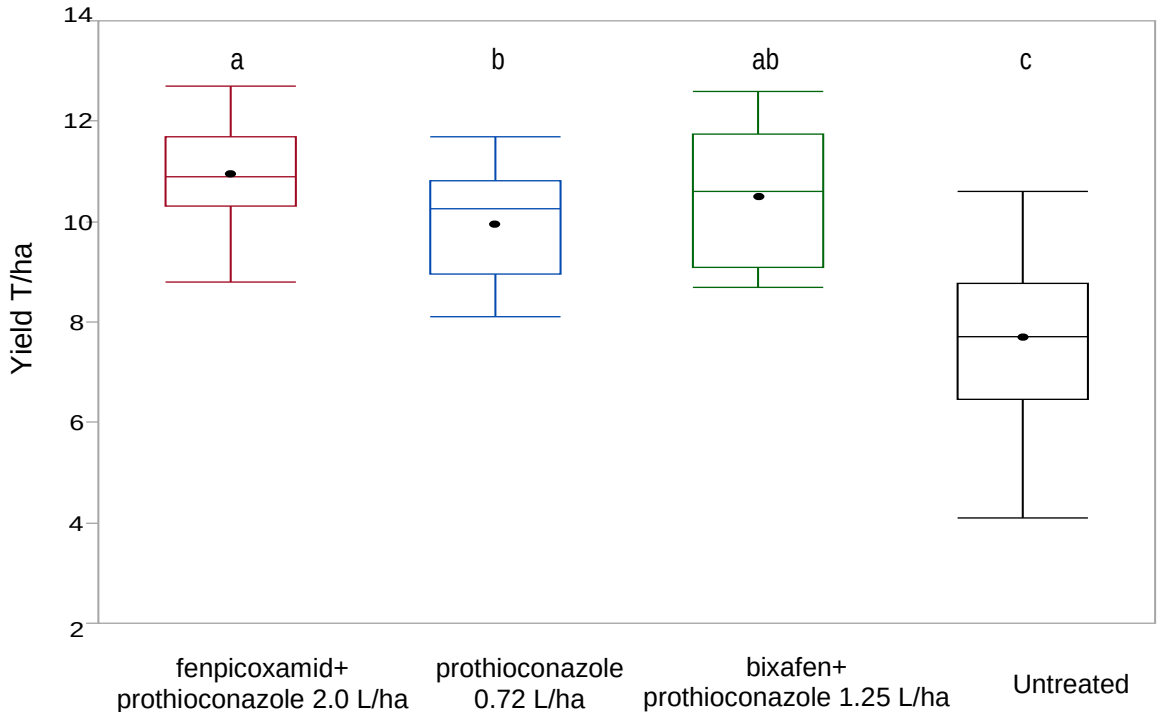
Crop yield in t/ha from treatments applied as a single application at T2 is shown in Figure 4.

Crop injury assessments were conducted in all trials but no phytotoxicity was observed from any treatment and no data are presented.



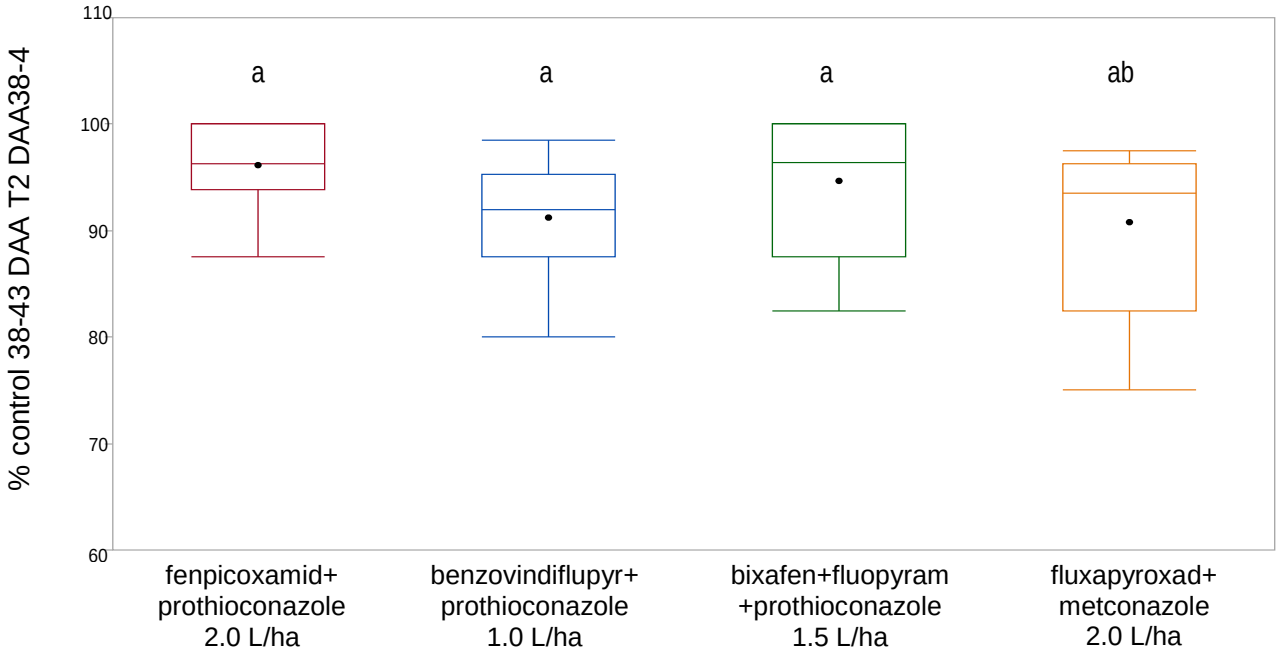
Mean of 18 trials, each treatment applied twice T1 and T2
Range of infection levels at each application – curative and protectant situations
Mean 52% infection in untreated at time of assessment, Tukey's HSD 2.40, P=0.05

Figure 1. Percent control of *Z. tritici* 35–42 Days after T2



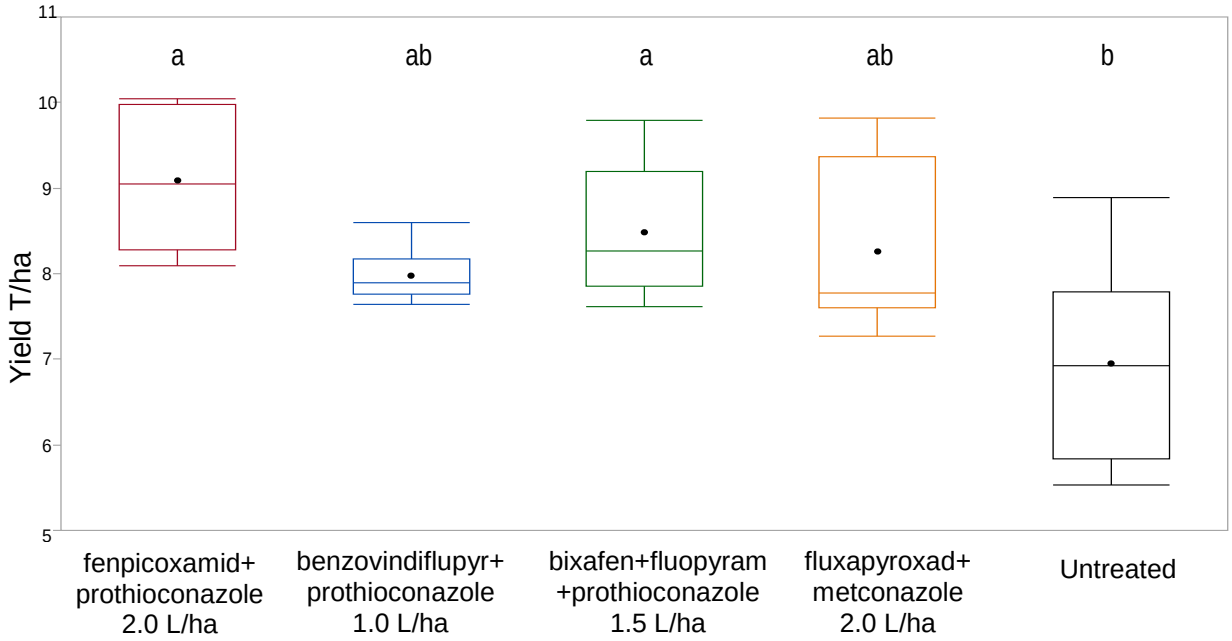
Mean of 18 trials, each treatment applied twice T1 and T2, Tukey's HSD 2.63, P=0.05

Figure 2. Crop Yield (t/ha)



Mean of 3 trials, each treatment applied once at T2,
Mean 24% infection in untreated at time of assessment, Tukey's HSD 2.68, P=0.05

Figure 3. Percent control of *Z. tritici* 38–43 Days after T2



Mean of 3 trials, each treatment applied once at T2, Tukey's HSD 2.90, P=0.05

Figure 4. Crop Yield (t/ha)

DISCUSSION

Fenpicoxamid + prothioconazole applied at its anticipated label rate of 2.0 litres/ha is statistically superior to both standards prothioconazole and bixafen + prothioconazole applied

at their respective label rates of 0.72 L/ha and 1.25 L/ha for the control of *Z. tritici* (92%, 65.8% and 82% mean control respectively, Figure 1) and delivers a statistically significant mean yield increase across the 18 trials of 1.0 t/ha over prothioconazole (10.9 and 9.9 t/ha respectively, Figure 2), and 0.4 t/ha over bixafen + prothioconazole (10.9 and 10.5 t/ha respectively, Figure 1) although not statistically significant.

Fenpicoxamid + prothioconazole applied at its anticipated label rate of 2.0 L/ha is more consistent than benzovindiflupyr + prothioconazole, bixafen + fluopyram + prothioconazole and fluxapyroxad + metconazole at their respective label rates for the control of *Z. tritici* (96.2, 91.2, 94.7 and 90.8% control respectively, Figure 3) and delivers a higher yield, although not statistically significant. Mean yield increases across the trials ranged from 0.7 t/ha over benzovindiflupyr + prothioconazole, 0.2 t/ha over bixafen + fluopyram + prothioconazole and 0.4 t/ha over fluxapyroxad + metconazole (8.7, 8.0, 8.5 and 8.3 t/ha respectively, Figure 4).

Fenpicoxamid is the first cereal fungicide from the picolinamide class of chemistry with a new and unique target site of activity in cereals. It provides cereal growers with superior control of *Z. tritici* across a range of curative and protectant situations, yield increases over current fungicide options and no cross resistance to current chemistry.

Fenpicoxamid is a single site inhibitor. To minimise the risk of resistance developing it will be formulated in mixture with prothioconazole and recommended as part of a programme.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Meiji Seika Pharma Co. Ltd., Dow AgroSciences Ltd colleagues as well as external researchers.

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GF-3447 - A NEW HERBICIDE CONCEPT CONTAINING HALAUXIFEN-METHYL FOR THE POST EMERGENCE CONTROL OF BROADLEAF WEEDS IN WINTER OILSEED RAPE

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Summary: GF-3447 (proposed trade name BELKAR™) is a new herbicide concept for use across Europe in winter oilseed rape as a post-emergence broad spectrum herbicide. GF-3447 (EC formulation) contains picloram at 48g ae/litre and Dow AgroSciences new active substance halauxifen-methyl (Arylex™ active) at 9.6g ae/litre. Both actives are HRAC Group O (synthetic auxin) with halauxifen-methyl belonging to a new family of 6-arylpicolinate herbicides. GF-3447 can be used at 0.25 litres/ha from two leaves of the crop and at 0.5 litres/ha from six leaves until beginning of side shoot development with a maximum total dose of 0.5 litre/ha. GF-3447 controls key broadleaf weed species including *Fumaria officinalis*, *Geranium pusillum* & *dissectum*, *Capsella bursa-pastoris*, *Papaver rhoeas* and *Galium aparine*. Robust control is maintained throughout this period irrespective of weather conditions. GF-3447 provides growers the flexibility to wait until a commercial crop stand is established before making a herbicide application.

INTRODUCTION

GF-3447 is a new herbicide concept for use across Europe in winter oilseed rape as a post-emergence broad spectrum herbicide. GF-3447 (EC formulation) contains picloram at 48g ae/litre and Dow AgroSciences new active substance halauxifen-methyl at 9.6g ae/litre. Both actives are members of the pyridine carboxylic (picolinate) family of herbicidal auxins classed as HRAC Group O (synthetic auxin). However, halauxifen-methyl differs from other members of the picolinate family as it contains a 6-phenyl substitution on the pyridine ring. Halauxifen-methyl is the first member of the new structural class of chemistry known as the arylpicolinate herbicides.

The active ingredients in GF-3447 are primarily foliar systemic herbicides that are translocated in the phloem and xylem to the meristems and other developing parts of the plant. Both halauxifen-methyl and picloram bind to specific receptor proteins involved with turning on and off vital plant processes causing a deregulation of plant growth metabolic pathways. Activity on sensitive species occurs through the subsequent uneven cell division and growth with various symptoms possible including cessation of growth, epinasty, chlorosis and stem thickening. Key species controlled include *Fumaria officinalis*, *Geranium pusillum* & *dissectum*, *Capsella bursa-pastoris*, *Papaver rhoeas* and *Galium aparine*. Proposed rates of use are 0.25 litres/ha from two leaves of the crop, a repeat application of 0.25 litres/ha at a minimum of two weeks from the first or, provided the crop has six leaves, an application at 0.5 litres/ha. Robust control of weed species is maintained throughout this period irrespective of weather conditions. GF-3447 therefore provides growers a wide window of application and the flexibility to wait until a commercial crop stand is established before making any herbicide applications.

Results presented in this paper summarize data across the Maritime, North-East and South-East EPPO Climatic Zones focusing on the key broadleaf weeds previously stated. Data reported are from over 100 efficacy trials with GF-3447 applied at the rates mentioned above.

MATERIALS AND METHODS

Trial Design

Over one hundred trials, on 50+ varieties, were conducted to GEP standards in Germany, France, Poland, UK, Czech Republic, Slovakia, Hungary, Romania, Denmark, Latvia, Lithuania and Sweden over a 4 year period from 2013 to 2017. All trials were designed as randomised complete blocks with 4 replicated plots per treatment and a minimum plot size of 12m^2. Weed populations were natural and growth stages and mean density (plants/m^2) of the key weeds discussed are presented (Table 1) along with number of trials per species. All applications were made post-emergence of the crop and weed species using a precision small plot sprayer calibrated to deliver 150-400 litres/ha.

Efficacy assessment

Assessments for % visual control relative to the untreated plots were made at regular intervals and data from the final assessment timing are presented (Tables 2 to 5 and Figure 1). Control was assessed on a linear scale where 0% represents no control and 100% represents complete plant death.

Application timing

Early autumn applications (earl Sept – early Oct) at 0.25 litres/ha were made to winter oilseed rape at BBCH 10-15. Later autumn applications (Oct-Nov) at 0.5 litres/ha were made to winter oilseed rape at BBCH 13-18. At both timings the majority of winter oilseed rape plants were at BBCH 12-14 or 16 respectively (as requested) with only a few plants in a few trials outside these stages. Trials with 0.25 litres/ha followed by 0.25 litres/ha had the second application made at the later timing. Weed growth stages and densities were as follows:

Table 1. Weed growth stages and densities at time of application with 0.25 litres/ha applied at the early timing and 0.5 l/ha applied at the late timing.

Application rate (litres/ha)	Species (number of trials in brackets)	Growth stage (BBCH)	Weed density plants/m^2
0.25	*Fumaria officinalis* (11)	10-30	14
0.5		11-32	19.5
0.25	*Geranium pusillum* (19)	10-16	35
0.5		10-61	47
0.25	*Geranium dissectum* (12)	10-21	45
0.5		10-29	50
0.25	*Capsella bursa-pastoris* (55)	10-16	18.8
0.5		11-51	20
0.25	*Papaver rhoeas* (38)	10-24	24
0.5		10-30	45
0.25	*Galium aparine* (33)	10-33	7.3
0.5		10-34	9

RESULTS

Control (% visual) of the key broadleaf weeds stated above from GF-3447 applied at 0.25 litre/ha once or twice and 0.5 litre/ha applied once, meaned across trials in the period 2013-2017, is presented (Figure 1). Control data are from the final efficacy evaluation conducted once growth had resumed in spring, typically in April. Trials were conducted in all countries mentioned above.

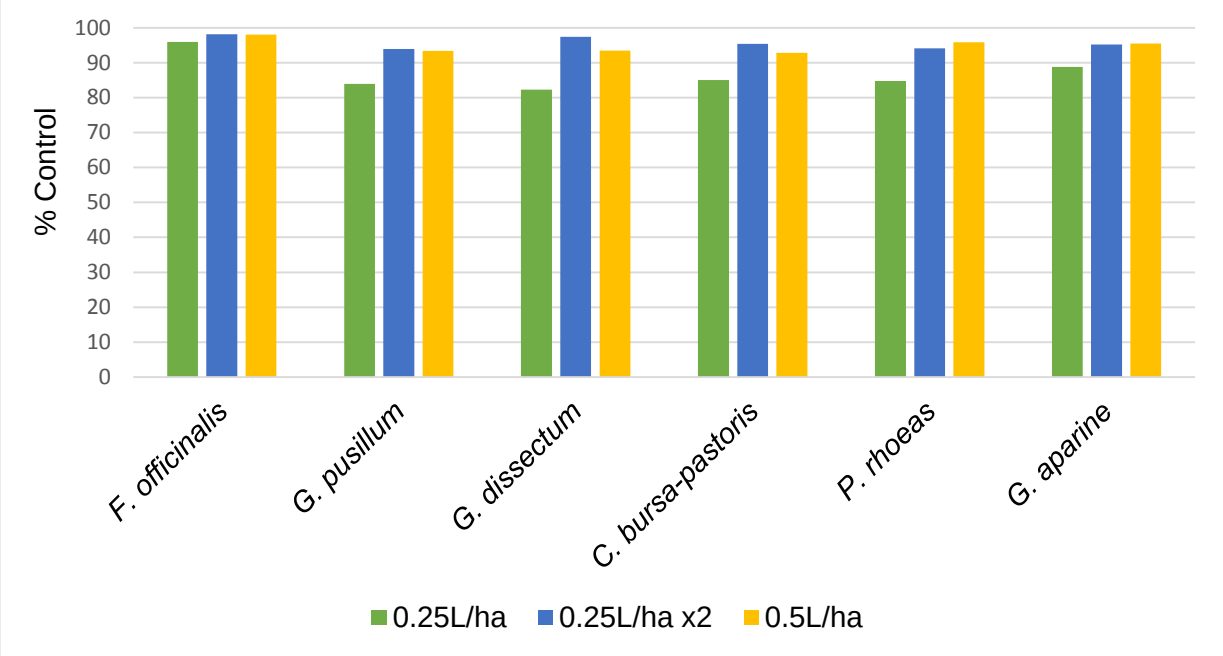

Figure 1. Mean % control of key weed species in oilseed rape 4-6 months after an application of GF-3447

Control of *Papaver rhoeas* from one trial carried out in the UK in 2016 / 17 comparing GF-3447 with dimethenamid-P + metazachlor + quinmerac is given in Table 2.

Tables 3 – 5 show control from specific trials, taken from the above mentioned trial set, from Hungary or the UK where there had been a period of adverse (cold) weather post late autumn application. The late autumn application in one trial (Table 5) was also preceded by a period of dry weather. Both cold and dry conditions are not conducive to growth and can impact herbicide performance.

Table 2. Mean % control of *Papaver rhoeas* 97 or 159 days after application

Treatment	Rate (litres/ha) date of application in brackets	Weed number, size and growth stage at application	% Control
GF-3447	0.25 (1/10/16)	120plants/m^2, 2cm diameter, BBCH 11-14	75.0
GF-3447	0.25 (both timings)		99.3
GF-3447	0.5 (2/12/16)	120plants/m^2, 10cm diameter, BBCH 14-19	94.0
Dimethenamid-P + metazachlor + quinmerac	2.5 (1/10/16)		48.3
1 trial UK		P=0.05, LSD = 11.47	

Table 3. Mean % control of *Geranium dissectum* and *Galium aparine* either 185 or 150 days after an application of GF-3447 with cold conditions after the later application

	Geranium dissectum		*Galium aparine*	
Rate (litres/ha) date of application in brackets	Weed number, size and growth stage at application	% Control	Weed number, size and growth stage at application	% Control
0.25 (20/10/14)	100 plants/m^2, 2cm diameter, BBCH 12-14	42.5	8 plants/m^2, 4cm diameter, BBCH 12-16	61.3
0.25 (both timings)		98.5		98.3
0.5 (24/11/14)	100 plants/m^2, 15cm diameter, BBCH 16-19	97.8	10 plants/m^2, 12cm diameter, BBCH 23-32	96
1 trial Hungary	P=0.05 LSD=5.58		P=0.05 LSD = 5.22	

Table 4. Mean % control of *Geranium molle* either 213 or 136 days after an application of GF-3447 with cold conditions after the later application.

	Geranium molle	
Rate (litres/ha) date of application in brackets	Weed number, size and growth stage at application	% Control
0.25 (18/9/14)	3% ground cover, 5cm diameter, BBCH 12-14	80
0.25 (both timings)		99
0.5 (4/12/14)	50% ground cover, 10cm diameter, BBCH 18-19	94.7
1 trial UK	P=0.05, LSD = 14.255.	

Table 5. Mean % control of *Geranium pusillitum* and *dissectum* 185 or 150 days after an application of GF-3447 with cold conditions after the later application (also preceded by dry weather).

	Geranium pusillum		*Geranium dissectum*	
Rate (litres/ha) date of application in brackets	Weed number, size and growth stage at application	% Control	Weed number, size and growth stage at application	% Control
0.25 (20/10/14)	100 plants/m^2, 2cm diameter, BBCH 12-14	61.25	100 plants/m^2, 2cm diameter, BBCH 12-14	81.25
0.25 (both timings)		98		98.8
0.5 (24/11/14)	100 plants/m^2, 20cm diameter, BBCH 16-19	96	100 plants/m^2, 15cm diameter, BBCH 16-19	95.8
1 trial Hungary	P=0.05 LSD=5.92		P=0.05 LSD = 5.23	

DISCUSSION

Levels of susceptibility relate to control levels according to the European Weed Research Society (EWRS) scale where highly susceptible = 95-100%, susceptible = 85-94.9%, moderately susceptible = 70-84.9%, moderately tolerant = 50-69.9% and tolerant = 0-49.9%.

GF-3447 applied once at 0.25 litres/ha, with the majority of crops at BBCH 12-14, provided susceptible control of *C. bursa-pastoris* and *G. aparine* and highly susceptible control of *F. officinalis*. A repeat application of 0.25 litres/ha, at the later timing, resulted in susceptible control of *G. pussillum* and *P. rhoeas* and highly susceptible control of *F. officinalis*, *G. dissectum*, *C. bursa-pastoris* and *G. aparine*. GF-3447 applied at 0.5 litres/ha (late timing) provided a susceptible level of control of *G. pusillum*, *G. dissectum* and *C. bursa-pastoris* and highly susceptible control of *F. officinalis*, *P. rhoeas* and *G. aparine*. Results were taken from over 100 trials across 12 European countries and highlight the broad spectrum efficacy of GF-3447.

Compared to current standard actives, GF-3447 provides greater efficacy on key species such as *Papaver rhoeas*, as evidenced from a trial in the UK (Table 2). Highly susceptible control was attained from the sequence of 0.25 litres/ha and susceptible control from 0.5 litres/ha applied at the later timing. This was compared to tolerant levels achieved by dimethenamid-P + metazachlor + quinmerac applied at the early timing.

Robust levels of control from GF-3447 were maintained throughout the autumn application window regardless of weather conditions, which can be very variable at this time of year, as shown in trials from Hungary and UK (Tables 3-5). In Hungary *G. dissectum* and *G. aparine* were highly susceptible to a sequential application of 0.25 litres/ha (mid Oct and late Nov) or a late November application of 0.5 litres/ha (Table 3). High levels of control were recorded despite cold conditions after the late November application with average maximum temperature 28 days post application of 6.5°C and an average minimum temperature of 1.9°C. In the UK, *G. molle* control further demonstrates the robustness of GF-3447 applied sequentially at 0.25 litres/ha (mid Sept and early Dec) or once at 0.5 litres/ha applied early

December, with highly susceptible or susceptible levels of control achieved (Table 4). Control was attained despite cold conditions after application with average maximum, minimum and daily average temperature 28 days post application of 7.9°C, 0.9°C and 2.3°C respectively. Cold conditions persisted through January (average maximum 7.5°C, minimum 0.1°C and daily average 1.3°C) and February (average maximum 6.9°C, minimum 0.0°C and daily average 1.0°C). Highly susceptible control of *G. pursillum* and *G. dissectum* was achieved from a sequence of 0.25 litres/ha (late Oct and late Nov) or a late November application of 0.5 litres/ha (Table 5). Highly susceptible control was attained despite dry conditions before the late November application and cold conditions after application. Only 1.8mm of rain was recorded 28 days pre-application and average maximum temperature 28 days post application was 6.5°C, average minimum 1.9°C and daily average 4.1°C. GF-3447 provided robust control over a broad range of environmental conditions when applied either as two sequential applications of 0.25 litres/ha or a single application of 0.5 litres/ha.

In conclusion, GF-3447 is a broad spectrum herbicide that will deliver control of key weed species in oilseed rape. It provides growers a wide window of application with performance regardless of weather conditions. Further, it gives growers the ability to wait until a commercial crop stand is established before applying herbicides.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Dow AgroSciences colleagues across Europe with this work.

HALAUXIFEN-METHYL + FLORASULAM A NEW POST EMERGENCE BROAD LEAVED WEED CEREAL HERBICIDE PROVIDING ROBUST CONTROL WHEN APPLIED DURING CONDITIONS OF POOR WEED GROWTH DUE TO SOIL MOISTURE DEFICIT AND COLD TEMPERATURES

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Summary: A series of trials were established to evaluate the impact of poor growth due to soil moisture deficit or low temperatures on the efficacy of Zypar[TM1] herbicide (halauxifen-methyl + florasulam 6 g a.e. + 5 g a.s. /L, OD) for the control of broad leaved weeds in cereals. Halauxifen-methyl + florasulam is a new post emergence herbicide for the control of an extensive range of broad leaved weeds in cereals. Both actives are post-emergence, foliar systemic herbicides. Halauxifen-methyl is a HRAC Group O herbicide which belongs to the new family of 6-arylpicolinates. Florasulam is a triazolopyrimidine HRAC Group B herbicide. Halauxifen-methyl+florasulam provided superior control of all weed species irrespective of the soil moisture content or temperature before and after application. Halauxifen-methyl showed robust and consistent efficacy of broadleaf weeds even during periods of poor or variable growth and provides the grower with true flexibility on application timing.

[1] Trademark of The Dow Chemical Company ("Dow") or an affiliated company of Dow.

INTRODUCTION

Halauxifen-methyl + florasulam is a new post emergence herbicide approved by the CRD in 2017 for use in winter and spring cereals. It can be applied from the 1st September to the 30th June, BBCH 13 to 45 of the crop inclusive and provides broad spectrum broadleaf weed control of commercially important weed species including; *Chenopodium album*, *Galeopsis tetrahit*, *Galium aparine*, *Geranium species*, *Fumaria officinalis*, *Lamium species, Polygonum convolvulus*, *Papaver rhoeas, Stellaria media* and small *Veronica species.*

Halauxifen-methyl is a new active substance discovered by Dow AgroSciences for the global cereal market. It is a post-emergence HRAC Group O herbicide (synthetic auxin) which belongs to the new family of 6-arylpicolinate herbicides. It is a foliar systemic herbicide with limited residual activity. It is translocated through the phloem and xylem to the meristematic areas and induces a phenotypic response on sensitive plant species similar to the natural/synthetic auxin herbicides. Halauxifen-methyl offers broad spectrum broadleaf weed control of commercially important weed species. Early trials work during the development of halauxifen-methyl showed that efficacy is expressed independent of variable weather conditions. Therefore it offers true flexibility on application timing and more reliable weed control. Furthermore, as a member of the HRAC Group O herbicides, halauxifen-methyl is considered to be a low risk herbicide for resistance and can be used as a resistance management tool. It is applied at low rates ($\leq$ 7.5 g a.e. /ha).

Florasulam is a post emergence HRAC Group B herbicide which belongs to the triazolopyrimidine family of chemistry discovered by Dow AgroSciences and first sold in

Europe in spring 1999. This class of herbicide is known to inhibit the plant enzyme acetolactate synthase (ALS). It is mobile in both the xylem and phloem accumulating in the primary and auxiliary meristems, providing control of commercially important broadleaf weeds. Environmental conditions that are not conducive to active plant growth impact the translocation of florasulam to the meristems and results in reduced herbicidal activity.

Cloquintocet-mexyl is included in the formulation at 6 g a.s./L to enhance crop selectivity.

This paper summarises trials specifically established during conditions of poor weed growth to determine what effect these conditions had on the efficacy of halauxifen-methyl + florasulam for the control of key broadleaved weeds.

MATERIALS AND METHODS

Trial design

Six replicated field trials were conducted in winter wheat to GEP standards in the UK over a 4 year period (2012 to 2016) to evaluate the efficacy of halauxifen-methyl + florasulam across a range of weed species when applied during low temperatures. All field trials were designed as randomised complete blocks with 4 replicated plots per treatment and a minimum plot size of 2 x 6 m. All applications were applied post-emergence of the crop and weeds using a precision small plot sprayer calibrated to deliver 150 to 250 L/ha. Details of application dates, weed species, weed density are detailed in Table 1.

In addition to the field trials two glasshouse trials were established to evaluate the impact of soil moisture on the efficacy of halauxifen-methyl + florasulam for the control of *Papaver rhoeas* and *Centaurea cyanus*. Both glasshouse trials were designed as randomised complete blocks with 4 replicated pots per treatment. Each pot was 75 mm square, filled with a light sandy loam soil, with 1-2% organic matter, pH of 6 and contained five plants. For each treatment two separate soil moisture regimes were followed 70 to 80% soil capacity for the duration of both trials and 30 to 40% soil capacity for the duration of the *P. rhoeas* trial. In the *C. cyanus trial* 40% soil capacity was maintained for first 3 weeks and then 70 to 80% for the remaining 2 weeks of the trial. All pots received 12 hours light and 12 hours dark, air temperature 8^{o}C in the night and 12^{0}C during the day. Treatments were applied 1 week after the pots were placed in the respective soil moisture regimes using a pot sprayer calibrated to deliver 200 L/ha. Details of application dates, weed species, weed density are detailed in Table 2.

Crop safety and efficacy assessments

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

Assessments for percent visual control relative to the untreated plots were made at regular intervals and the final assessment data are presented in Figures 1 to 6. Control was assessed on a linear scale, where 0% represents no control and 100% represents plant death.

Trials Details

Table 1. Details of Field Trials

Weed species present	Weed growth stage (BBCH)	Weed density/ m^2	Weed height/diameter (cm)	Date of application	Crop growth stage (BBCH)
Papaver rhoeas	10-16	11	0.5 / 2.5	16 Dec12	12-21
Vicia faba	12-13	23	0.5 / 0.5	9 Jan 13	11-12
Galium aparine	22-23	5	3 / 10	22 Mar 16	22-26
Galium aparine	21-24	11	5 / 15	29 Feb 16	22-24
Fumaria officinalis	18-21	22	3 / 7	26 Feb 12	13
Fumaria officinalis	15-16	6	2 / 5	15 Feb 13	21-22

Table 2. Details of Glasshouse Soil Moisture Trials

Weed species	Weed growth stage (BBCH)	Weed height/diameter (cm)	Soil moisture capacity
Centaurea cyanus	12	5 / 7	70-80%
			30-40%
Papaver rhoeas	16-18	8 / 10	70-80%
			30-40%

FORMULATION DETAILS

Details of all formulations tested can be found in Table 3.

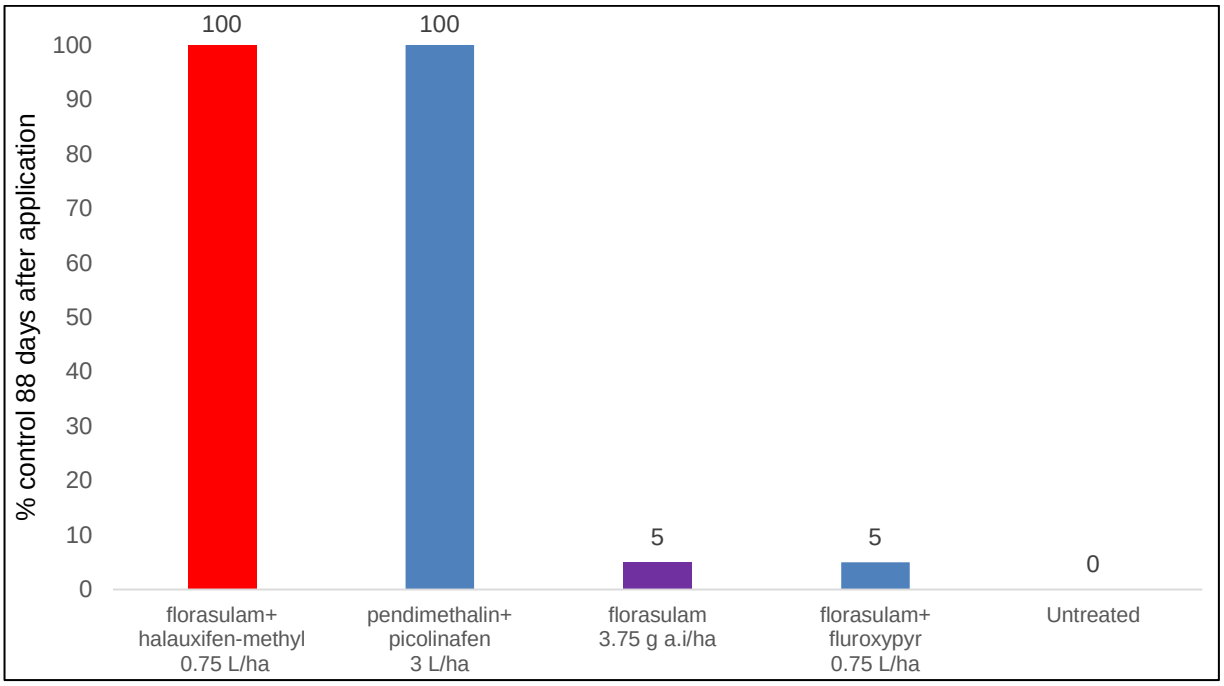
Table 3. Product and Formulation Details

Treatment	Active substance concentration	Formulation type
halauxifen-methyl + florasulam + cloquintocet-mexyl	6+5+6 g a.s./L	OD
Florasulam + fluroxypyr	5+100 g a.s./L	EC
pendimethalin + picolinafen	320+16g a.i./L	SC
florasulam	50g a.s./L	SC
fluroxypyr + metsulfuron-methyl + thifensulfuron-methyl	135+5+30 g a.s./L	OD
metsulfuron-methyl	20 % w/w	SG

RESULTS

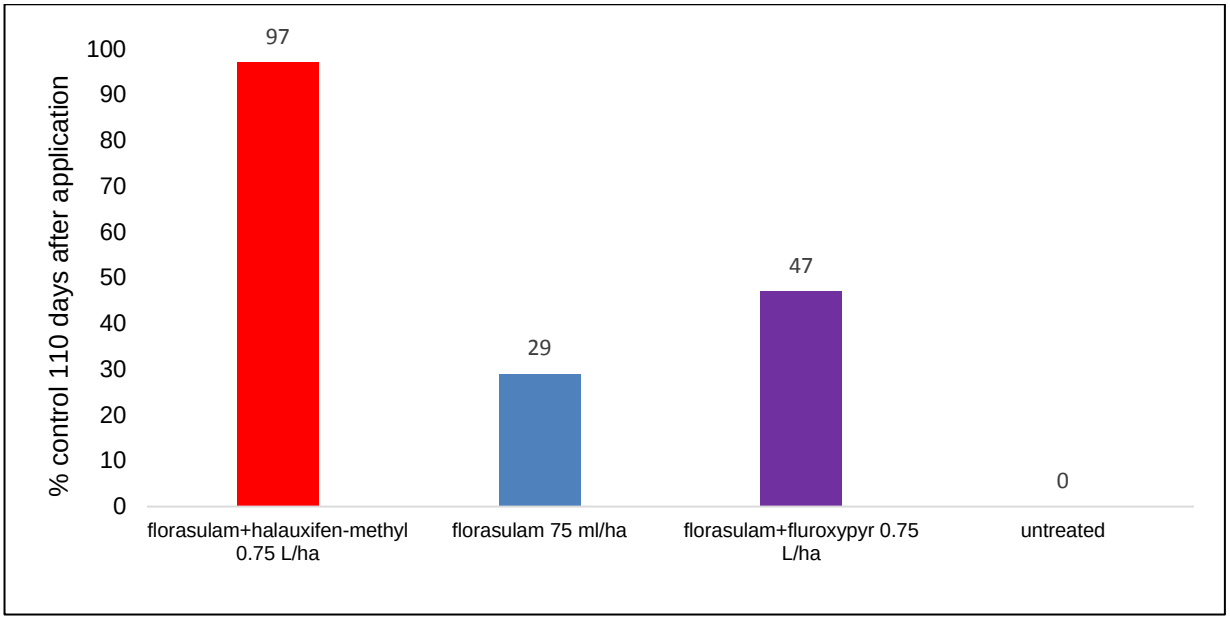
Crop injury assessments were conducted but no phytotoxicity was observed from any treatment and no data are presented.

Visual percent control for each specie is shown in Figures 1 to 6.



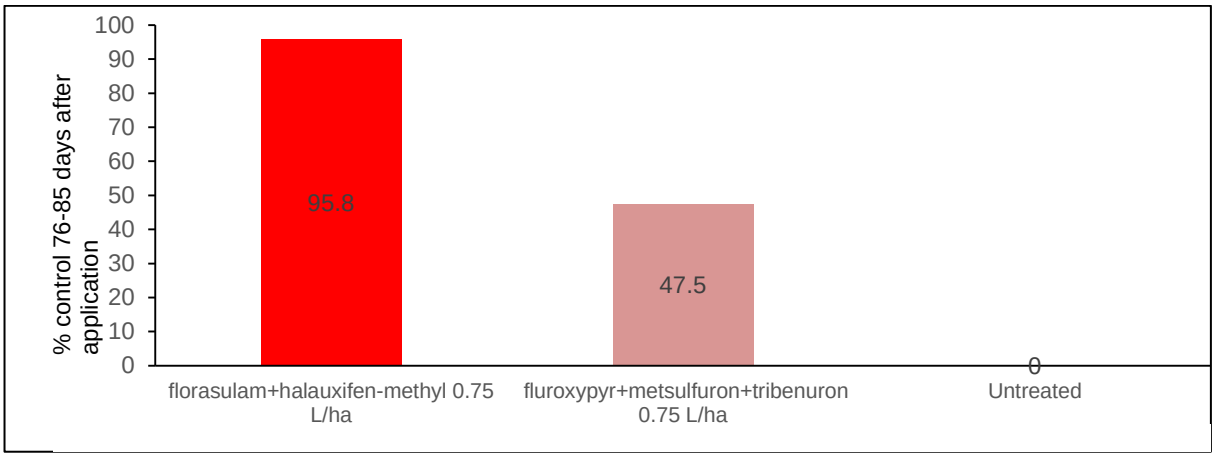
Papaver rhoeas BBCH 10-16 Mean of 1 trial, LSD 4.5, P=0.05

Figure 1. Percent control of P. rhoeas, herbicides applied 16th December 2012



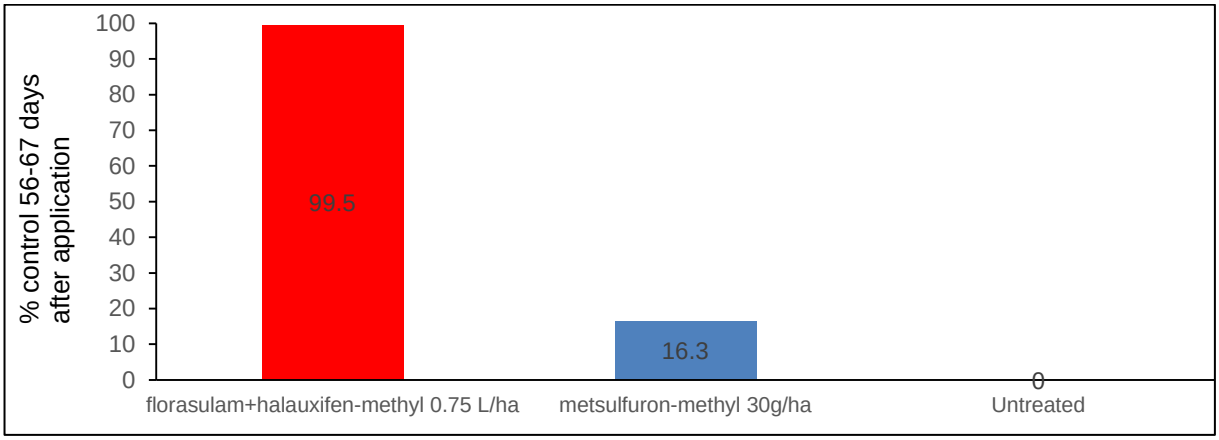
Vicia faba BBCH 12-13 Mean of 1 trial, LSD 2.4, P=0.05

Figure 2. Percent control of V. faba, herbicides applied 9th January 2013



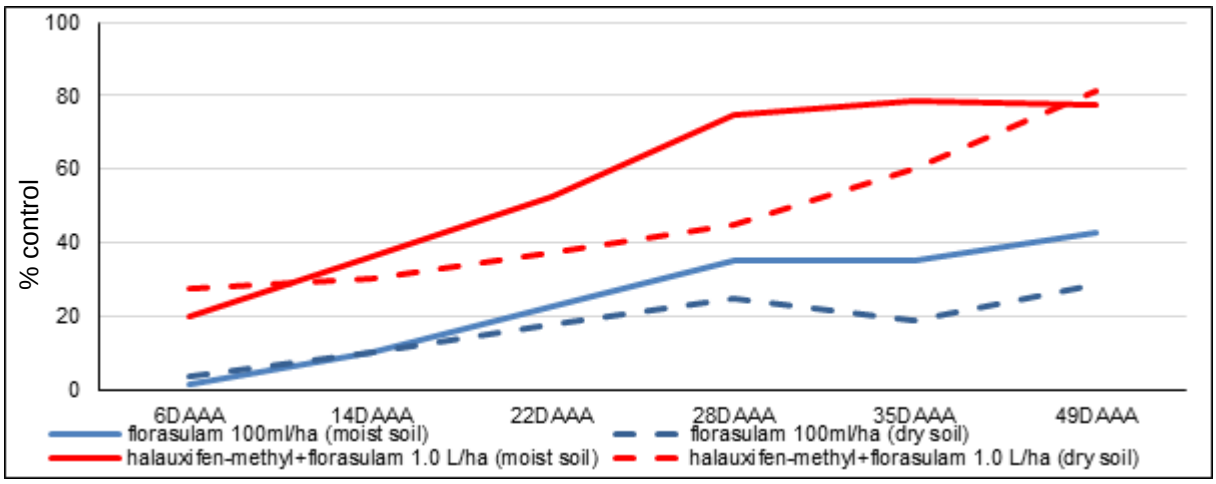
Galium aparine BBCH 21-24 Mean of 2 trials, LSD 2.52, P=0.05

Figure 3. Percent control of G. aparine, herbicides applied 29th February & 22nd March 16



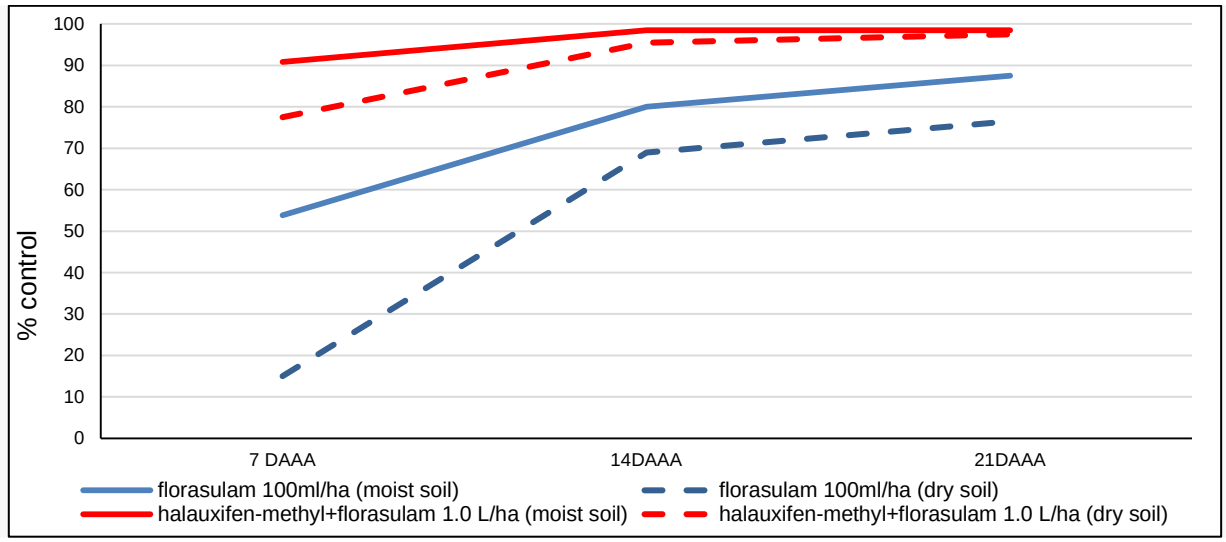
Fumaria officinalis BBCH 15-21 Mean of 2 trials, LSD 2.52, P=0.05

Figure 4. Percent control of F. officinalis, herbicides applied 26th Feb 2012 & 15th Feb 2013



Centaurea cyanus BBCH 12 Mean of 1 trial, Tukey's HSD 22, P=0.05

Figure 5. Percent control of C. cyanus, Moist Soil and Dry Soil



Papaver rhoeas BBCH 1-18 Mean of 1 trial, Tukey's HSD 38, P=0.05

Figure 6. Percent control of P. rhoeas, Moist Soil and Dry Soil

DISCUSSION

The weather conditions prior to application and subsequently were typical for the time of year with periods of frost. December 2012, January, February and March 2013 was especially cold with prolonged periods of frost. Average air temperature across the trials sprayed in this period (Figures 1, 2 and 4) was 1oC for up to 1 month after application with an average range of 0.010C to 6.90C. Halauxifen-methyl + florasulam applied during prolonged cold periods in December, January and February provided excellent control (> 96 %) of V. faba G. aparine, F. officinalis and P. rhoeas. (Figures 1-4). Efficacy on all tested species was significantly superior to the standards, florasulam, fluroxypyr-meptyl, metsulfuron-methyl + tribenuron-methyl and metsulfuron-methyl (P=0.05). Halauxifen-methyl + florasulam applied to soils with different moisture contents provided robust control of C. cyanus (>80%) and P. rhoeas (>97%) and was significantly superior to that of the standard florasulam (P=0.05).

In conclusion halauxifen-methyl + florasulam is a new flexible broad spectrum herbicide that can be used in winter and spring cereals from 1st September to 30th June, offering robust weed control during poor growing conditions, with a true wide window of application.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Dow AgroSciences Ltd colleagues.

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PROPYZAMIDE – AN ACROSS YEARS SUMMARY OF *ALOPECURUS MYOSUROIDES* AND *LOLIUM MULTIFLORUM* RESISTANCE TESTING

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Summary: The number of cases of resistance to ALS and ACCase herbicides is increasing in *Alopecurus myosuroides* and *Lolium multiflorum* across Europe. To maintain profitability, it is crucial that these weeds can be controlled in the rotation. The use of non-chemical methods of control as part of an integrated pest management (IPM) programme is imperative and will provide a measure of control. However, chemical means still remain an integral tool and it is vital that reliable and effective actives are protected. Propyzamide, used for controlling weeds including *A. myosuroides* and *L. multiflorum* in oilseed rape and beans, is one such active. This paper presents a summary of resistance testing of *A. myosuroides* and *L. multiflorum* seed samples collected in the UK and France. Data confirms the lack of resistance to propyzamide, demonstrates its efficacy on *A. myosuroides* and *L. multiflorum* and supports its use as an effective resistance management tool.

INTRODUCTION

Annual grassweeds can be a significant problem in autumn sown crops if not properly controlled. Two such grassweed species are *Alopecurus myosuroides* (blackgrass) and *Lolium multiflorum* (Italian ryegrass). Across North-western Europe *A. myosuroides* is a major weed in winter cereal crops (Chavvel et al 2002) and in England is now the most prevalent arable weed (Gosling, 2015). Control of both these weed species, particularly *A. myosuroides*, in cereals is becoming more challenging due to the increasing resistance to the commonly used acetolactate-synthase (ALS) and acetyl Co A carboxylase (ACCase) herbicides (Heap 2017). Blackgrass resistance to ALS and/or ACCase herbicides has been confirmed in Belgium, Czech Republic, Denmark, France, Germany, Italy, Netherlands, Poland, Spain, Sweden, Turkey and the UK (Heap 2017). Cases of resistance in *L. multiflorum* have been confirmed over a broader geographical range compared to blackgrass and include the European countries of Denmark, France, Italy and the UK (Heap 2017). It is therefore vital that, along with non-chemical control methods, growers have actives available in their rotation that provide effective control of these problematic weeds. One such active in oilseed rape and winter beans is propyzamide (Kerb™ Flo 500, Dow AgroSciences) where there is no reported case of resistance.

Propyzamide is a soil active systemic herbicide and member of the benzamide chemical family (HRAC Group K_1). Propyzamide binds to tubulin preventing its assembly into microtubules ultimately inhibiting cell division leading to plant death. It has a multi-site mode of action which is a key factor in why resistance has not yet developed.

Results in this paper present a summary across years of resistance testing of both blackgrass and Italian ryegrass seed samples collected in the UK and France also including a known susceptible accession. Plants grown from seed samples were treated with propyzamide, an acetolactate-synthase (ALS) inhibitor (HRAC group B) and two acetyl Co A carboxylase (ACCase) herbicides (HRAC group A), one belonging to the aryloxyphenoxy-propionates (Fops) and one belonging to the cyclohexanediones (Dims); actives were chosen to demonstrate the lack of any cross resistance between propyzamide and the ALS and ACCase chemistries.

MATERIALS AND METHODS

Seed samples

Samples of *A. myosuroides* seed were collected from farms in the UK and France in the seasons 2013/14, 2014/15 and 2015/16 with samples of *L. multiflorum* collected across the same seasons but from France only (detail given in Table 1). The susceptible standard was obtained from Herbiseed in the UK. Before planting seeds were stored in a cold room maintained at 4°C.

Table 1. Seed samples tested for sensitivity to propyzamide, ALS and ACCase herbicides

Country of Origin	Year of collection	Species	Number of samples tested per season
UK	2013	*A. myosuroides*	3
	2014		2
	2015		3
	2013-15		Susceptible standard
France	2013	*A. myosuroides*	2
	2014		5
	2015		2
France	2013	*L. multiflorum*	1
	2014		1
	2015		3
UK	2013-15	*L. multiflorum*	Susceptible standard

Plant propagation

Plastic pots (26 cm diameter) were filled with a clay loam soil (sand 21%, silt 60%, clay 19%) with an organic matter content of 5% and pH of 7.4. Seed was sown (0.35 g/ pot) and mixed in the top two cm of soil.

Experimental design

Two replicate pots were used per treatment for each seed sample with pots arranged in fully randomised blocks. All applications were made at a water volume of 200 litres/ha using a Lurmark OIE80 Even spray nozzle.

Application timing

The target application timing for propyzamide was once soil temperature at 8-10 cm was below 8°C with plants at the two to three leaf stage. In each year (except one) application was made mid-November with plants and soil at the desired stage. In the 2013/14 season application was made in the second week of November with plants at the correct growth stage but with soil at 11°C; however, soil temperature declined to below 8°C shortly after application. In all years ALS and ACCase treatments were applied under conditions suitable for them to work effectively – active growth of target weeds. The details of the treatments tested are presented in Table 2. Throughout the experimental period, pots were exposed to rainfall except prior and post herbicide application were they were kept under cover 12 hours pre and 12 hours post application.

Efficacy assessment

Assessments of % visual control relative to the untreated pots were made at regular intervals with a final assessment timing at 11 or 12 weeks post application. Control was assessed on a linear scale where 0 % represents no control and 100 % represents complete plant death.

Table 2. Treatments tested for control of *A. myosuroides* and *L. multiflorum* including test year, dose rate examined and adjuvant rate where used.

Test year	Active ingredient(s)	Dose rate (g a.i/ha)
2013-15	Propyzamide	840
2013-15	Iodosulfuron-methyl-sodium + mesosulfuron-methyl + adjuvant (Biopower 1L/ha)	14.4* 18**
2014 & 15	Cycloxydim + adjuvant (Dash 1L/ha)	200
2014 & 15	Propaquizafop + adjuvant (Biopower (2014) / Actirob B (2015) 1L/ha)	60
2013-15	Untreated (water only)	

*Rate used 2013 **Rate used 2014-15

RESULTS

Control of the susceptible standard of both species by all treatments averaged > 98% confirming application conditions were suitable for the tested herbicides to work effectively.

Control (% visual) achieved by the herbicides stated in Table 2 from the accessions indicated in Table 1 is presented in Figures 1-3 for *A. myosuroides* (one Figure per year) and Figure 4 for *L. multiflorum* across years. Control data presented is from the final efficacy assessment carried out at 11 or 12 weeks after application. Data for both the 14.4 g a.i/ha (2013) and 18 g a.i/ha (2014-15) rate of iodosulfuron methyl-sodium + mesosulfuron-methyl is combined for *L. multiflorum* in Figure 4.

The susceptible standard of *A. myosuroides* was controlled at levels between 97.5% and 100% across all years and herbicides tested. The susceptible standard population of *L. multiflorum* was 100% controlled by all herbicides for years 2013 and 2015. In 2014 the susceptible standard was controlled at levels of 90% from propyzamide, 97.5% from iodosulfuron methyl-sodium + mesosulfuron-methyl and 100% from cycloxydim and propaquizafop.

Treatment rates for Figures 1-4 are given in Table 2.

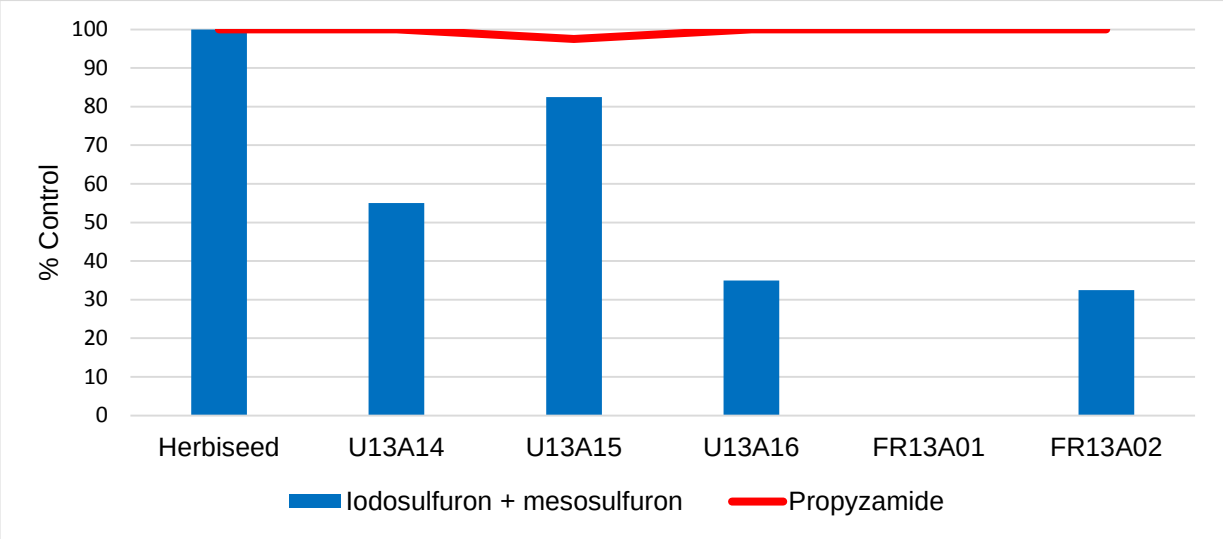

Figure 1. Control (% visual) of *A. myosuroides* seed samples collected season 2013-14 from the UK and France 12 weeks after application and including a susceptible accession

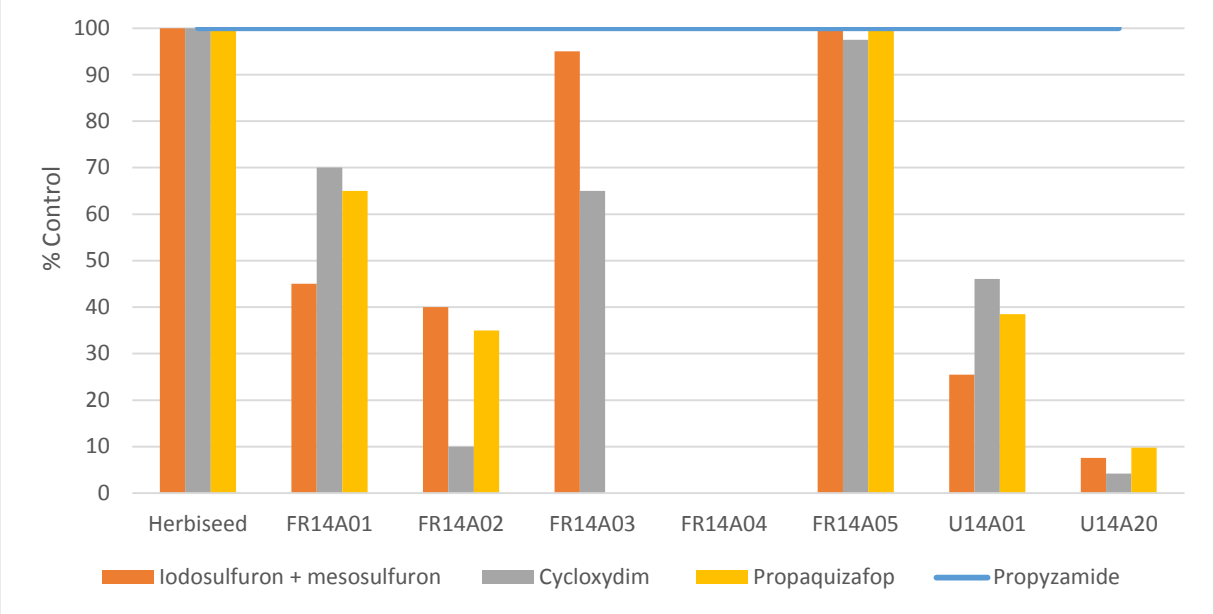

Figure 2. Control (% visual) of *A. myosuroides* seed samples collected season 2014-15 from the UK and France 11 weeks after application and including a susceptible accession

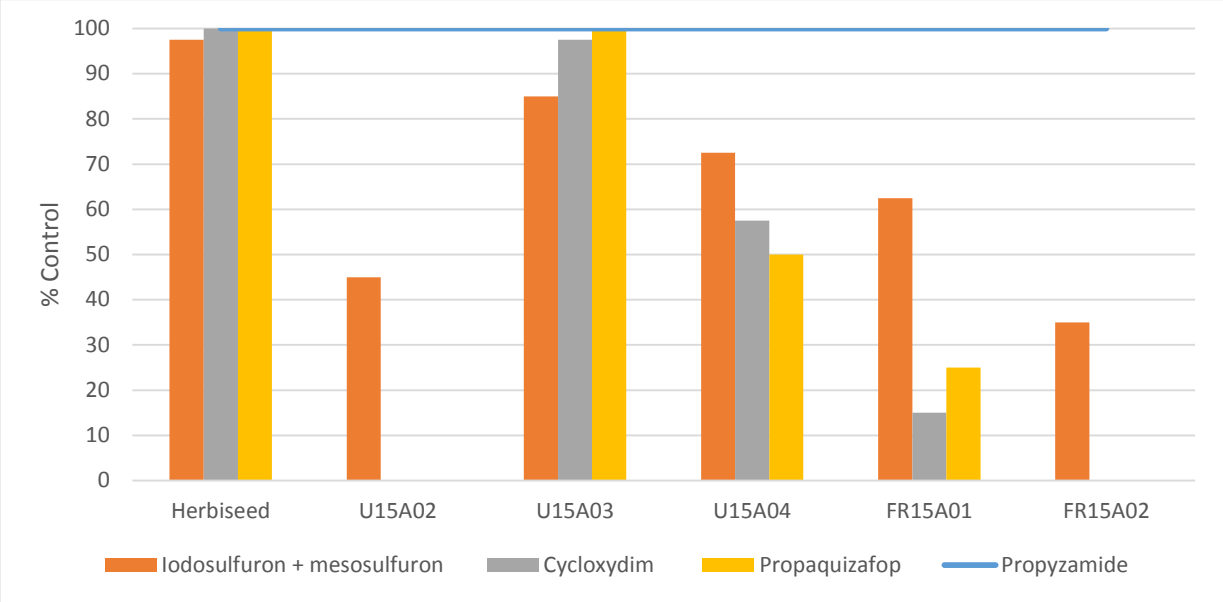

Figure 3. Control (% visual) of *A. myosuroides* seed samples collected season 2015-2016 from the UK and France 11 weeks after application and including a susceptible accession

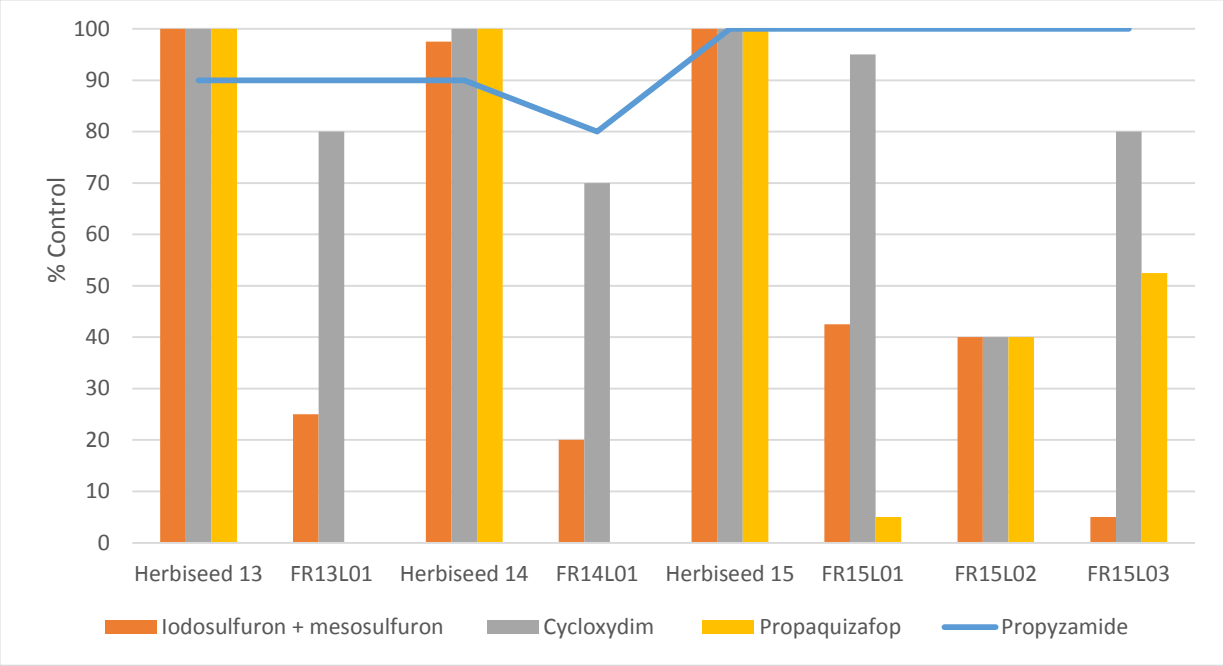

Figure 4. Control (% visual) of *L. multiflorum* seed samples collected seasons 2013-2014, 2014-2015 and 2015-2016 from France 11 weeks after application and including a susceptible accession

DISCUSSION

In this paper the method for categorising the resistance 'R' rating of a seed sample (Moss *et al*.,1999) has been employed but % reduction in foliage fresh weight has been substituted for % visual control. Samples within 20% of the susceptible standard are categorised as susceptible with those outside this classed as resistant.

All *A. myosuroides* samples tested across all years were susceptible to propyzamide. Of *A. myosuroides* accessions tested 77 % were resistant to ALS (iodosulfuron methyl-sodium + mesosulfuron-methyl) and 83 % resistant to ACCase (cycloxydim or propaquizafop). Accessions of *A. myosuroides* with complete resistance (0-5 % control) to ALS chemistry (FR13A01 & FR14A04) or ACCase chemistry (U15A02 & FR15A02) were susceptible to propyzamide.

All *L. multiflorum* samples tested were susceptible to propyzamide. Of the *L. multiflorum* accessions tested 100 % were resistant to ALS (iodosulfuron methyl-sodium + mesosulfuron-methyl), 40 % were resistant to cycloxydim and 100 % resistant to propaquizafop.

Data presented clearly demonstrate the ability of propyzamide to control ALS and ACCase resistant accessions of both *A. myosuroides* and *L. multiflorum* and its vital role in any IPM programme for these species. Despite its use over many years and over a broad geography there has not been a reported case of resistance to propyzamide. To protect the activity of propyzamide and secure its role as a resistance management tool it is essential growers make use of the appropriate non-chemical methods of control in line with the sustainable use of pesticides directive. This will help ensure populations of problematic weeds like *A. myosuroides* and *L. multiflorum* can be managed at levels that protect commercially viable farming.

ACKNOWLEDGEMENTS

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EMERGENCE AND SEEDLING LOSS DUE TO *MICRODOCHIUM* SPECIES ON SPRING OATS AND BARLEY AFTER A COLD PERIOD

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Summary: Laboratory experiments were carried out to examine which *Microdochium* species, *M. nivale* or *M. majus* was responsible for the reduced emergence in spring oats and barley and to determine if a period of 0°C would increase seedling loss. A host preference in oats for *M. nivale* was previously observed by McEwan & Cockerell (2016) and *M. nivale* was proven to be responsible for all seedling losses due to *Microdochium* species in oats. In contrast, 90% of the seedling losses due to *Microdochium* species in barley were caused by *M. majus*. Incubation at 0°C for 24 hours made no difference to seedling emergence in oats but reduced seedling emergence in barley by 10%.

INTRODUCTION

Previous experiments in 2014 and 2015 showed that there was a host preference in *M. nivale* for oats, and that both *M. majus* and *M. nivale* were found on barley seed (McNeil *et al*, 2014 and McEwan and Cockerell, 2016). In 2015 further laboratory experiments to define which pathogen was responsible for seedling loss were carried out. PCR analysis was carried out on *Microdochium* colonies isolated from dead seeds and abnormal seedlings after growth at 15°C for 2 weeks. Although there was seedling loss in spring oats (26% caused by *M. nivale*), very little loss of emergence was seen in spring barley (7% caused by *M. majus*), even at infection levels up to 70% (McEwan & Cockerell, 2016).

In 2012, Haigh and Hare showed that exposure of seeds to 0°C and sub-zero temperatures after planting, increased the severity of Microdochium seedling blight on naturally infected winter wheat. This work was carried out to see if holding spring barley and oat seeds for a short period after sowing, at a temperature near freezing, would increase the amount of pre-emergence seedling loss in Microdochium infected seed.

MATERIALS AND METHODS

Seed lots

Three lots each of spring oats and spring barley naturally infected with *Microdochium* species were selected (Table 1), one lot for each, with nil or very low infection, was included. The *Microdochium* species levels selected for the spring barley were very high due to previous experiments showing very little effect of *Microdochium* species on seedling loss (M. McNeil *et al.*, 2014).

Table 1. Seed lot test results

	Seed lot	% infection *Microdochium*	% Germination
Oats	A	0	91
	B	39	90
	C	65	91
Barley	D	1	100
	E	86	94
	F	84	96

Agar plate and germination tests

Agar plate test to ascertain the level of *Microdochium* spp. (*M. nivale* and *M. majus*) on the seed lots were conducted in accordance with the International Seed Testing Association Seed Health Method 7-022 (2016).

Germination tests were conducted in accordance with International Rules for Seed Testing 2016 (ISTA, 2016), using the rolled-paper towel or organic growing medium methods.

Seedling emergence experiment

Two x 50 seeds from each seed lot were surface sterilised, rinsed in sterile H_2O and planted on wet paper towelling. They were placed in fridge at 7ºC for 48 hours then into an incubator at 0°C. After 24 hours the seeds were planted in soil trays with Levingtons F2, marked out with a grid and labelled. The soil trays were placed in a fridge at 3°C for 7 days, then into an incubator at 15°C for a further 11 days. A further 2 x 50 seeds from each seed lot were used as controls. Control seeds were treated as experimental seeds but were not held at 0°C and were planted in soil trays after 48 hours at 7°C and placed directly in the fridge at 3°C.

Soil trays were checked daily for seedling emergence and emerging or non-emerged seedlings recorded until maximum emergence was achieved. Seedling growth was assessed by trained seed analysts as either; normal, abnormal or dead (not emerged) according to the International rules for seed testing 2016 and descriptions of the abnormal seedlings recorded.

All abnormal and dead seeds from each seed lot were surface sterilised, rinsed in sterile H_2O, plated onto PDA plates and incubated at 20°C for up to 7 days. Twenty normal seedlings from each seed lot were also plated up using the same method.

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

Plates were assessed for colonies of *Microdochium* species. Any *Microdochium* isolates observed were sampled and grown into pure cultures. Pure cultures were sampled and DNA extracted from the mycelium in 1x TE buffer. The samples were frozen at -60°C for 20 minutes, heated at 90°C for 20 minutes then centrifuged before PCR testing. PCR analysis was carried out using the primers designed by Glynn *et al*., 2005. The *Microdochium* species were assigned as either *M. nivale* or *M. majus*.

RESULTS

Spring Oats Emergence

The emergence of spring oat seedlings with and without incubation at 0°C is shown in Figure 1.

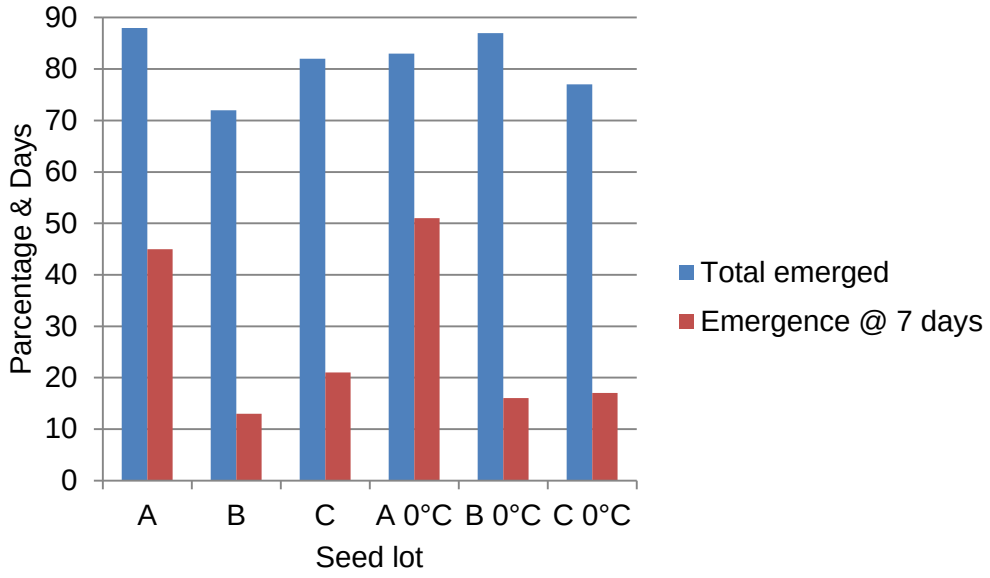

Figure 1. Percentage total emergence and emergence @ 7 days of *Microdochium* infected oats with and without 24 hours incubation at 0°C

Adding a 24 hour period at 0°C has not led to a significant change in total emergence in spring oats (p-value 0.63). There was a 5% greater loss in total emergence for seed lot A (control 0% infection) and C, but a 15% increase in emergence for seed lot B when compared to control seed lots without a 24 hour cold period. Differences in emergence of nil infected and infected seed lots were more obvious after 7 days (Figure1).

Spring Oats *Microdochium* species infection

The percentage of normal and abnormal oat seedlings, and dead seeds infected with *M. nivale* is shown in Figure 2. Only one normal seedling was found infected with *M. majus*, all others were infected with *M. nivale*.

Seedling loss in the oat seed lots is caused in part by an increase in the number of dead and abnormal seedlings infected with *M. nivale* after a 24 hour incubation at 0°C. As individual seedling losses were variable, all seed lots were examined as one (Table 2).

Table 2. Percentage oat seedling loss

	Total % loss from all oat seed lots	Total % loss due to *Microdochium* species	% loss due to *M. majus*	% loss due to *M. nivale*
Control	55	29	0	29
With cold treatment	55	45	0	45

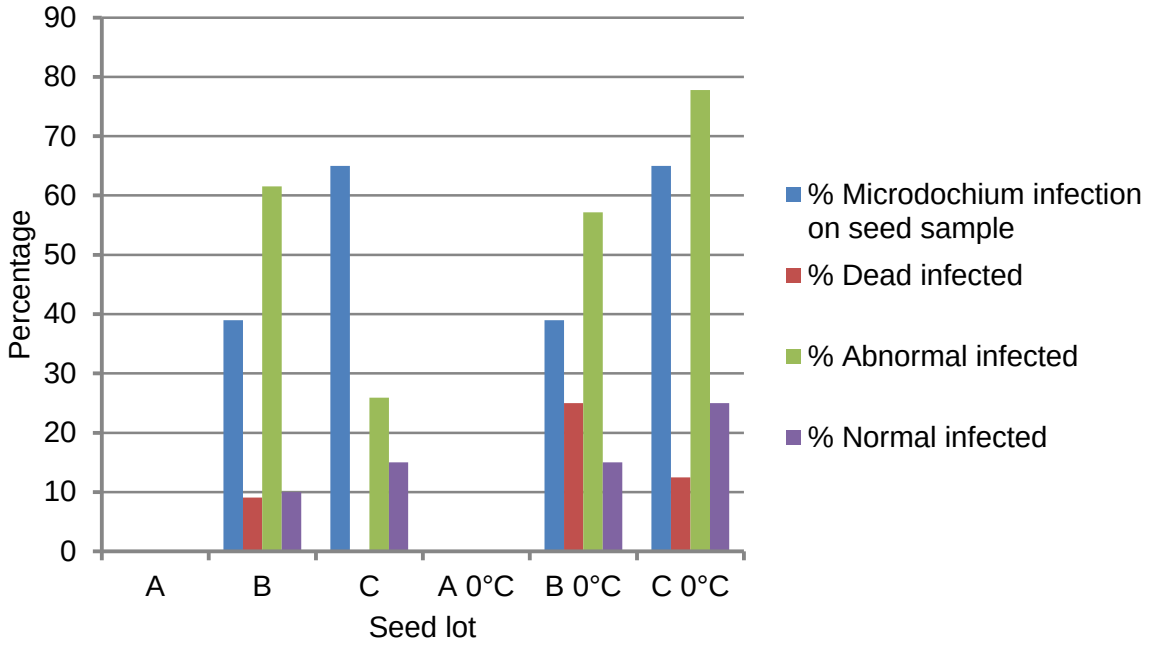

Figure 2. Percentage of oat dead, abnormal and normal infected with *Microdochium* species with and without 24 hours incubation at 0°C

Spring Barley Emergence

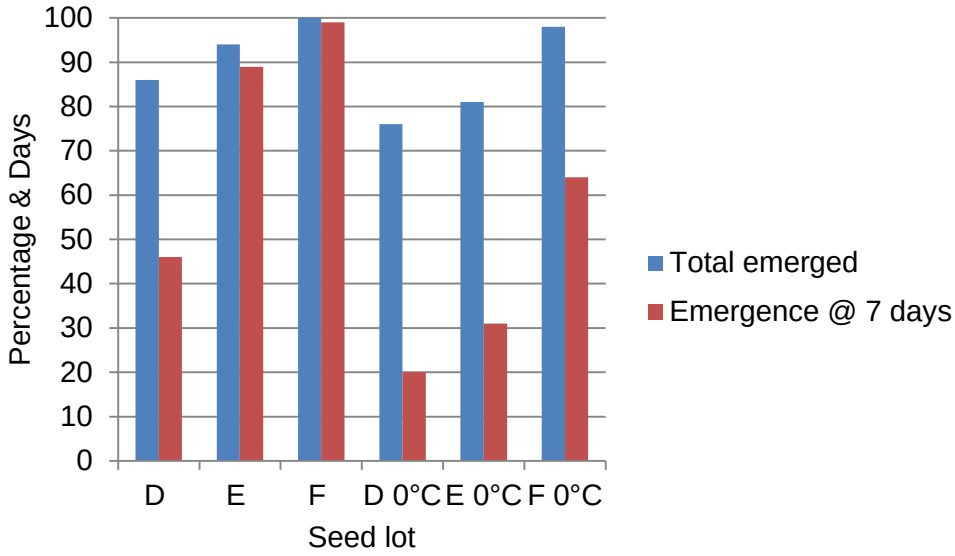

Figure 3. Percentage emergence of *Microdochium* infected barley with and without 24 hour incubation at 0°C

Adding a 24 hour period at 0°C has led to a significant reduction in the total emergence in spring barley (p-value <0.001), and seedlings emerged more slowly (emergence @ 7 days). There were 10, 13 and 2% greater losses in total emergence respectively in samples F, E and D when compared to control seed lots without a 24 hour incubation at 0°C (Figure 3).

Spring Barley *Microdochium* species infection

The percentage of normal and abnormal barley seedlings, and dead seeds infected with *M. majus* is shown in Figure 4. 15% of normal barley seedlings in seed lot F were found to be infected with *M. majus* and this increased to 25% after incubation at 0ºC. No infected normals were found in seed lot E.

The 55% abnormals confirmed with *Microdochium* species infection for seed lot F, increased to 100% after incubation at 0°C. The converse was found for seed lot E, where 100% of abnormals were found to be infected with *Microdochium* species, but after incubation at 0°C no *Microdochium* species was detected.

This seedling loss is caused in part by an increase in the number of dead seeds and abnormal seedlings infected with *Microdochium* species. Seed lot losses were examined as one lot. The majority of the *Microdochium* species detected were *M. majus* with only 10% of the total *Microdochium* species (*majus* + *nivale*) found being *M. nivale* (Table 3).

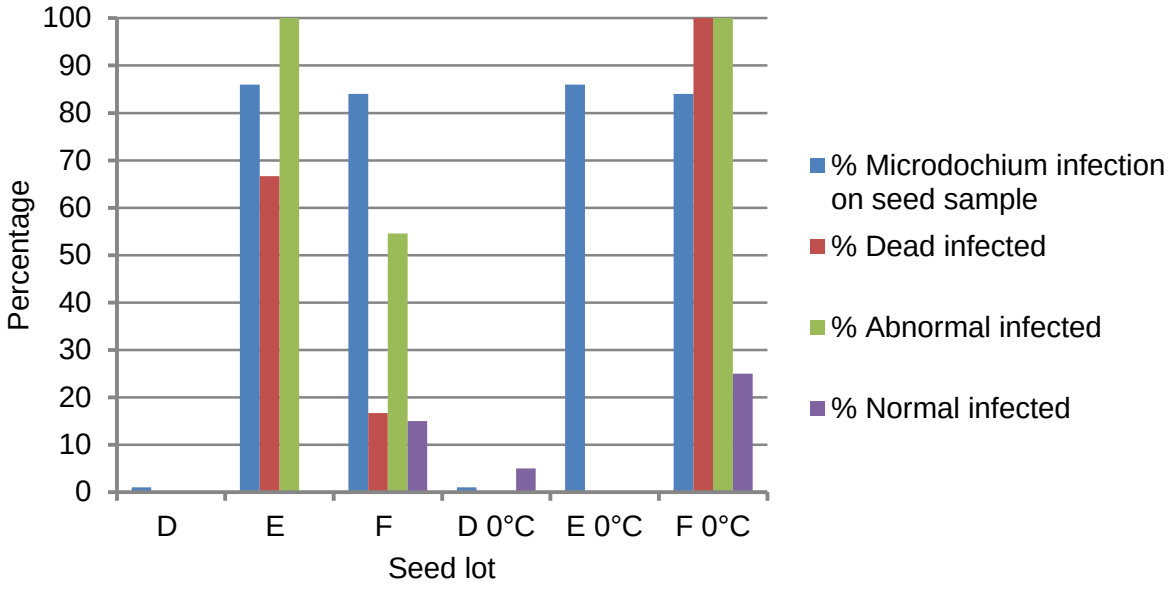

Figure 4. Percentage of barley dead, abnormal and normal infected with *Microdochium* with and without 24 hours incubation at 0°C

Table 3. Percentage barley seedling loss

	Total % loss from seed lots	Total % loss due to *Microdochium* species	% loss due to *M. majus*	% loss due to *M. nivale*
Control	30	19	17	2
With cold treatment	40	35	31	4

CONCLUSIONS

A 24 hour incubation at 0°C did not significantly reduce the emergence of spring oats compared to no cold snap. However although the same number of seedlings were lost, the percentage of losses attributable to *M. nivale* increased (Table 2).

A period of 24 hours at 0°C pre-emergence did give a significant reduction in emergence in all three spring barley seed lots, where the majority of the infections found were caused by *M. majus*. Seed lot F showed an increase in seedling losses attributable to *Microdochium* species, but seed lot E had reduced losses (Figure 4). This was despite both seed lots having a similar percentage seed infection. The species infecting the seed was not characterised before commencing the experiment. It could be that the infecting species differ between the 2 lots.

Table 3 shows that overall the incubation at 0°C did increase the total seedling losses for all barley samples from 19% to 35% causing the overall seedling losses to be 10% greater.

ACKNOWLEDGEMENTS

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THE IMPACT OF CURRENT AND PROPOSED NEONICOTINOID RESTRICTIONS ON SCOTTISH CROPS AND PESTICIDE USAGE PATTERNS

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Summary: The use of three neonicotinoid insecticides, clothianidin, thiamethoxam and imidacloprid, are currently restricted on crops attractive to pollinators. EC draft regulations propose to further restrict their use to crops grown under permanent protection. The existing restrictions have made cultivation of winter oilseed rape challenging for some Scottish growers, although the impact has been far less severe than in other regions of the UK. In the absence of neonicotinoid seed treatments to control insect pests at crop establishment, use of foliar insecticides, primarily pyrethroids, has increased. The impact of the proposed further neonicotinoid restrictions on potato, cereal and vegetable crops are discussed. It is predicted that whilst these withdrawals may not have major operational impacts in the short term, they are likely to further increase pyrethroid use and resistance pressure on the remaining insecticides.

INTRODUCTION

In 2012, the European Commission (EC) asked the European Food Safety Authority (EFSA) to peer review the risk assessments for neonicotinoid insecticides in relation to pollinators. In early 2013, EFSA published reviews for seed treatments and granules containing the active substances clothianidin, imidacloprid and thiamethoxam. In response, in December 2013, the EC implemented restrictions on the use of these three compounds as seed treatments on crops considered to be attractive to bees and on spring-grown cereals. In Scotland the main impact of this was the loss of insecticidal seed treatments for winter oilseed rape.

In 2015, EFSA published reviews of the risk assessments of all other uses of these three compounds, including foliar applications; and in March 2017 the EC issued draft regulations proposing further restrictions on their use. These drafts, which at the time of writing have not been voted on by member states, propose that the aforementioned neonicotinoids be authorised only for use in permanently protected systems, where the crop spends its lifecycle within a protected structure and is not replanted outside. In Scotland, these restrictions would mainly affect insect control options on winter cereal, potato, vegetable and fodder crops.

This paper reports on the impact of the second year of the neonicotinoid restrictions on Scottish winter oilseed rape cultivation and foliar insecticide use patterns. It also considers the potential impact that the EU draft regulations, if implemented, may have on Scottish crops.

MATERIALS AND METHODS

Survey of the impact of the second year of the neonicotinoid restrictions on Scottish winter oilseed rape cultivation

This survey is a follow-up to one conducted during the first year of the restrictions (Hughes *et al*., 2016). The survey sample consisted of 50 of the 96 growers who participated in the

previous survey, supplemented with 54 new participants. Both groups were recruited from a random sample of arable farms, stratified by farm size and geographic region, drawn from the agricultural census. These 104 growers collectively cultivated 5,553 ha of winter oilseed rape (WOSR) which represented 18% of the 2016 crop. Growers were contacted twice for information, once during the winter of 2015 and once post-harvest in 2016. At the first data point information was collected about; crop cultivation, perception of autumn insect pressure and damage, insecticide use and perception of insecticide efficacy. At the second data point, growers were asked about *Turnip yellows virus* (TuYV) incidence and 2016 yields. The reported data represent only those growers surveyed and full methodological details, including statistical methods, and results can be found in the technical report (Hughes *et al*., 2017).

Changes in foliar insecticide use on winter oilseed rape detected in the Scottish arable pesticide usage survey

Surveys of pesticide use on Scottish arable farms are conducted biennially. The two most recent surveys relate to crops harvested in 2014 (Monie *et al*., 2015) and 2016 (Monie *et al*., 2017). These surveys include data about pesticide use on WOSR crops planted immediately before the neonicotinoid approvals were amended and during the second crop season following the restrictions. These arable pesticide usage surveys are based on a random sample of farms, stratified by farm size and geographic region, drawn from the agricultural census. Data were collected from 114 farms growing 4,596 ha WOSR in 2014 (13% of census area) and 79 farms growing 2,416 ha in 2016 (8% of census area). Scottish pesticide use estimates were produced from the sample data by ratio raising, a standard statistical technique for producing estimates from a sample.

Potential impact of further EU restrictions on neonicotinoid pesticide use

The potential impact of the draft EC regulation to restrict neonicotinoid use to fully protected environments was investigated by comparing current use patterns reported in the SASA pesticide usage dataset with the availability of alternative plant protection products.

RESULTS

Survey of the impact of the second year of the neonicotinoid restrictions on Scottish winter oilseed rape cultivation

Growers were asked to rate their perception of aphid and flea beetle populations during emergence and establishment of the 2016 crop. The majority reported that aphid populations were either low or not seen (86%) with 6 and 1% ranking them as moderate and high respectively (remainder unknown). In relation to cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*) presence, the majority of growers (82%) also ranked populations as low or not seen, 13% ranked levels as moderate and 2% as high. The proportion of growers reporting both aphid and flea beetle numbers as moderate or high in this survey was significantly lower than in the 2015 crop ($p<0.001$).

In our 2015 survey we encountered an increase in autumn foliar insecticide applications of almost 50% from the previous year (Hughes *et al.*, 2016). However, in line with the reduced pest pressure encountered in this survey, significantly fewer autumn insecticide applications were applied to the 2016 crop than had been in 2015 ($P<0.01$). Forty seven, 61 and 44% of growers sampled applied an autumn insecticide in 2014, 2015 and 2016 crop seasons respectively (Figure 1). The average number of autumn insecticide sprays per grower was 0.48, 0.71 and 0.54 in 2014, 2015 and 2016 respectively. Therefore the average number of

spray applications per grower in 2016 was 13% greater than in 2014, the last crop season before the restrictions.

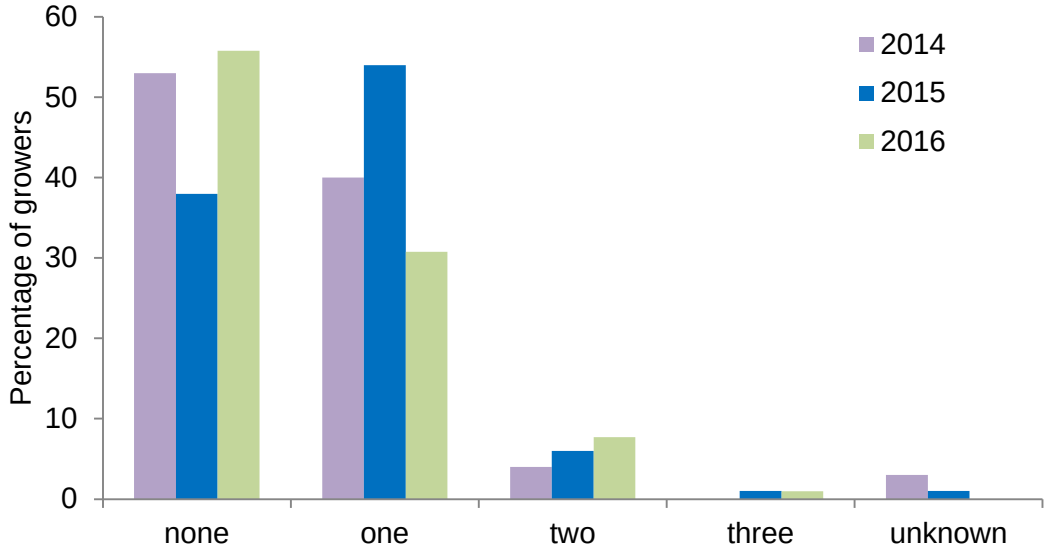

Figure 1. Number of autumn insecticide applications made by surveyed WOSR growers in 2014, 2015 and 2016 seasons

The main targets of autumn insecticides on the 2016 crop were flea beetles (65% of sprays, mainly CSFB but also *Phyllotreta spp*.). Aphids were the target of 9% of sprays and rape winter stem weevil (***Ceutorhynchus*** *picitarsis*), which is not an approved target of neonicotinoid seed treatments, was the focus of 25%. As in the previous survey, the insecticides encountered were almost exclusively pyrethroid compounds. The only exception was a single spray of the pyridine insecticide pymetrozine, which has approval for autumn use against *Myzus persicae*, the aphid vector of TuYV. Despite concern about the pyrethroid resistance status of both *M. persicae* and CSFB, the majority of growers who sprayed (87%) did not report problems with pesticide efficacy. Those that did encounter issues mostly cited operational problems with foliar control (e.g. weather conditions, lack of time to spray, lag between pest detection and spraying). One grower stated that they could not achieve control with alpha-cypermethrin against CSFB due to poor spray efficacy.

Autumn insect damage to the 2016 crop was rated as low by the majority of respondents; 82% of growers, collectively growing 83% of the sampled crop, reported no or low insect damage. Fifteen percent of growers reported moderate damage and 1% reported high damage levels. CSFB was the most common species cited to be responsible for crop damage (61% of growers). In the previous survey, the proportion of growers reporting damage as moderate (28%) or high (6%) was significantly greater than in this survey ($p=0.001$). No crop failure was attributed to insect damage in 2016, in contrast to the 2015 survey where 1.1% of the crop was redrilled due to CSFB grazing. The majority of growers (85%) visually checked for TuYV during the growing season, 5% reported viral symptoms but only one crop was tested and the results were negative. However, as TuYV may be asymptomatic and can only be definitively diagnosed by serological testing these data may underestimate viral presence.

The average 2016 WOSR yield reported in this survey was 3.46 t/ha. This is similar to the 2016 Scottish average yield of 3.31 t/ha (Anonymous, 2016). The 2016 survey yield is 19% lower than 2015 and this decrease is statistically significant ($p<0.001$). A range of adverse weather conditions were reported by growers to have been the main factors influencing yield decline. Two growers, both based in the south of Scotland, representing 5% of those

experiencing yield losses, reported that the CSFB damage had contributed to yield decline in 2016.

Changes in foliar insecticide use on winter oilseed rape detected in the arable pesticide usage survey

The biennial arable pesticide usage surveys for 2014 and 2016 covered WOSR crops sown just before (autumn 2013) and two years after (autumn 2015) the neonicotinoid restrictions came into effect. Unlike the results described in the previous section, these are not presented as sample data but have been used to estimate total Scottish insecticide use and are presented as totals with associated Relative Standard Errors (RSE).

An estimated 47,987 ha (±9% RSE) of insecticides, with a weight of 886 kg (±14% RSE) were applied to WOSR in 2014. In contrast, 43,782 ha (±12% RSE) of insecticides, with a weight of 805 kg (±14% RSE) were applied in 2016, an overall decrease in insecticide use on WOSR of 9% for both area treated and weight applied. However, there was a 17 per cent decrease in the area of WOSR grown between the two surveys (36,419 and 30,142 ha in 2014 and 2016 respectively). When crop area is taken into account, there was a 10 per cent increase in foliar insecticide applications to WOSR crops in 2016, in relation to both spray area and weight, in comparison with 2014. There was no difference in the insecticide spray area applied during the summer months of the 2014 and 2016 crops; therefore this increase in use relates only to autumn insecticide applications. In the 2014 crop, all autumn insecticide use encountered consisted of pyrethroid compounds. In 2016, 92% of autumn spray area was of pyrethroid insecticides and 8% was thiacloprid, a neonicotinoid compound unaffected by the current restrictions. Thiacloprid has approval for a single autumn application for control of *M. persicae*.

Potential impact of further restrictions on neonicotinoid pesticide use

Restriction of outdoor use of thiamethoxam would result in its loss as a foliar aphicide for potatoes, particularly seed crops, where it is an integral part of aphid control regimes to combat virus transmission. Thiamethoxam was applied to 16 and 7% of the 2014 and 2016 Scottish seed potato crops respectively. Other aphicides are available, including pyrethroids, pyridine and non-restricted neonicotinoid compounds; these are used in combination with thiamethoxam in current control and resistance management strategies. In 2016, 79% of the Scottish seed potato crop was treated with a foliar insecticide, with crops receiving an average of 5.6 insecticide sprays. Following the loss of pirimicarb in 2017, restriction of thiamethoxam would further decrease the range of insecticides available. Removing this control option may increase use of pyrethroids which have known aphid resistance issues, particularly in relation to *M. persicae*, an important vector of potato viruses. Currently pyrethroids account for almost three quarters of the total insecticide spray area applied to seed potato crops (73% in 2016).

Another impact of the proposed thiamethoxam restrictions would be loss as a seed treatment on vegetable and fodder crops. In Scottish vegetable production, thiamethoxam seed treatments are mainly used on turnip/swede and, as an extension of use, on carrot crops (27 and 11% treated respectively in 2015). They are also used on fodder crops such as kale, fodder rape and forage turnip/swede. These seed treatments are aphicidal and also provide control of selected soil pests. Whilst there is an alternative pyrethroid seed treatment (tefluthrin) which provides control for carrot fly and soil pests in carrot, there are no alternative seed treatments for turnips or for the fodder crops. As with potatoes, alternative foliar insecticides are available for these crops for control of aphids and other foliar pests at crop establishment. These include pyrethroids, non-restricted neonicotinoids and spirotetremat. Currently the majority of foliar pesticides used on these vegetable crops are pyrethroids (representing 80 and 66% of the carrot and turnip/swede crop insecticide spray area in 2015).

As there is no commercial sugar beet production in Scotland, the main impact of the loss of outdoor use of clothianidin would be in relation to seed treatments for winter cereals. In Scotland, clothianidin is used on winter barley, winter wheat and winter oats. It was applied to 4, 7 and 18% of the 2016 crop respectively, for control of aphid virus vectors and soil pests such as wireworms and slugs. There are alternative pyrethroid seed treatments for control of wireworms for winter wheat and winter barley (tefluthrin and cypermethrin) and winter oats (telfluthin only) which are also active against wheat bulb fly. In relation to control of aphid vectors of viruses such as *Barley Yellow Dwarf Virus* (*BYDV*), the loss of clothianidin would leave only foliar control options. Following the withdrawal of the organophosphate chlorpyrifos in 2016 and the carbamate pirimicarb in 2017, both of which were used for summer aphid control, the remaining approved insecticides for winter barley and winter oats are exclusively pyrethroids, with no alternative mode of action available. In relation to winter wheat, whilst the pyridine flonicamid, the organophosphate dimethoate and the neonicotinoid thiacloprid have approval for aphid control later in the season, none can be used in autumn, therefore; again control at crop establishment is limited to pyrethroid compounds. There are acknowledged pyrethroid resistance issues for some aphid species including for the grain aphid (*Sitobion avenae), a vector of BYDV*. In 2016, 27, 28 and 55% of the winter wheat, barley and oats crops received a foliar insecticide respectively, with an average of 1.2, 1 and 1 spray per crop. In the same year, pyrethroids accounted for 100% of insecticide sprays encountered on winter barley and oats, and 97% of the spray area on winter wheat (the remainder being chlorpyrifos). The loss of clothianidin is likely to increase this foliar insecticide use in winter cereals with additional sprays, primarily of pyrethroids, during crop emergence.

In relation to imidacloprid, the approval of this compound, and subsequent usage pattern, has become increasingly restricted in recent years. Current UK approvals are only for protected crops and therefore the draft restrictions will have little impact on Scottish crop production.

DISCUSSION

It is clear, from our two seasons of Scottish winter oilseed rape cultivation surveys, that the current neonicotinoid restrictions have introduced additional challenges for some growers. This has had economic and operational impacts for those affected and may discourage some growers from cultivating the crop in future. However, the overall impact of the restrictions has, so far, been far less severe in Scotland than in other regions of the UK. Our surveys suggest that most Scottish growers have continued to successfully cultivate WOSR during the neonicotinoid restrictions, influenced by Scotland's lower pest pressure and continued pyrethroid efficacy against CSFB.

However, the loss of any active substance has an impact on alternative pesticide usage patterns. In the first year of the neonicotinoid restrictions our survey detected a 50% increase in WOSR sprays per grower (Hughes *et al.*, 2016). Pest pressure was significantly lower in 2016 and was associated with a 13% increase in sprays compared to pre-restriction levels. Similarly, the arable pesticide usage survey, which estimates total Scottish pesticide use based on sample data, detected a 10% increase in foliar insecticide use on WOSR between 2014 and 2016. In all three of these surveys the majority of autumn sprays, applied to control insect pests in the absence of an insecticidal seed treatment, were pyrethroids.

The EU draft proposals to further restrict the approvals of clothianidin, imidacloprid and thiamethoxam will have an impact on control options for Scottish potato, vegetable and winter cereal crops. Initial analysis of current usage patterns and alternative controls suggest that these withdrawals are unlikely to have major operational impacts in these sectors in the short term. Alternative pesticides are available for most of the restricted uses, and there are no major control gaps as there have been with other recent pesticide withdrawals such as

chlorpyrifos. It should be noted however, that the majority of the alternative chemical controls are pyrethroid compounds. Scottish agriculture and horticulture is very reliant on pyrethroids for insect control in nearly all sectors. The withdrawal of outdoor use of clothianidin and thiamethoxam will further increase reliance on this mode of action for insect control. These restrictions, coupled with other recent insecticide losses and predicted future losses due to active substance review under Regulation (EC) No 1107/2009, are likely to exacerbate the existing pyrethroid resistance issues. Therefore, whilst the impact of these draft restrictions themselves will not prevent the successful commercial production of these crops in the short term, it adds to the direction of travel of having fewer tools in the crop protection tool box and more pressure on those remaining.

It is also important to consider the environmental profile of the alternative pesticides used when others are withdrawn or restricted. Generally pyrethroids have high aquatic toxicity and require buffer zones around water courses to mitigate this risk. This toxicity varies with compound, but is exemplified by cypermethrin which has recently been reclassified from a specific pollutant to a priority hazardous substance under the Water Framework Directive (2000/60/EC). Currently, cypermethrin is approved for use in all of the scenarios for which neonicotinoids are proposed to be restricted and was applied to 1, 2, 3 and 3% of Scottish winter wheat, seed potato, winter barley and winter oilseed rape crops respectively in 2016 (Monie *et al.*, 2017). Almost all pyrethroid compounds also have high toxicity to bees, which is mitigated by label restrictions to avoid sprays to flowering crops, where bees are actively foraging or where flowering weeds are present.

Only chemical control methods have been discussed in this paper. With the current pressure on pesticide approvals and availability it will be increasingly important to target use of pesticides within an Integrated Pest Management (IPM) framework and develop and adopt non-chemical control methods in all crop sectors, particularly in arable systems where the majority of pesticides are used. In 2016, 24% of arable farmers had an IPM plan for their crops (Monie *et al*, 2017) and uptake of a range of IPM activities was reported.

ACKNOWLEDGEMENTS

We acknowledge the support of the growers who provided data for the surveys reported in this paper.

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MONITORING SCOTLAND'S PESTICIDE USAGE: THE WORK OF SASA'S PESTICIDE SURVEY UNIT

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Summary: There is a statutory requirement to monitor agricultural pesticide usage as part of regulatory post-approval surveillance. This paper describes SASA's pesticide monitoring programme and presents summary data describing Scottish usage patterns and integrated pest management uptake. It also discusses how the dataset is used to inform a range of Scottish stakeholders.

INTRODUCTION

The pesticide authorisation process requires post-approval monitoring to be conducted. This surveillance is designed to collect information about how pesticides are used, the effects that this use may have on wildlife, humans and the environment and to check that pesticides are behaving as predicted by the risk assessment process. Collectively, these data provide feedback to the regulation system to ensure that the authorisations granted are appropriate. A range of monitoring measures are conducted, these include surveillance of pesticide residues in water bodies and food and investigation of the effects of pesticide exposure on wildlife and humans. In addition, data about pesticide sales and usage patterns are collected. This paper describes the Scottish usage monitoring conducted by the Pesticide Survey Unit (PSU) at Science and Advice for Scottish Agriculture (SASA), a division of the Scottish Government's Agriculture and Rural Economy Directorate.

STATUTORY DATA COLLECTION

The collection of data about plant protection product (PPP) usage patterns is a statutory requirement of both domestic (Food and Environment Protection Act, 1985) and, more recently, European (Regulation EC No 1185/2009) legislation. The UK programme is overseen by the Working Party for Pesticide Usage (WPPUS). This is a sub-group of the UK Expert Committee on Pesticides (ECP), a Defra committee providing independent scientific advice to Government on authorisation of pesticides in the UK.

The UK pesticide usage surveys are conducted by three organisations; data from Scotland, Northern Ireland and England & Wales is collected by SASA, the Agri-Food and Biosciences Institute (AFBI) and Fera Science respectively. The UK data are collated and published by Fera Science, whilst SASA and AFBI produce reports for Scotland and Northern Ireland respectively. The range of crops surveyed has varied over time depending on the specifications of the WPPUS. Since 2010 the data collection series has consisted of a rolling cycle of surveys of pesticide use on arable, vegetable, soft fruit, orchard, grass and fodder crops and edible crops grown under permanent protection. Currently, all crop sectors are surveyed biennially, except grass and fodder crops which are surveyed every four years. It should be noted that although SASA contribute data to the UK dataset for edible protected and orchard crops, we do not currently publish Scottish reports as crop areas are very low.

Methodology

Each survey records all pesticide use from a random sample of farms in the targeted crop sector. This sample is drawn from the agricultural census and is stratified by farm size and geographic region to ensure that it is representative of how pesticides are used. National estimates of pesticide use are produced by ratio raising, a standard statistical technique for producing estimates from a sample. These estimates are presented alongside relative standard errors to indicate statistical uncertainty. The same sampling and estimation methods are used by all of the UK survey teams. It should be noted that, whilst it is a legal requirement to keep records of pesticide use, data provision to SASA is not mandatory. There has been an increasing trend towards non-participation in recent years (12, 23 and 36% of those contacted in the 2012, 2014 and 2016 arable surveys respectively). If this continues it could influence the quality of the estimates produced by reducing sample size and accuracy of the estimates and by increasing non-response bias.

Scottish pesticide use

SASA has been collecting information about how pesticides are used in Scottish agricultural and horticultural systems since the 1970s and an electronic database of Scottish pesticide use has been maintained since 1992. This dataset allows SASA to produce estimates of pesticide use over time (Figure 1), in relation to different crop sectors and pesticide types (Figure 2) and to compare Scottish use patterns with other regions of the UK (Figure 3). The SASA dataset allows analysis of how pesticides are used on different crop groups and on individual crops. It also allows collation of usage data in relation to specific chemical groups and modes of action as well as for individual active substances.

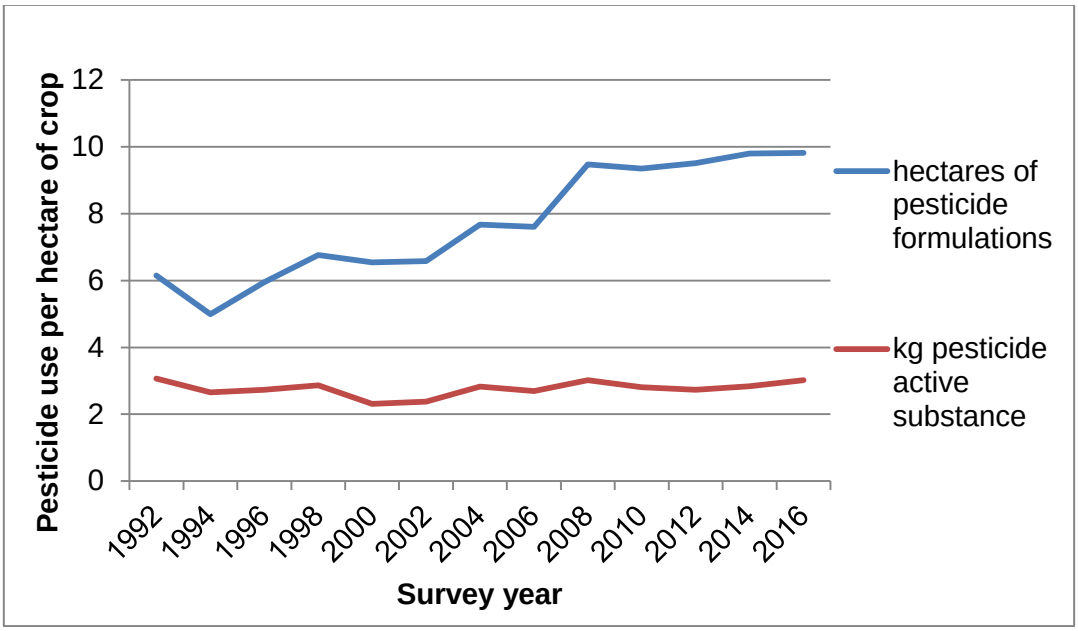

Figure 1. Total estimated pesticide use (excluding sulphuric acid) on Scottish arable crops over time. The area of pesticide formulations applied has increased by 60% over this timescale. Weight is almost unchanged; this reflects increased use of modes of action which are active at low dose rates

Approximately 1,600 to 1,700 tonnes of PPP active substances are applied per annum to the crops surveyed (Figure 2). Just under 500,000 ha of arable crops (cereals, potatoes, oilseeds and combinable legumes) are grown in Scotland each year and they account for almost 90% of pesticide use, with an average pesticide input of 3.0 kg/ha active substance. Vegetable and soft fruit crops, which account for approximately 17,000 and 1,900 ha respectively, have higher pesticide input rates per hectare (4.0 and 7.9 kg/ha respectively). In contrast, grass and fodder crops, the dominant crop system in Scotland with around 4.4 million hectares, receive very little pesticide input (average of 0.02 kg/ha). Despite Scotland having 30% of the UK's agricultural area (including grassland) and 13% of the cropped area, Scotland uses ca. 10% of total UK pesticides (Figure 3). These differences in pesticide input are influenced by differences in the types of crop cultivated, climate and pest pressure.

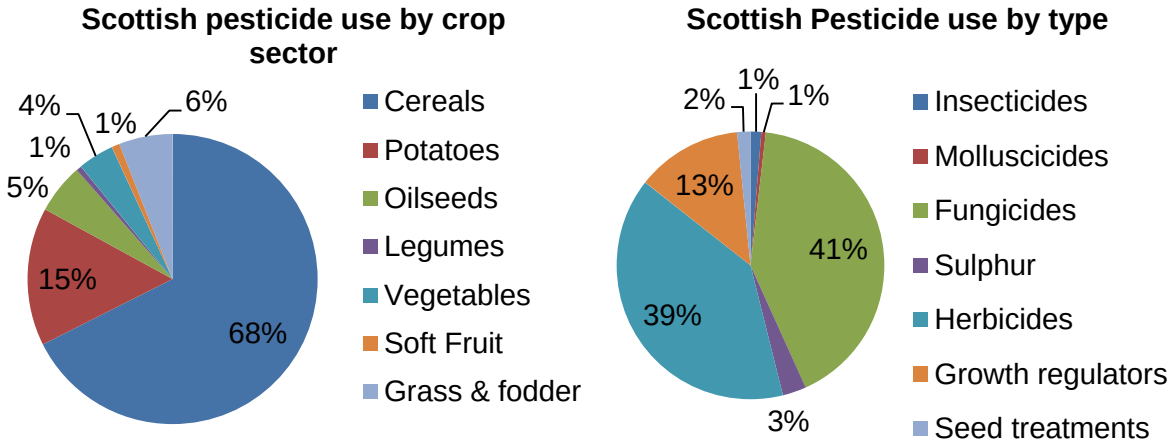

Figure 2. Current Scottish pesticide use, by weight, in relation to the crop sectors surveyed and pesticide type

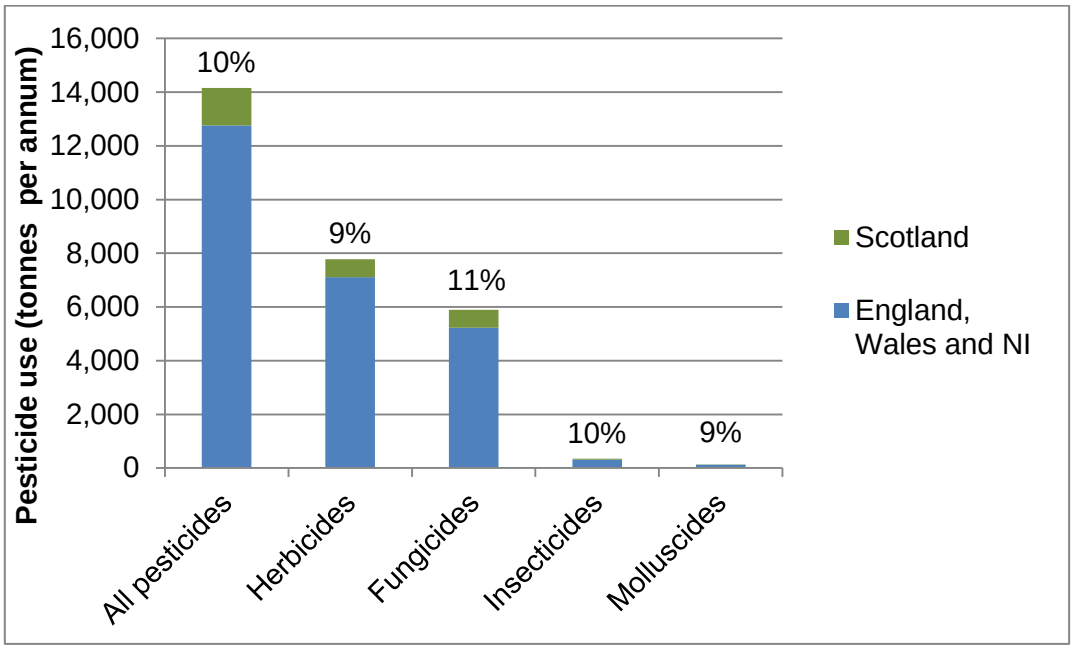

Figure 3. Comparison of estimated pesticide use on all crop sectors surveyed (2013-2015)

NON-STATUTORY DATA COLLECTION

In addition to the statutory surveys, SASA also collects data about other aspects of pest control and crop protection. For example, we conduct a programme of surveys of Scottish rodenticide use (Figure 4). This data collection series was historically part of the UK programme but was discontinued by the rest of the UK in the early 2000s. The Scottish rodenticide dataset has been used to investigate the relationship between rodenticide usage and occurrence of residues in non-target species (Hughes *et al.*, 2013) and, more recently, to investigate the impact of the 2016 rodenticide stewardship scheme on usage patterns and best practice (Wardlaw *et al.*, 2017).

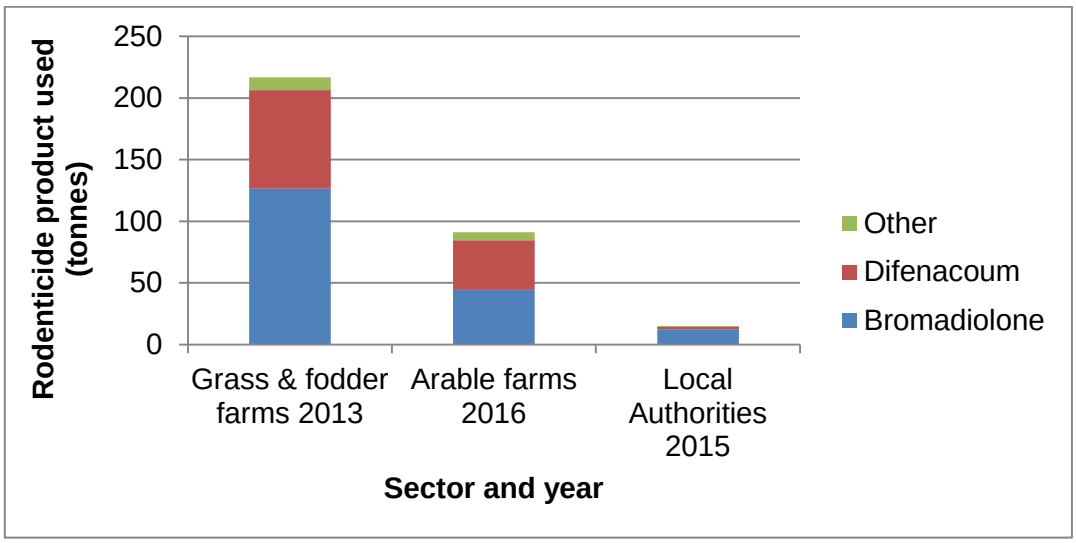

Figure 4. Estimated rodenticide use in most recent surveys of arable farms (surveyed biennially since 1992), grass and fodder farms (surveyed quadrennially since 1993) and local authorities (surveyed for the first time in 2015)

SASA also conducts *ad hoc* surveys to fill data gaps related to Scottish pesticide use. For example, two recent surveys were conducted to investigate the impact of the current restrictions of neonicotinoid insecticides on Scottish winter oilseed rape cultivation (Hughes *et al.*, 2016; Hughes *et al.*, 2017). These surveys were designed to inform the Scottish Government (SG) about the effect of the loss of these seed treatments on pest damage, alternative foliar insecticide use, crop yield and grower intentions about future crop cultivation.

Another area where SASA are currently conducting surveillance is in relation to uptake of Integrated Pest Management (IPM) by Scottish growers (Figure 5). It is a requirement of the EU Sustainable Use of Pesticides Directive (2009/128/EC) that member states should promote low pesticide input pest management, in particular IPM. The SG promotes IPM through a number of activities, including the development and hosting of a Scottish IPM plan and via funding of their strategic research programme and farm advisory service.

In order to measure current uptake of IPM, we asked farmers to respond to a supplementary IPM questionnaire alongside selected statutory surveys of pesticide use, to provide an overview of all crop protection practices that these growers implement, not just their chemical control. Information about the three general principles of IPM (risk management, pest monitoring and pest control) was collected under 16 different sub-categories. Unlike the pesticide usage surveys, these data represent responses from the sample and are not raised

to be representative of the population. Baseline data has been collected for vegetable (Monie *et al.*, 2016) and protected crops (Reay *et al.*, 2016) in 2015 and arable (Monie *et al.*, 2017) and soft fruit crops (Reay *et al.*, 2017) in 2016. IPM uptake in grass and fodder crops will be surveyed in 2017 and reported in 2018. The frequency of future data collection is yet to be decided but monitoring will continue to allow comparison over time. This surveillance is designed to help to inform the SG about the effectiveness of their IPM related activities.

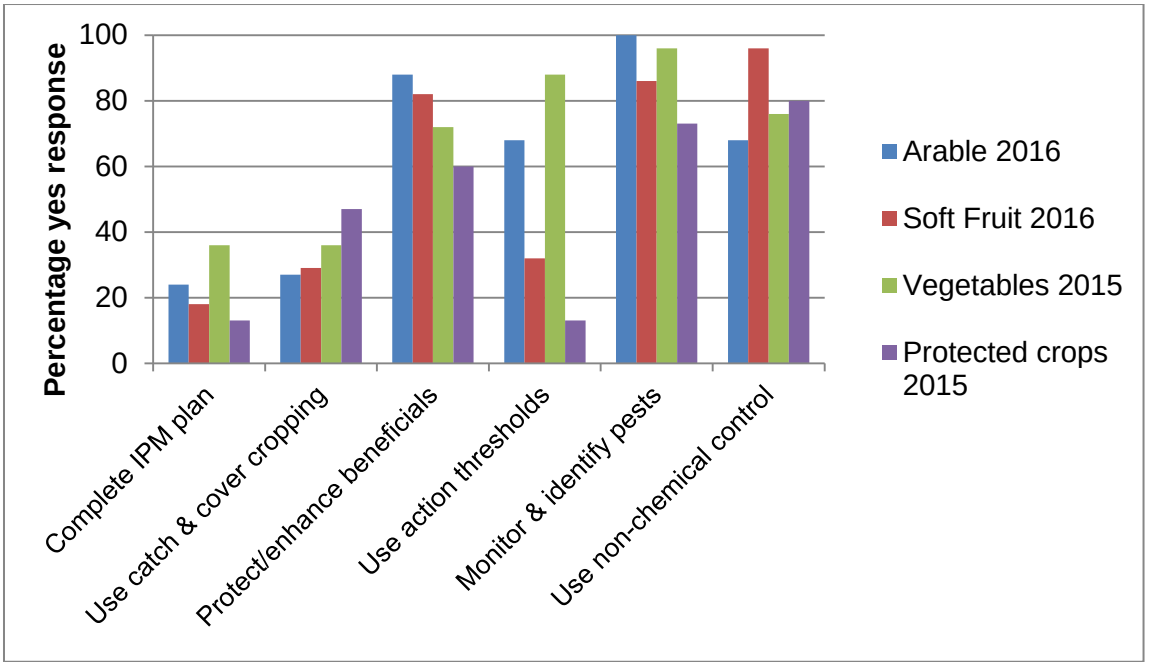

Figure 5. Responses to selected questions asked during IPM surveys conducted with growers of arable, soft fruit, vegetable and protected crops (full results available in pesticide use reports)

DATA USERS

Whilst the UK pesticide usage data are primarily produced as a feedback mechanism to the pesticide regulatory system, the Scottish dataset is used by a wide range of other data users.

The PSU data supports Scottish Government (SG) policy officials and ministers. The provision of independent data and advice is essential to allow evidence-based policy and decision making. Scottish officials can access information about how pesticides are used in Scotland and take into account specific differences in use related to Scottish crop patterns, climate and pest pressure; thus assessing the direct impact of regulatory decision making on Scottish agriculture. The dataset helps to inform the SG about the impact of pesticide losses as they occur, *e.g.* the 2016 withdrawal of chlorpyrifos which leaves no chemical control options for leatherjackets. It also allows the SG to pre-empt the effect of potential changes and consider where support and research may be needed in future to mitigate for gaps in crop protection solutions. A recent example where PSU data have supported SG policy is analysis of the potential impact if glyphosate was not to gain reauthorisation, or if it was to have its approved uses amended to exclude pre-harvest applications. Glyphosate is used for crop desiccation to a greater extent in Scotland than in other regions of the UK. Similar analyses have been conducted in relation to the proposed draft regulations to restrict the use of neonicotinoid insecticides to crops grown under permanent protection. As well as looking at

individual active substances, the PSU dataset has also been used to conduct a series of impact analyses focussing on the range of potential pesticides losses which may result from pesticide reauthorisation under the Approvals Regulation (EC 1107/2009). These analyses help to outline the predicted impact that this may have on Scottish pest control options and crop production.

In addition to supporting Scottish Government, the Scottish pesticide usage dataset is used by a variety of other stakeholders. The pesticide usage reports and public access database are presented in a clear and concise manner to allow accessibility to a range of data users. In addition, the PSU receives approximately 60 to 70 requests for advice and data each year and can provide custom datasets on request. Our data are used to inform and complement research conducted by Scottish agricultural research institutions and Universities and also to help shape the risk assessments and monitoring strategies of Scottish environmental regulators and water monitoring bodies. Pesticide data are also accessed by commercial agricultural organisations such as farming unions and by environmental and wildlife organisations to help inform their position on crop protection. The data are also an educational resource, used by schools and universities for teaching and student research projects. The pesticide industry also uses the information to complement their own sales data and to gain an insight into the use patterns of their own and competitor's products.

Overall, the data collected by the PSU are an important resource for pesticide regulators, the SG and a wide range of other stakeholders. They allow an understanding of how pesticides are used currently in Scotland, and also help to predict how changes to pesticide availability might affect future crop protection and production.

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We are grateful for the support of the growers who provided data for the surveys reported in this paper.

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MONITORING RHYNCHOSPORIUM COMMUNE IN SCOTLAND FOR THE APPEARANCE OF RESISTANCE TO STROBILURIN FUNGICIDES

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Summary: *Rhynchosporium commune* is the fungal pathogen that causes leaf scald in barley. The disease is controlled by a number of fungicide active groups including the strobilurins. The efficacy of this group of fungicides has been shown to be declining since their introduction at the turn of the century. A mutation on the cytochtrome b gene has been implicated in the appearance of resistance and the decline in efficacy. The mutation has been reported sporadically across Europe. In this study we have shown the efficacy is declining but the G143A mutation may not be responsible for this decline. The implications for growers is discussed

INTRODUCTION

Rhynchosporium remains the most damaging disease of barley in the north and west of the UK, especially Scotland. The disease can cause losses of up to 40% (Fitt *et al*., 2012) and is highly favoured by the cool, wet weather throughout Scotland (AHDB, 2016). This fungal causal pathogen, *Rhynchosporium commune,* is an anamorphic ascomycete and infection significantly reduces photosynthesis and results in a reduced grain quality and quantity. Symptoms are scald-like lesions on leaves, ears and leaf sheaths (see Figure 1). These lesions can merge to form large area of yellowing on the leaf which can result in chlorosis and eventual leaf death in the crop and yield losses worth £7.2 million annually despite treatment (Henly, 2015).

Figure 1. Symptoms of *Rhynchosporium commune* infecting spring barley

Rhynchosporium is currently controlled using various fungicides, such as strobilurins and triazoles (Walters *et al*., 2014). Cases of resistance have been noted throughout the world, including Ireland and France, with the most detrimental resistance observed being towards the strobilurins. Although the resistance has not yet been documented in Scotland (Fountaine, 2011), the increase of resistance in other locations has increased the potential for this to occur in Scotland. The pathogen resistance may be at level 8 (medium-high) according to AHDB. Fungal populations can change rapidly, thus defeating new barley resistance genes and fungicides after only a few seasons of commercial use. The development of sustainable management strategies relies on an enhanced understanding of biology of *R. commune*, and in particular the interactions between the barley host, the pathogen and the fungicides (Avrova & Knogge, 2012). All current findings indicate that global *R. commune* populations have significant potential to evolve rapidly in response to environmental changes, including the use of resistant cultivars, fungicide applications and climate change (McDonald, 2015). Resistance was first detected in the strobilurin fungicide group in Germany after only being used commercially for a few years. The resistance was due to a single point mutation (Fountaine, 2011) found in the mitochondrial-encoded cytochrome b gene (*cytb*) (Torriani *et al*., 2009). Further target site mutations in *cytb* gene (G143A, F129L) and additional mechanisms can also result in resistance to this group of fungicides (FRAC, 2015). Performance of the strobilurin fungicides in the UK had remained relatively good but the selection of strobilurin sprays with high levels of control is highly recommended, along with using various fungicides with varying modes of action (FRAG, 2016). The G143A substitution was confirmed at low frequencies in *R. commune* samples in 2012 (France), 2014 (UK) and 2015 (Spain). The mutation was also detected in 2014 in Ireland. Fortunately, the frequency of the substitution in these samples was low (2–18%) (Phelan *et al*., 2017).

The aim of this study was to monitor *R commune* samples from Scotland to see if the mutation had appeared since the last major study was undertaken (Fountaine, 2011).

MATERIALS AND METHODS

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Efficacy Trials

Efficacy trials were carried out at a number of sites across the UK every year to monitor the performance of fungicides against a target pathogen. A susceptible cultivar is chosen, based on resistance ratings on the AHDB recommended list. The crop is sown in 2m x 10m plots and four replicates were used per treatment. Fungicides were applied at GSZ32 and at four different rates (0.25, 05, 1.0 and 2.0 of the manufacturers recommended dose). Disease symptoms and green leaf area (GLA) were scored at the time of spraying and then 3 and 6 weeks after this on each leaf layer. Data was collated by AHDB and used to produce fungicide performance curves on an annual basis. Trials were taken to yield so that the effect of disease control on yield could be quantified.

Sensitivity assays

R. commune infected leaves were collected from a spring barley (cv. Concerto) trial carried out at SRUC trial site in Midlothian in 2016. One of the trials had a cover spray of pyraclostrobin (pyr), which gave poor control of disease symptoms. Attempts were made to produce single spore isolates using the method described in Fountaine 2011. Diseased leaf samples were also taken from the JHI long-term trial at the Centre for Sustainable Cropping (CSC) trial at Balruddery. Single-spore isolates were produced from three different winter barley cultivars Retreiver, Saffron and Cassata, managed under a conventional or sustainable system. Isolates were to be tested for sensitivity to pyraclostrobin using the multiwell plate assay described in Oxley *et al.* (2007).

Mutation testing

DNA extracted from single-spore isolates of *R. commune* were used for this simple test for the G143A mutation following the procedure described by Torriani *et al.* (2009). DNA concentration was initially measured on a Nano drop spectrophotometer (Thermo-Scientific Ltd) and diluted to 2.5 ng/µl. The PCR amplifications were carried out under the following conditions; initial denaturation at 96°C for 2 min, followed by 35 cycles of 96°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were separated on gel red stained (Biotium, CA, USA) 1.3 % (w/v) agarose gels run in Tris-borate-EDTA buffer (TBE: 89 mM Tris base, 89 mM Boric acid, 2 mM EDTA, pH 8.0) and exposed to UV light to visualise DNA fragments. A portion of each PCR product (10 µl) was digested using 1U *Fnu4*HI (New England Biolabs, England) for 4 h at 37°C. This restriction enzyme specifically cuts at the mutated sequence found at codon 143 (GCT), but will not cut in the presence of the wild-type sequence (GGT). The digests were then visualised on a 1% agarose gel with 1x TRIS-borate-EDTA. Each time this procedure were carried out for Scottish *R. commune* samples, DNA from a resistant isolate collected from Ireland were run as a control.

RESULTS

Efficacy Trials

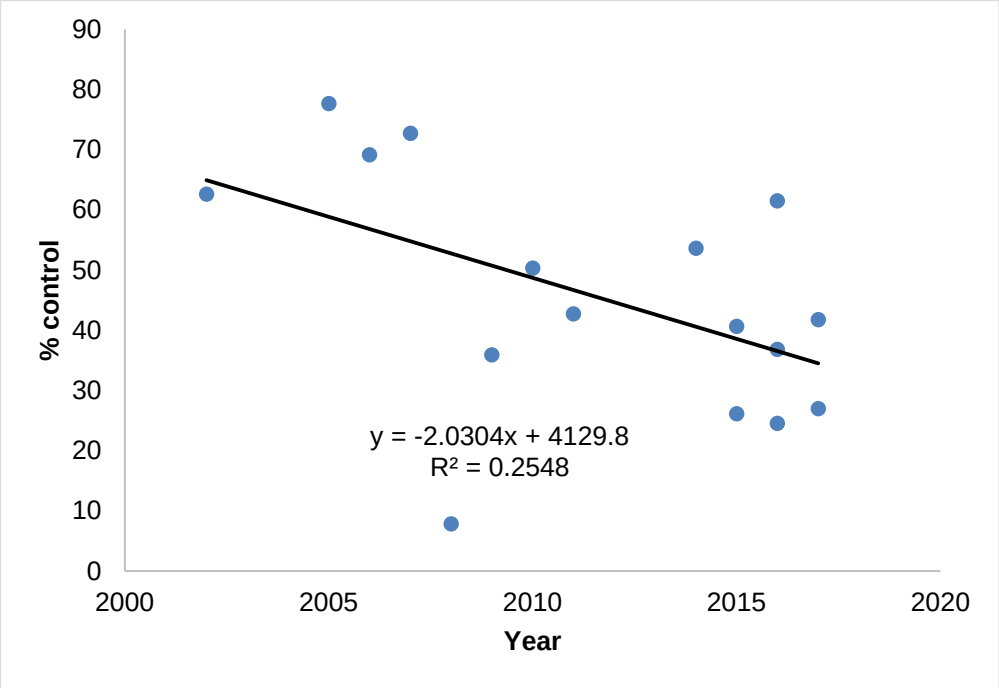

Figure 2. Protectant activity of 0.5 rate of pyraclostrobin against *R. commune* in spring barley in AHDB Fungicide Performance trials.

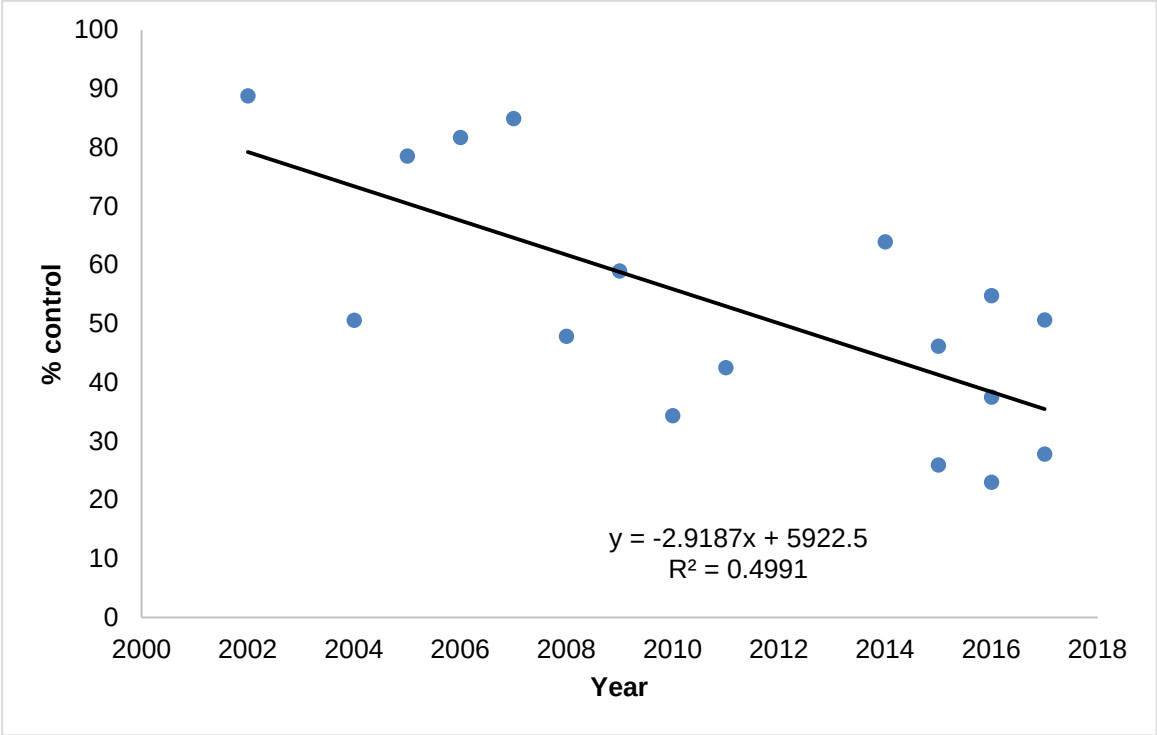

Figure 3. Protectant activity of 1.0 rate of pyraclostrobin against *R. commune* in spring barley in AHDB Fungicide Performance trials

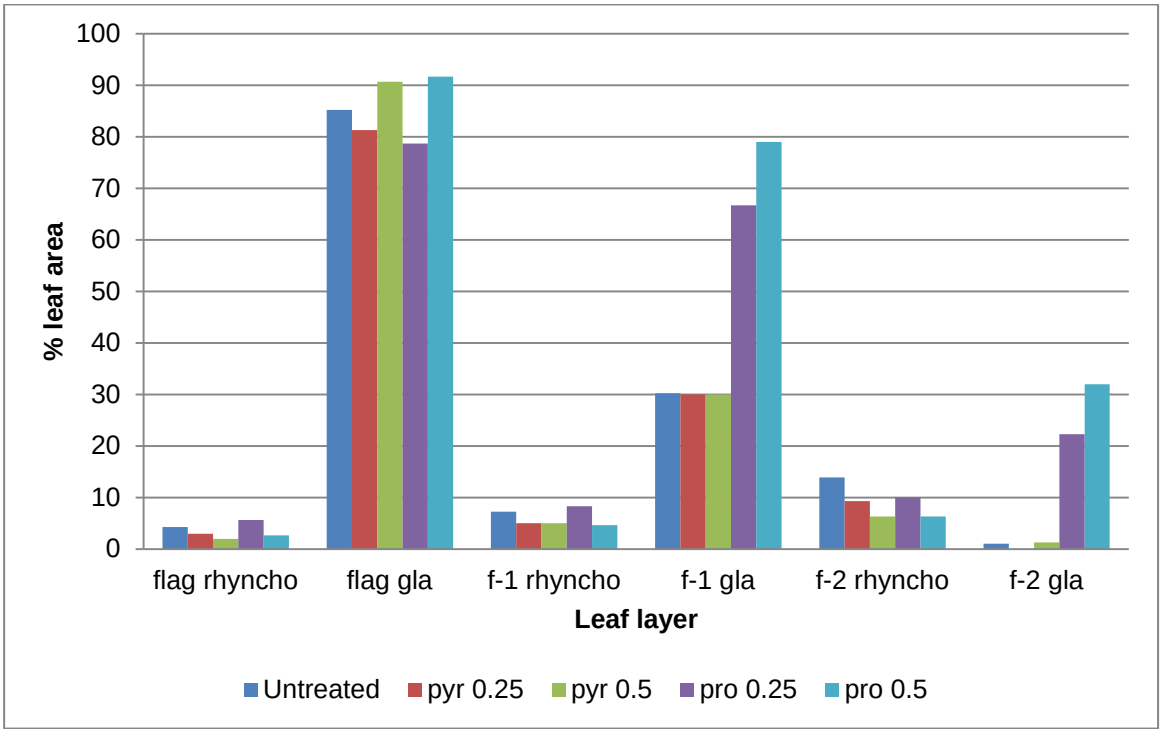

Figure 4. Levels of control from pyraclostrobin and prothioconazole (pro) in an AHDB Fungicide Performance trial in winter barley (Scotland 2017)

Sensitivity assays

Issues with bacterial contamination during the isolation procedure at SRUC meant no sensitivity assays were able to be performed in the 2017 study. Leaves were taken from the crop in the late season and the surface sterilisation methods used previously were unable to control the leaf microbes.

Mutation testing

Twenty four single-spore isolates produced from the winter barley crop at Balruddery were tested for the presence of the G143A mutation. None of the isolates produced bands similar to the positive control after the restriction digest indicating none carried the mutation.

DISCUSSION

The data presented in Figures 2 and 3 indicates that a significant decline has occurred in the activity of the strobilurin fungicide pyr against the pathogen *R. commune* in Fungicide Performance trials. In 2001 a 0.5 and 1.0 dose gave 62% and 88% control respectively. By 2017 these figures had dropped to approx. 30% and 40% respectively in the same trials. In the 2017 winter barley trial (Figure 3) the strobilurin fungicide appears to be giving similar levels of control to the azole fungicide pro at 0.25 and 0.5 dose rates. In the flag and f-1 leaf layers the strobilurin fungicide appears to be giving better protectant activity than the azole. However, in a more eradicant situation on leaf 2 there is no difference between the fungicides. Although the strobilurin fungicides have showed activity against the pathogen they have not helped maintain green leaf area as well as the azole fungicide. This may be related to the more robust general disease control shown by the azole fungicide.

This decline in activity has serious implications for growers. In order to maintain the activity of the strobilurin fungicide it is important that they are used in mixtures with another fungicide with better or at least equivalent activity against *R. commune* (FRAG, 2016).

The G143A mutation has been reported in low frequencies in Ireland in recent years (Phelan *et al.,* 2017). Although this study only tested isolates from one site it appears to be still relatively uncommon in Scotland. There are some reports that the cytochrome b gene structure in *R commune* means that the mutation incurs a severe fitness penalty and will make resistant lines non-viable (Deising *et al.,* 2008). The results presented in this paper indicate a decline in fungicide efficacy but it seems that the G143A mutation may not be responsible for this gradual decline. The appearance of the G143A mutation in other barley pathogens lead to rapid and complete resistance in the population (Fountaine & Fraaije, 2009). The drop in field efficacy may be due to other changes in the pathogen such as alterative mutations. Isolates with F129L or G137R mutations express moderate (partial) resistance. Strobilurin fungiicdes applied at manufacturers' recommended rates are shown to provide effective control of diseases with the F129L or G137R mutation. In contrast, a severe loss in disease control is always seen in populations where G143A predominates and strobilurins are used alone. (FRAC, 2017). Further work is needed to assess the distribution of mutations within the *R. commune* population in Scotland.

ACKNOWLEDGEMENTS

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RAMULARIA LEAF SPOT OF BARLEY: THE IRISH SITUATION

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Summary: *Ramularia collo-cygni*, the causal agent of Ramularia leaf spot, is a seed- and wind-borne pathogen known to be widespread throughout Europe. As disease symptoms are often only visible post-heading a reliable decision support system is required to identify whether the disease should be a specific target for a GS 39-49 fungicide application. In 2017 spring barley trials were conducted at two Irish sites differing in disease pressure to assess a decision support system developed in the UK for its relevance to Ireland.

INTRODUCTION

Ramularia collo-cygni has become a significant threat on both winter and spring barley crops throughout Europe over the last two decades as this disease reduces both quality and quantity of harvested grains. Currently, control is based upon the application of fungicides with efficacy against the pathogen at GS39-59, where fungicide applications have previously shown the most activity (Burke *et al*., 2001). However, work in Scotland by Oxley and Havis (2010) found disease development to be directly related to leaf wetness in early June (for spring barley), which can be used as a tool for disease forecasting. If the crop is deemed to be at high risk in this period, fungicide mixtures with known efficacy against the pathogen can deployed at the GS45 application. The aim of this research was to assess whether the decision support system (DSS) tool is relevant to Irish field conditions.

MATERIALS AND METHODS

Field trials were conducted at two sites in 2017; at Oak Park, Carlow (considered a medium disease pressure environment) and in Kildalton, Co. Kilkenny (considered a high disease pressure environment). A completely randomised split-plot design was implemented with four commercially relevant spring barley cultivars differing in susceptibility to Ramularia leaf spot (RLS); RGT Planet, Propino, Irina and Olympus. These cultivars received 5 different treatments at T2; 1) a 'standard' of prothioconazole (Proline) and chlorothalonil (Bravo) applied at 50% the recommended rate, 2) 'QoI' pyraclostrobin (Modem) to let RLS develop but not other major barley pathogens, 3) 'DSS product' of chlorothalonil (Bravo), bixafen and prothioconazole (Siltra Xpro) selected due to high levels of leaf wetness at the start of stem extension, 4) 'DSS rate' with increased rates (75%) of the standard treatment also due to high forecasted risk and 5) an 'untreated' control. At GS75 percentage RLS and green leaf area (GLA) were visually assessed on leaf 2 of 10 main tillers per plot. All plots were harvested and yields calculated as t/ha at 15% moisture combine. The statistical analysis consisted of a split plot ANOVA which was determined using Genstat 14th Edition software.

RESULTS

Mean disease levels of RLS on L2 and yield are presented in Table 1. Both sites were deemed as high risk, however Kildalton had a significantly higher level of disease (data not shown). The variety RGT Planet had the lowest disease level while Irina had the highest (data not shown). Whilst both DSS programmes provided the best control, neither was significantly different in either control or yield to the standard programme (Table 1). There was a significant programme x cultivar interaction (P=0.01), while the programme x site interaction was non-significant (P=0.9).

Table 1. Means of yield and % RLS in spring barley (2017)

Programme	Yield (t/ha)	% RLS (L2)
Untreated	5.48	12.08
Standard (Proline 0.4l/ha + Bravo 1l/ha)	6.84	6.1
QoI (Modem 0.625l/ha)	6.21	8.07
DSS product (Siltra Xpro 0.5l/ha + Bravo 1l/ha)	6.99	4.63
DSS rate (Proline 0.6l/ha + Bravo 1.5l/ha)	7.02	4.74
LSD (P=0.05)	0.45	1.64
s.e.	0.20	0.75

DISCUSSION

Although both sites were deemed high risk based on levels of leaf wetness recorded early in the season neither DSS programmes, that were altered to reflect this risk, provided significantly better disease control or yield compared to the standard programme. This may be due to the superior activity provided by chlorothalonil against RLS. Alternatively given concerns surrounding the sensitivity of *R. collo-cygni* populations to both the azoles and SDHIs, the lack of difference between the treatments may reflect inferior activity provided by the mix partner(s) in all three programmes.

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FACTORS INFLUENCING THE RADICLE EMERGENCE TEST FOR SWEDE RAPE

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Summary: The radicle emergence test is a relatively new test used to assess seed vigour. We show that the radicle emergence test can discriminate between samples with high germination and discuss the suitability of the RE test for assessing seed vigour. Radicle emergence is affected by seed storage moisture content, and may be genetically controlled to a certain extent.

INTRODUCTION

The climate is becoming increasingly variable and less predictable, resulting in variable and unpredictable field conditions. As a result the need for high quality, high vigour seed and demand for tests of seed vigour is increasing. Traditionally tests for seed vigour have involved assessing germination after a period of seed deterioration (e.g. Controlled Deterioration or Accelerated Ageing tests), or assessing seed performance when subject to ageing or stress. These tests tend to be fairly time consuming as germination is slowed down as a result of the treatments applied. The International Seed Testing Association has recently introduced radicle emergence (RE) as a seed vigour test. The RE test is based on the theory that as seeds age they accumulate damage. When seeds take up water they become able to respire and metabolise. One of the first things to happen in the earliest stages of germination, before radicle emergence, is the repair of any damage that has occurred while seeds have been in the dry state. The more damage accumulated, the longer the time required for repair and the longer the lag period between imbibition and radicle emergence. Hence the time between imbibition and radicle emergence gives an indication of the extent of damage in a seed lot.

The radicle emergence test has been shown to give results that are repeatable across laboratories (Powell *et al*., 2014) and to be predictive of field emergence (Matthews *et al*., 2012). The RE test is quick; a result is available after 30 hours, and is inexpensive; all that is required is the equipment for germination testing.

The aim of this work was to investigate the use of a radicle emergence test as a test that can provide greater information on the quality of a sample than the germination test alone. The majority of the testing that has been conducted to verify the performance of the RE test has been done on a single variety, therefore we also wanted to investigate whether RE was influenced by genetics.

MATERIALS AND METHODS

Thirty swede rape seed lots were tested for germination, radicle emergence, moisture content, equilibrium relative humidity (eRH) and longevity. Germination (two replicates of 100 seeds), radicle emergence (two replicates of 100 seeds) and moisture content (two replicates of 4.5 – 5 g of seed) tests were carried out according to the International Rules for Seed Testing (ISTA, 2017).

A Rotronic HygroPalm 23-AW-A water activity meter was used to measure the eRH of two independent samples of seed from each seed lot. The chamber was filled full of seeds, and an eRH reading was taken once the measurement had stabilised.

To measure seed longevity seed moisture content was adjusted by placing seeds in a sealed plastic electrical box (Ensto, Finland) at 20°C over a non-saturated solution of LiCl giving 47% RH (Hay *et al*., 2008). After 10 d equilibration, seeds were transferred to experimental storage conditions of 45°C and 60% RH (in a second sealed plastic box over a different concentration of non-saturated LiCl solution). Seed moisture content should be similar for the two environments, thus on transfer to 45°C and 60% RH the seeds only have to equilibrate to the higher temperature. Sub-samples of seeds were removed at 7 d intervals and tested for ability to germinate. Germination tests were carried out on one replicate of 100 seeds, as described above. Probit analysis was carried out to estimate p_{50} (the time taken for viability to fall to 50%).

RESULTS

Germination of seed lots was generally high, with 28 out of the 30 seed lots tested having germination of 90% or greater, and only one seed lot having a germination of below 85%, the standard for germination of certified seed in Scotland. Radicle emergence was more variable, with 29 out of the 30 seed lots tested having between 58 and 97% radicles emerged after 30 hours at 20°C, and a single seed lot having just 5% radicle emergence. Fifteen seed lots (all with germination between 95 and 100%) were tested for longevity, and the p_{50} values for these seed lots varied considerably – from 4.7 to 14.5 weeks. For seed lots with high germination, there is a range in the radicle emergence results and variation in the seed longevity, measured as p_{50} (Figure 1).

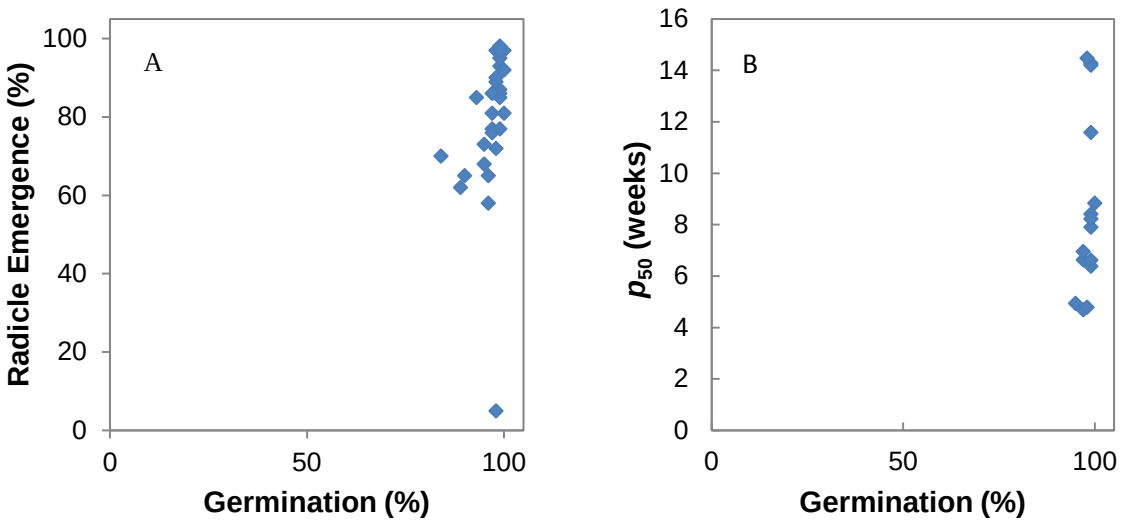

Figure 1. The relationship between germination and (A) radicle emergence, (B) seed longevity in swede rape.

There is a difference in the radicle emergence and p_{50} values between varieties. Of the varieties tested INV1030 and Extrovert had notably higher RE and p_{50} values, with Mentor and Picto having lower RE and p_{50} results (Figure 2).

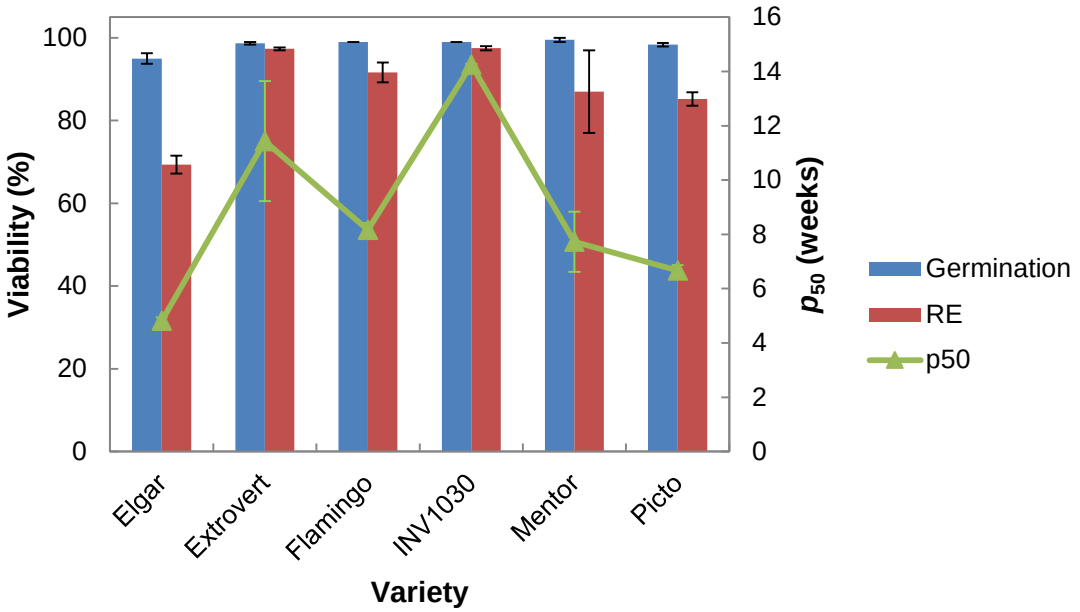

Figure 2. Germination (blue bars), radicle emergence (red bars) and p_{50} (green triangles) of six different varieties of swede rape.

A clear relationship between seed quality and seed moisture status was observed for both moisture content and equilibrium relative humidity. Seeds with a higher moisture content or eRH tended to have lower quality. There was a slight decrease in germination in seeds with an eRH of above 60%, and a more obvious decrease in radicle emergence in seeds with an eRH of above 55%. The relationship between p_{50} and eRH is linear, with p_{50} decreasing sharply with an increase in eRH (Figure 3).

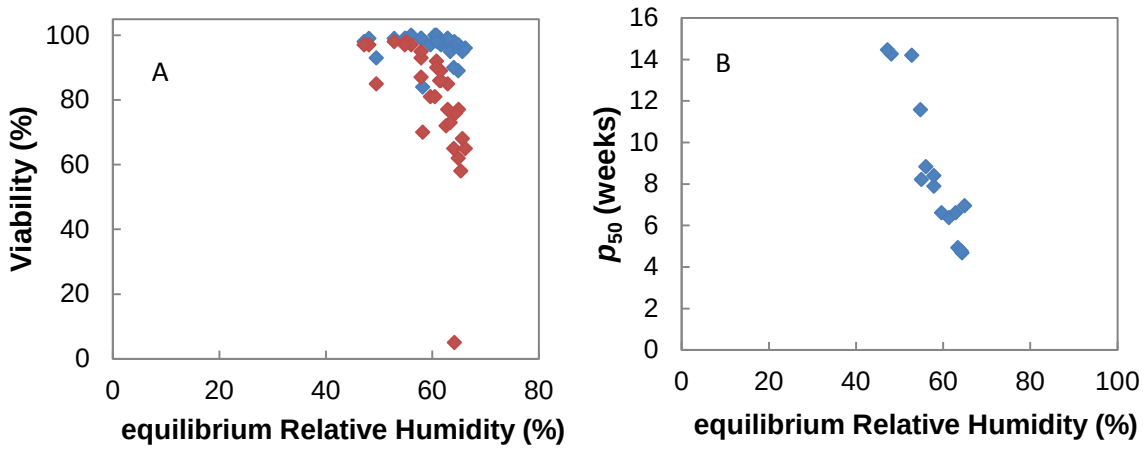

Figure 3. The relationship between equilibrium relative humidity and (A) germination (blue) and radicle emergence (red), and (B) p_{50} in swede rape seed.

DISCUSSION

It is often presumed that germination is as good an indicator as any test of the quality of a sample, but these results indicate that the radicle emergence test is able to discriminate

between seed lots with high (>90%) germination. Previous work has shown that rate of germination is correlated with field emergence (Bowden and Cockerell, 2013). The radicle emergence test gives a direct indication of the rate of germination, and therefore it is very likely that radicle emergence will give a good indication of field emergence and seed vigour. It is likely that p_{50} also gives a very good indication of seed vigour, and we have shown that even in samples with a germination of 95% or higher, there is considerable variation in p_{50}. This suggests that p_{50} could potentially be a better indicator of seed vigour than RE, however longevity testing is time consuming, and the RE test is quick, simple to carry out, and the results are easy to interpret.

The clear relationship between seed moisture status and seed quality highlights that if seed is to be stored, then seed moisture status is critical for maintaining seed quality, with a lower storage moisture content being better for seed quality. This also suggests that a major reason for lower seed quality – lower radicle emergence and lower p_{50} values is a direct result of seed moisture content. Moisture is critical to seed quality, in particular to seed storability, and it is known that a 1% reduction in seed moisture content will approximately double seed storage life. It is also possible that the reason for differences between varieties could be attributed to differences in moisture content. At any given humidity, if seeds are in equilibrium with their environment then moisture content will vary depending on seed oil content. Of the varieties tested, INV1030 had the highest RE and p_{50} values, and has a high oil content, Mentor and Picto had lower quality and lower oil contents in comparison with the other varieties.

This work has shown that the radicle emergence test is a useful test of seed quality and can provide more information on the vigour of a sample than a germination test. The work has also highlighted that if swede rape seed is to be stored for any length of time, then storage at a low moisture content is critical for maintaining seed quality. As a recommendation, storage at a moisture content of lower than approximately 6%, or lower than approximately 55% eRH, will ensure that seed quality is maintained. Control of seed moisture may be particularly important for varieties with lower oil contents which will have a higher moisture content at any given relative humidity. In conclusion the RE test is able to discriminate between samples with high germination. We recommend radicle emergence as a test for seed vigour.

ACKNOWLEDGEMENTS

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RESEARCH ON INTEGRATED PEST MANAGEMENT (IPM) FOR POTATO

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Summary: Potato crops are adversely affected by many pests and pathogens. Research at The James Hutton Institute contributes to IPM through the development of effective control methods, population monitoring, disease forecasting, genetic improvement of crops, diagnostics and improved management of inputs and resources. Control options can be integrated to achieve the most effective and sustainable control of pests and pathogens of potato. We describe some of the challenges and successes in our progress towards achieving a balance between adequate control of disease and minimising chemical inputs to prevent economic losses due to pests and pathogens. IPM for blackleg, late blight and soil-borne pests and pathogens, including virus-vector free living nematodes is discussed.

INTRODUCTION

Integrated Pest Management (IPM) is an environmentally and economically sustainable approach to managing the impact of crop pests, pathogens and weeds. IPM strategies combine available methods (IPM tools) for pathogen and disease monitoring, risk prediction and control of pest, pathogen and weed populations into programmes where the tools operate synergistically to reduce pests and disease with minimal environmental impact and economic risk.

The European Union Directive on the Sustainable Use of Pesticides (2009/128/EC) established a framework to achieve more sustainable use of pesticides by reducing the risks and impacts of pesticide use on human health and the environment and promoting the use of IPM and alternative approaches or techniques. Member states are required by law (1107/2009/EC) to implement IPM plans under their National Action Plans (NAP).

The James Hutton Institute carries out multidisciplinary research to develop, improve and demonstrate the value of specific IPM tools. Such component tools are then deployed in combination to create effective, flexible and durable solutions for sustainable IPM. We will focus on the progress made for IPM of blackleg, late blight, and soil-borne pests and pathogens. Additionally, we will describe some new early disease detection technologies being developed to aid IPM in the future. Further information on IPM research at The James Hutton Institute can be found at http://ipm.hutton.ac.uk/.

IPM FOR BLACKLEG

Blackleg (including soft rot) is caused by the bacterial pathogens *Pectobacterium* and *Dickeya*. There are no chemical treatments for its control and, pre 1960, blackleg was a major problem to the potato seed industry. Disease incidence has been much reduced due to considerable improvements in controlled ventilated storage, seed certification, the use of micro-propagation, good hygiene and more recently, managing seed imports (Safe Haven

scheme and Government legislation). However, over the last 5 years blackleg disease appears to be on the increase across the whole of Europe but the reasons for this are not clear. At the James Hutton Institute, and in association with colleagues in SRUC, SASA, Fera and SBCSR, we are focussing on six control measures to be included in the blackleg IPM strategy;

1) I**mproved diagnostics:** computer software that uses whole genome sequences to investigate the relationships between species and between strains has been developed and used to make novel diagnostics for use by researchers, industry and statutory agencies (Toth *et al.*, 2015; Pritchard *et al.*, 2016).

2) **Disease resistance:** as there are no known resistance genes (R-genes) for these bacterial pathogens, there is also no simple screen for resistance. We have identified resistant and susceptible potato accessions within the Commonwealth Potato Collection (CPC) and have produced crosses to investigate potential mechanisms and markers for this resistance.

3) **Reducing pathogen spread:** the spread of the pathogen and routes to contamination of high grade seed in both commercial and experimental environments are being investigated and this knowledge is being used to develop control strategies.

4) **Biocontrol:** we are working with industry to test the use of bacteriophages (phages - viruses that attack and kill bacteria) as potential biocontrol agents in the field. Different application methods and timings are being tested in order to determine the most effective phage control options.

5) Forecasting: the use of powerful modelling tools, together with national databases for seed certification, climate and soil structure, is allowing us to examine the spread of disease at a national scale, and to search for factors that may be associated with blackleg disease.

6) Precision agriculture: together with colleagues at SRUC, we are examining the use of multispectral imaging to identify blackleg incidence in the field to help develop non-intrusive ways to monitor and then act on disease incidence.

IPM FOR LATE BLIGHT

Potato late blight caused by the oomycete *Phytophthora infestans* is one of the most destructive and economically important crop diseases. In a typical growing season, UK potato farmers apply late blight fungicides prophylactically in a 7-10 day programme, resulting in 10-15 applications on average. Although chemical control is effective in most cases, crop losses are not always completely prevented and conversely, some fungicide applications may be unnecessary in a low disease risk year. The cost of late blight control in the UK is approximately £350/ha, with costs of up to £72M p.a. during high pressure blight seasons. Across Europe, the total financial loss associated with late blight is estimated at €1bn (Haverkort et al, 2008), representing 15% of total farm gate price.

Control options include the use of host resistance, improved disease risk forecasting and optimal fungicide use, alone and in combination. A genotypic, and more critically phenotypic, understanding of the local and international *P. infestans* populations, gained through initiatives such as the AHDB ‘Fight Against Blight’ campaign and the Euroblight network is crucial for the ongoing development of practical control decisions, and ultimately IPM programmes.

Late blight disease risk forecasts typically assume that viable inoculum is ubiquitous, and make a prediction based on the suitability of weather conditions for infection. The Hutton criteria are a recently devised set of weather conditions which reflect the characteristics of the current pathogen population and improve upon the accuracy of previously used infection risk criteria. However, viable inoculum is not always present and therefore such risk forecasts provide a conservative estimate of risk, potentially leading to unnecessary chemical applications. This is particularly the case at the start of a growing season when accurate information on risk is required to inform the initiation of a spray programme. Accurate prediction of high risk periods is also essential throughout the growing season in order to inform the use of the most effective products, which tend to be more expensive with constraints on their permitted application frequency. Although challenging, the aim of late blight IPM is to combine robust components in order to optimise the targeted and sustainable use of fungicides for effective disease management.

IPM FOR SOIL-BORNE PATHOGENS

Soil-borne pathogens of potato cause a number of serious blemish diseases. By employing appropriate soil sampling strategies in conjunction with a method for soil DNA extraction (Brierley *et al*., 2009), and real-time PCR assays to detect and quantify target pathogens, (e.g. Cullen *et al*., 2002; van de Graaf *et al*., 2003) the relationship between soil-borne inoculum and disease risk can be validated. The relationship between pathogen detection and disease risk for black dot (Lees *et al*., 2010; Brierley *et al*., 2015) and powdery scab (Brierley *et al*., 2013) have been determined. Furthermore, these studies included the impact of soil-borne inoculum on disease in conjunction with host resistance. By quantifying soil inoculum prior to planting, growers can target appropriate cultivars to fields. Utilizing host resistance remains the most effective strategy for controlling powdery scab, whilst for black dot other crop management options can be employed to further reduce risk, e.g. reduced irrigation, early harvest and, where appropriate, in-furrow application of azoxystrobin.

IPM FOR SOIL-BORNE PESTS - FREE LIVING NEMATODES

Free-Living Nematodes (FLN) are a major problem for the UK potato industry (Dale & Neilson, 2006), exacerbated in the short term by removal of approved nematicides (91/414/EEC) and in the long-term by expected population increases due to a changing climate (Neilson & Boag, 1996). Globally, FLN have been calculated to cause annual economic losses valued at over $125 Billion (Chitwood, 2003). Species of *Paratrichodorus* and *Trichodorus* typically feed directly on roots or root hairs resulting in reduced yields. Furthermore, some species vector Tobacco Rattle Virus (Taylor and Brown, 1998) affecting tuber quality through visible spraing symptoms. *Pratylenchus*, are semi-endoparasites, thus in potato can briefly enter root cells and on egress, the entry/exit point forms a wound that is frequently utilised by secondary pests and pathogens which has been well characterised for potato (MacGuidwin and Rouse, 1990; Forge *et al*., 2015).

Long-standing management of FLN has been the application of synthetic nematicides (e.g. a.i. oxamyl or fosthiazate). In stark contrast to Europe and North America, UK agriculture has been slow to uptake alternative forms of in-field FLN management although since 2015 field grown mustards, commonly used in North America to control FLN, have become increasingly prevalent. Having a potential single solution replicates the current situation of reliance on synthetic chemicals and is unsustainable in the long-term. Thus, a range of achievable strategies are required to maintain long-term sustainable potato production.

Potential interventions as an integrated strategy for the management of FLN are available for deployment such as the extension of the rotational period between each potato crop and ensuring that the crops used within the rotation are non-hosts of FLN and effective control of weeds that can act as a reservoir for virus. Monitoring is one of the key principles of effective IPM and cannot be implemented effectively without accurate estimates of target FLN abundance. Such data can provide key information for the grower, for example, to make decisions on cultivar to be grown. Using recently developed DNA diagnostics for four trichodorid species (InnovateUK project TP 292-249), high-throughput, rapid and cost-effective monitoring can be achieved.

Whilst other IPM strategies for FLN have been suggested, for example, application of soil amendments; deployment of biological control; conservation tillage; production of biofumigant (a perceived sustainable strategy to manage FLN by the growing and incorporation of *Brassica* species) or cover (a perceived strategy to manage soil erosion, quality, biodiversity and soil-borne pathogens including FLN by the growing of a range of plant species such as legumes, brassicas and grasses) crops, only the latter has gained recent traction. There is a considerable barrier to general uptake of these strategies as the limited research to date has yielded inconsistent results under UK conditions.

NEW TECHNOLOGIES

One of the most important sources of inefficiency in commercial agriculture (with regards to crop protection), is the treatment of crops as if they are homogeneous over large areas. The result is that protection measures are usually applied uniformly to crops, including on regions that do not need treatment. This inefficiency carries both an economic cost to the grower (and ultimately consumer), and can be a source of unnecessary environmental impact. This situation has arisen from the fact that it has not been economically feasible to map the spatial variability in crops (with regards to factors such as disease risk, pathogen inoculum distribution and disease symptom expression) with high enough spatial and temporal resolution to provide growers with the quality of information required to segment their crops and apply crop protection measures with a more targeted approach. However, the increasing availability and affordability of technologies that facilitate remote monitoring of crops with high frequency and levels of spatial detail (Mahlein, 2016) and use of machine learning methods (Behmann *et al*., 2015) will likely lead to on-farm interventions.

There are several ongoing projects at the James Hutton Institute developing remote sensing technologies for crop disease detection and discrimination; one of which is ‘In-field optical detection of potato disease (Poptical). Poptical is exploring the use of a combination of multispectral and RGB sensors mounted on Unmanned Aerial Systems (drones) for the early detection of, and differentiation between several diseases of potato; late blight, blackleg, early blight, black scurf/ stem canker, black dot, powdery scab, *Potato Mop Top Virus* and *Tobacco Rattle Virus*. It is the intention that, once mature, the remote sensing tools developed at the Hutton will provide growers with the knowledge they need to help them to deliver effective IPM programmes.

BARRIERS TO UPTAKE

A key challenge for the realisation of IPM for potato from research into practice is to understand and overcome the barriers to uptake. IPM research commonly focuses on single alternative control options (IPM tools). However, it is necessary for researchers to demonstrate that individual IPM tools can be combined into a package (or toolbox) from which an effective control strategy, not wholly reliant on chemical control, can be constructed specific

to grower needs. This is currently being addressed through collaborative research (Hutton and SRUC) developing IPM toolboxes for key Scottish crops, including potato, funded by the Scottish Government's Rural and Environment Science and Analytical Services (RESAS) Division.

Additional barriers to uptake of IPM approaches are disease specific. Where little or no chemical control options are available, for example for the control of blackleg, IPM strategies are routinely employed, even if not traditionally perceived as such. However, as pathogen populations are often dynamic in terms of species/strain abundance and relative pathogenicity, IPM strategies require timely updating. This is indeed the case for blackleg control, where once effective control strategies now appear to be less so, and new research (as described in this paper) is underway to better understand how and why this has occurred and what can be done to improve and maintain disease control.

Where existing chemical control methods are available and considered effective, for example in the control of late blight, there is less motivation to adopt alternatives. Whilst in theory, IPM approaches which could potentially reduce inputs and costs are very attractive, in practice the perceived risk of total crop failure should alternative approaches to disease control fail is prohibitive in the uptake of IPM. Late blight control also highlights the need for monitoring of pathogen populations to maintain effective control options. Knowledge of the *P. infestans* population is necessary as the predominance and distribution of strains with varying pathogenicity and insensitivity to particular active compounds varies, sometimes on an annual basis. Understanding the pathogen population can therefore prevent the application of ineffective chemical control agents and identify effective controls.

For other pests, such as FLN, the need for IPM approaches has been magnified by the removal of active ingredients, in this case nematicides, which constituted the sole method of FLN control for many years. Recently developed diagnostics to detect key FLN species is proving an effective new tool in FLN management, whereas for other soil-borne pathogens, the cost of predictive diagnostics may be prohibitive to uptake, especially in the absence of cost/benefit analysis relating to their use, for example soil testing for the pathogens causing black dot and powdery scab.

An important barrier to uptake for any new technology by growers is how easily the information they receive can be incorporated into existing decision support systems (DSS) for pest and disease prevention and control. Alternatively, if new DSS are developed, this must be a process that engages fully with end users so that what is provided aligns with grower's needs, to maximise the likelihood of uptake.

A focus on knowledge exchange and demonstrating the effectiveness of IPM strategies in-field remains a priority for both researchers and funders to ensure that barriers to uptake are adequately addressed.

ACKNOWLEDGEMENTS

The work at The James Hutton Institute is partly funded by the Scottish Government's Rural and Environment Science and Analytical Services (RESAS) Division. In addition we would like to thank InnovateUK, the AHDB and all who have previously supported and continue to support IPM research at The James Hutton Institute.

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DEVELOPMENT OF NEW APPROACHES FOR PBTC MINI-TUBER PRODUCTION

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Summary: Mini-tubers are the first tubers in the potato production chain and preservation of their high health status is of paramount importance to the industry. At present, mini-tuber production in Scotland mainly relies on peat-based growing media, which is largely considered to be free from pathogens. However, there is a drive to reduce peat use by amateur growers by 2020 and by professional growers by 2030 to address environmental concerns. Growing media producers are reacting to this and introducing peat alternatives. Additionally, research suggests that it is possible for peat to harbour plant pathogenic microorganisms. Thus, there is a need to find peat alternatives and treatments that are suitable for raising mini-tubers in order to safeguard the future of the industry. SASA has initiated a project in close collaboration with Scottish growers, backed by AHDB funding, to test alternative growing media and treatments for PBTC production that will ensure continued supply of healthy Scottish mini-tubers.

INTRODUCTION

The Seed Potatoes (Scotland) Regulations 2015 advocate use of appropriate husbandry methods to keep mini-tubers free from disease and pre-basic tissue culture (PBTC) growers take every care to ensure optimal health of their mini-tubers. The Scottish Government works closely with them: monitoring health, providing advice and supporting the industry at its very foundations. Plants are grown under protection, strict hygiene procedures are implemented and they are often grown in peat, which is widely considered to be pathogen-free. However, there is a UK Government backed drive to reduce the use of peat by 2030 for professional growers (Anonymous, 2011) and many growing media producers are developing alternatives. In the horticulture sector, a growing media study assessing alternatives to peat is currently underway (DEFRA/HDC/industry funded project SP1215; Mulholland, 2017) and it is vital that the specific needs of mini-tuber production are considered, allowing growers to make informed choices as to the growing media they use.

PBTC mini-tuber production

Seed (and ware) potatoes produced in the UK are descendants of PBTC mini-tubers and it is important that their high health status is maintained as their health can influence the health of future generations in the potato production multiplication chain as depicted in Figure 1.

There are currently five PBTC growers in Scotland who produce mini-tubers from sterile micro-plants produced in Scottish Government laboratories. These microplants are multiplied to required numbers in growers' own laboratories before being planted in secure polytunnels or glasshouses, protected from the outside environment, pests and diseases. PBTC facilities are subject to regular inspections by Scottish Government inspectors to ensure they meet the required standards to fulfil the demands of the Seed Potatoes Scotland (2015) Regulations. In addition, an annual mini-tuber monitor is carried out in government laboratories whereby

randomly selected stocks undergo a rigorous testing schedule to test for a comprehensive range of fungal, bacterial and viral diseases.

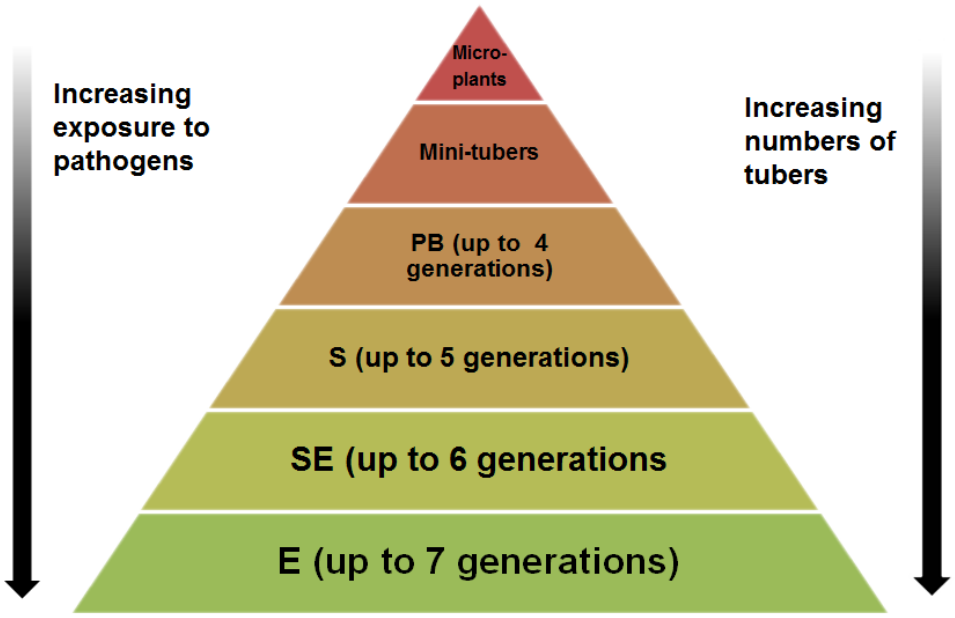

Figure 1. Mini-tubers are the first tubers in the potato production chain. Their health is important to future generations as seed-borne pathogens might be multiplied in the production system.

The use of growing media in PBTC production

Microplants are usually transferred from culture medium to peat-based growing media to produce mini-tubers. At this stage, they could, potentially, acquire pathogens from the growing medium and some pathogens could persist in the multiplication chain. Hence it is important to consider the pathogen potential of the growing medium used.

Use of peat and alternatives by professional growers

It is clear that the use of peat alternatives, in both professional and, especially, amateur markets, has increased in recent years. Waller (2015) reviewed peat use from 2012-2014 inclusive and found that, during that period, just over a third of growing media used by professional growers were peat alternatives whilst alternatives constituted around half of all amateur use. This contrasts to the relative amounts used in 1999 (Alexander, 2014) where peat alternatives were used much less frequently. Peat use by amateur growers was 15 times greater than alternatives and 19 times greater than alternatives in professional use.

There has been much publicity in the popular press over the past two decades urging reduction in use of peat, such as Diacono (2011), who notes that peat has only been used on a large scale since the 1970s. Before then, growers relied on inclusion of loam in growing media. In 2011, a government white paper called for elimination of peat use for professional growers by 2030 (Anonymous, 2011). The aims were to preserve a non-renewable resource, to protect the natural environment, conserve habitats and to protect carbon stores, the destruction of which might adversely affect climate change. Growing media producers are now exploring the use of alternative growing media and many have introduced a range of peat-free alternatives to their portfolios.

Peat is widely thought to be a sterile growing medium. However, research has shown that it can harbour a host of fungi, bacteria and yeasts. Whilst some of these may promote disease suppression (Hunter *et al*., 2006), there are also instances of plant pathogens being detected within peat such as *Pythium* (Hunter *et al*., 2006), *Plasmodiophora brassicae* (Staniaszek *et al*., 2007) and, in France, the potato pathogen *Spongospora subterranea* was detected in Baltic peat (Andrivon, 2000). To date, Scottish PBTC production has had no major problems with acquisition of pathogens from peat (mostly sourced within the UK and Ireland) but the potential for this is another reason to seek alternatives.

FINDING ALTERNATIVES TO PEAT FOR PBTC PRODUCTION

Introduction

The four most popular alternatives are: wood-based alternatives such as wood fibre (33% of alternatives per volume supplied in 2014), green compost (22%), coir (20%) and bark (17%) (Waller, 2015). This project will consider the use of these and more novel alternatives such as wool fibre. The properties of each medium will be fully researched and a program of testing will be conducted. Coir, for example, has been shown to be rich in suppressive microorganisms (Hyder *et al*., 2009) that can counter some soilborne pathogens, such as *Fusarium solani*. Other properties such as sustainability, physical characteristics and environmental impact will be considered.

Materials and methods

This project has three main phases of experimental work. During the first phase, a range of growing media and growing media constituents will be screened in microcosm studies (Figure 2), whereby each growing medium to be tested is added to a sterile polypropylene vessel. Next, a sterile microplant is planted in to the growing medium within a laminar flow cabinet. The growing medium is moistened with sterile distilled water. A second polypropylene vessel is placed on top of the first vessel and secured with micropore tape before placing in a plant growth chamber (18ºC; 16 hours light; see Figure 2). After 12 weeks, their roots will be harvested and their DNA will be extracted and tested for a range of pathogens by PCR.

Figure 2. Microcosm studies will be used in an initial screening process to determine the pathogen status of growing media.

COX primers (potato specific) will be used as a control to ensure presence of DNA. Media that are pathogen-free will be used in the second phase of the study - glasshouse trials at SASA. They will be assessed for suitability factors such as good handling and yields. The progeny tubers will be subject to a rigorous testing program to identify any pathogens acquired from the growing media. The third phase will be trials at PBTC growers' premises using the best media from the phase two trials. Performance of the growing media will be assessed within the growers' own production systems in terms of handling, structure, water retention, plant growth, yield, skin finish, size and tuber quality. Many growing media, by their very nature, contain microorganisms, some of which may be pathogenic. Therefore, the efficacy of a range of control strategies such as the use of sterilisation, fungicide treatment and the application of biocontrol agents and plant growth promoting bacteria will also be considered.

Project aims

At present, growers have little information available to them about the potential pathogen status of any growing medium or strategies for counteracting such a threat when it exists. This project aims to address this by evaluation of a range of growing media to determine the pathogen potential of each alongside a range of control measures to enhance the ability of the growing medium to eliminate pathogens. PBTC growers will be consulted throughout the project to ensure that the results of this work are pertinent and highly applicable to their production systems. A full account of results will be discussed at CPNB 2020.

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We acknowledge the support of the AHDB in providing funding for this project; the Scottish PBTC growers who willingly share their time, knowledge and expertise with us and UK and Northern Irish growing media producers who have been very open and accommodating, helping us learn about their industry. Thanks to colleagues Niall Ormiston for laboratory assistance and Sandra Goodfellow for supply of microplants and useful conversation.

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DEVELOPMENT OF A DECISION AID TO SUPPORT THE USE OF CURATIVE LATE BLIGHT FUNGICIDES

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Summary: Curative fungicides, which act within a pathogen's incubation period, are an important component of many late blight control programs. Growers and agronomists would benefit from a simple decision aid to provide justification for the use of curative products. This study aims to ultimately produce such an aid, and interim results which form the basis of its parameters are presented here. Data from glasshouse bioassays with a representative curative fungicide (fluopicolide + propamocarb) and a susceptible potato variety (King Edward) suggest that curative control rapidly declines from 20 hours post inoculation with an aggressive isolate, and from 56 hours with a less aggressive isolate. Further results from bioassays and field trials confirm that post infection temperatures increase the rate of pathogen development up until an optimum, after which it falls, and that varieties with higher resistance ratings increase the time period for curative control.

INTRODUCTION

Late blight (*Phytophthora infestans*) remains a persistent threat to potato crops, particularly in mild, wet climates such as that of northern Britain. Late blight's potential to cause explosive and highly destructive epidemics necessitates the use of routine fungicide applications, usually at 7 to 10 day intervals (Hansen *et al.*, 2016). There is a well developed market for late blight fungicides, and growers and agronomists have access to many active ingredients (a.i.s) with differing modes of action and formulations. A crucial element of a blight spray program is the selection of the most appropriate product based on considerations such as the growth stage of the crop, the local disease pressure, and the prevailing climatic conditions.

Whilst all late blight fungicides are applied with the aim of preventing infection within the crop, several of the a.i.s in widespread use have some mobility *in planta* and have the potential to give control in the pathogen's early developmental stages. This curative activity is commonly referred to as 'kickback' (Genet *et al*., 2001), and can be an important component of a late blight spray program, especially when there is a high probability that infection has taken place due to high risk weather conditions.

The toolkit that can be used to inform the choice of late blight control product is expanding: e.g. fungicide efficacies and characteristics are summarized by the EuroBlight table (Bain, 2016), the most up to date version of which is on the EuroBlight website; weather alerts are issued based on infection cycle criteria (Hutton Criteria); and outbreak warnings are provided by AHDB's 'Fight Against Blight' outbreak mapping service. The aim of this study is to add to this toolkit by producing a simple decision aid for the optimization of curative fungicide use against late blight.

The rapid life cycle of *P. infestans* coupled with the fact that curative fungicides are only effective within the pathogen's incubation period (which in some circumstances can be as little as 3 days) highlights the importance of the fungicide application timings. Treatments outside of a short 'curative window' are unlikely to provide control. Previous studies have explored the decline in the efficacy of curative applications with increasing disease development time (Johnson *et al*., 2000), but there is limited information on how this curative window can be modified by additional factors such as pathogen genotype (Cooke *et al*., 2012) or varietal resistance.

In order for the decision aid to be useful it should be based on empirical data, and to this end a series of bioassays and field trials have been conducted in order to explore the relationship between the duration of disease development and the control given by curative fungicide treatment. Experiments have also been conducted on factors thought likely to modify the curative window, to assess the value of their inclusion within the decision aid. Of particular importance is the developmental temperature because this is known to significantly alter *P. infestans* growth rates (Shakya *et al*., 2015) and it is intended that the temperature profile during the incubation period will be one of the inputs for the decision aid.

MATERIALS AND METHODS

A single representative fungicide product was used in experiments where infected material was treated curatively: Infinito (Bayer CropScience; 62.5 g fluopicolide + 625 g propamocarb l^{-1}). This fungicide is rated by EuroBlight as having 'good (++)' curative activity (Bain, 2016). In all experiments detailed below, a 'curative treatment' refers to a single application of fluopicolide + propamocarb at the manufacturer's recommended label rate, i.e. 1.6 l product in 200 l water ha^{-1}, using an AZO compressed air, precision sprayer.

Curative threshold bioassays

Foliage was collected from 7-week-old, glasshouse-grown King Edward (foliage resistance rating 3) potato plants. Leaf discs (12 mm diameter) were cut from this material using a cork borer. The discs were then placed within a 170 mm x 170 mm Perspex frame, into which holes had been drilled, each frame accommodating 64 discs. Cut edges were covered by Parafilm strips leaving a 1 cm^2 area of disc tissue exposed. Discs were then individually inoculated with a 20 µl droplet of *P. infestans* sporangial suspension (isolate 2012_10290A, genotype 7-A1 for the first run and 2012_9922C, genotype 13-A2 for the second), prepared from 7-day-old infected leaflets and adjusted to 10^5 sporangia ml^{-1}. The two isolates were from Great Britain. Inoculated discs, and non-inoculated controls, were sealed within transparent plastic boxes lined with damp tissue paper. Boxes were in turn placed within a controlled climate chamber (16h / 8h day/night cycle, 18^o C). At timings corresponding to 4-hour intervals between 8 and 72 hours post inoculation, selected frames were removed from the climate chamber and treated curatively – one frame containing 64 discs were treated at each time-point. Two frames of 64 discs were inoculated, but left untreated as controls. Frames were returned to incubation conditions immediately following treatment. Seven days from the initial inoculation discs were assessed for disease development. A disc that was completely necrotic or showed signs of sporulation was classified as a successful infection, whilst one that showed no symptoms or small arrested lesions was categorized as effective control.

Isolate response to temperature

Detached leaves from 7-week-old, glasshouse-grown King Edward potato plants were placed within transparent plastic boxes which had been lined with damp tissue paper. Two boxes were used for each experimental run at each temperature, each containing 8 leaflets. Each

leaflet was then inoculated with a 20 µl droplet of *P. infestans* sporangial suspension (isolate 2012_9922C), adjusted to 10^5 sporangia ml^{-1} as described in the previous section. There were 8 non-inoculated leaflets that served as controls, included in an additional box. The plastic boxes were then sealed and placed within a climate controlled chamber. Conditions in the chamber were set to begin with a 12-hour period at 18 ^oC to maximize the number of leaflets which were successfully infected, followed by 6 days at the experimental temperature (6, 10, 14, 18, 22, 26 or 30^oC). A 16h / 8h day/night cycle was maintained throughout. At 120, 144 and 168 hours post inoculation infected leaflets were removed from their boxes and digital images taken. Lesion size was quantified from these images via the program ImageJ (Schneider *et al*., 2012). Necrotic and/or sporulating tissue was measured using the 'polygon' function. Linear lesion growth rates were then estimated by linear regression of the square roots of these three observations (Visker *et al*., 2003) against time. The experiment was run twice for each experimental temperature.

Varietal resistance field trials

Potato plants of varieties King Edward (foliar resistance rating of 3) and Cara (foliar resistance rating of 5) were grown in small propagation pots within a poly-tunnel for approximately 7 weeks. When high risk weather was forecast (i.e. the Smith criteria, the experiment was conducted before the publication of the Hutton criteria) plants were transported to a trial field where a late blight epidemic was in progress. Plants were placed within open trays on ridges and were left exposed for 2 hours. The plants were then sealed within plastic sheeting and placed within a climate chamber (16h / 8h day/night cycle, 18^oC). After 1, 2 & 3 days, 12 plants per cultivar were treated curatively, or left untreated as controls, and returned to the climate chamber. Plants that had not been exposed in the trial field served as controls. Seven days after exposure to inoculum, the number of late blight lesions per plant was counted.

RESULTS

The time period during which curative fungicide efficacy was high was substantially affected by the aggressiveness of the *P. infestans* isolate, the temperature post infection and also varietal resistance to leaflet colonisation.

Curative threshold bioassays

Figure 1 shows the results of two runs of the leaf disc bioassay with (A) a highly aggressive isolate (2012_9922C) and (B) an isolate that had slower growth rates in other bioassays (2012_10290A). At early time points (approx. 8 – 20 hours) curative sprays generally offered good control on the leaf discs infected with isolate 2012_9922C. This control was then rapidly lost from approx. 20 – 40 hours, and at time points greater than 40 hours curative sprays rarely prevented more than 30% of infection sites from developing into lesions. In contrast for the second run of the bioassay, with isolate 2012_10290A, 40 – 100 % of the leaf discs did not develop expanding lesions after curative sprays in time points 8 – 56 hours.

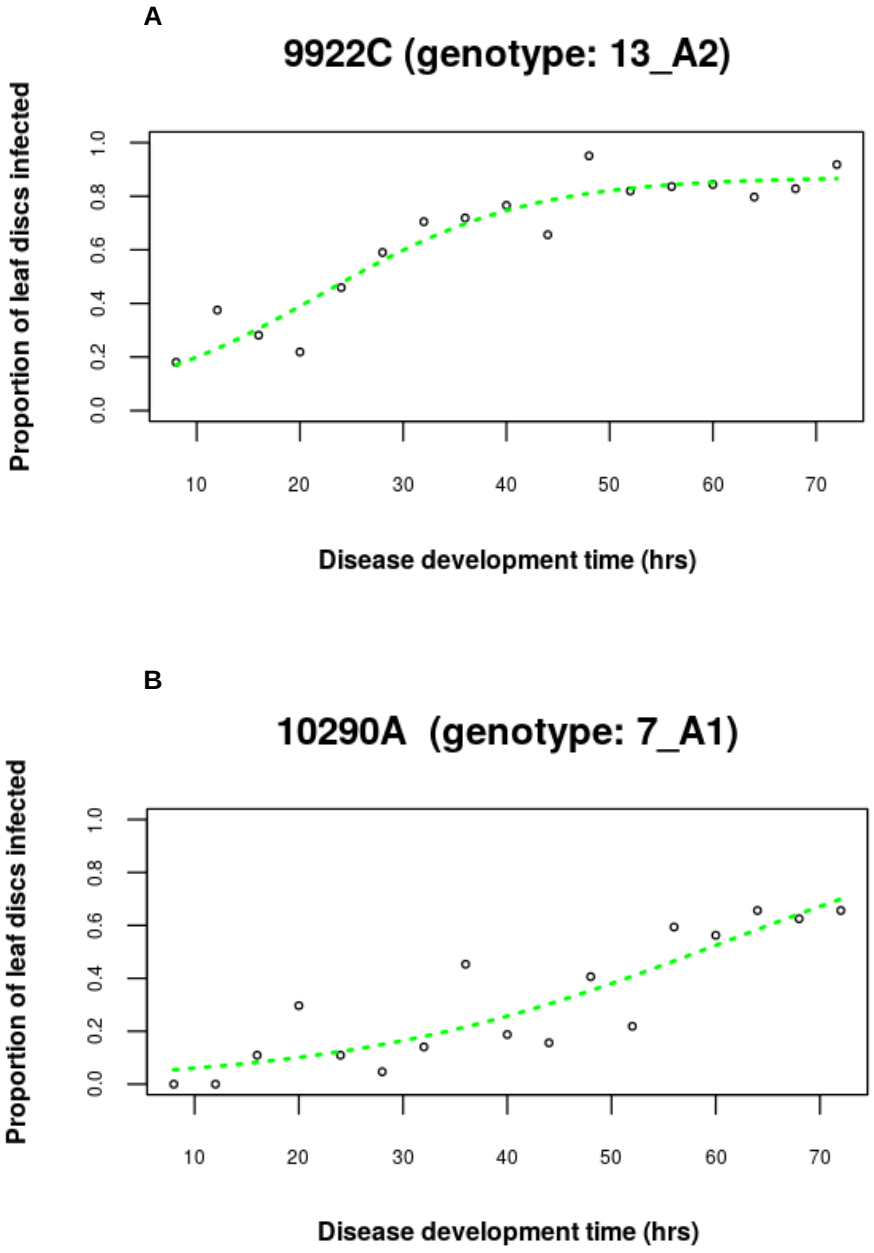

Figure 1. Proportion of leaf disc infection (n=64) in relation to curative fungicide timing (hours at 18 ^{o}C from inoculation to treatment with fluopicolide + propamocarb) for the isolates (A) 2012_9922C and (B) 2012_10290A. Sigmoid curves are fitted to the data (adjusted-R^2 for A = 0.90; adjusted-R^2 for B = 0.78).

Isolate response to temperature

For the lower part of the tested temperature range (6 – 18^{o}C) an increase in temperature was associated with an increase in the linear growth rate of lesions on leaflets. This rate plateaued between 18 and 22^{o}C, and then reduced as temperature increased. No lesion growth was observed at 30^{o}C.

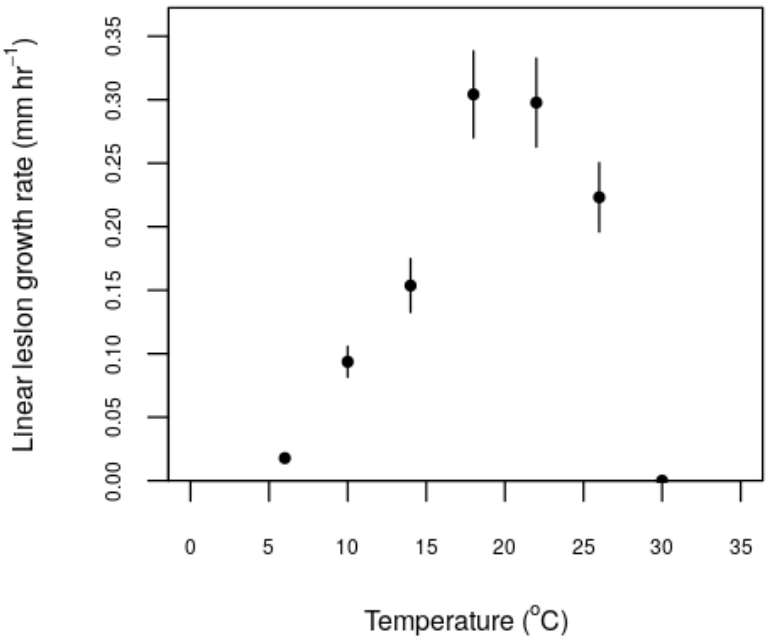

Figure 2. Mean linear lesion growth rates (bars represent 95% confidence intervals) of *P. infestans* (isolate 2012_9922C) on detached leaflets, incubated at 6, 10, 14, 18, 22, 26 or 30 ^{o}C.

Varietal resistance field trials

In this experiment differences between both varietal resistance scores and spray timings were statistically significant ($p<0.01$). On Cara plants lesion numbers were significantly lower for all three curative treatment times compared with untreated control plants. In contrast, only King Edward plants treated after a single day of disease development had significantly lower lesion counts. A full presentation of these results is available (Maloney *et al*., 2018), although they should be interpreted with caution as aggressiveness and genotype data are not available.

DISCUSSION

The curative activity displayed by some fungicides is effective over a relatively short period after infection, after which infections are not well controlled. The results presented here will form the basis of a simple decision aid to assist selection of the most appropriate treatment following a period where infection is suspected. Results from both glasshouse bioassays and field trials suggest that modifying factors can play an important role in altering the duration of the curative window, and these factors should ideally be taken into account when assessing the appropriateness of a curative treatment.

With the susceptible variety King Edward, the reduction in developed lesions of more aggressive isolates was obtained early in both the glasshouse/leaf disc bioassay and the field experiment: less than 20 hours from inoculation in the leaf disc assay, and 1 day from exposure in the field. These times are broadly consistent considering it is possible that the inoculum density and isolate differed between the two experiments. For the more resistant variety Cara, lesion number was reduced for all three fungicide treatment times (1, 2 and 3 days post exposure) in the field trial. The response of the two isolates used in the leaf disc bioassay also differed, with the more aggressive isolate (2012_9922C) reaching a point of limited curative response well before the less aggressive isolate (2012_10290A). This result should not be generalised because these two isolates probably represent extremes in aggressiveness that don't currently exist in the competitive pathogen population. For the decision aid it is probably best in the short term to take the more risk-averse approach of

assuming a crop is under pressure from the most aggressive strain. It may be possible to include genotype aggressiveness as a factor in later versions, once very rapid genotyping of air-borne *P. infestans,* or very early lesions in neighbouring outbreaks, is possible on a large scale. Temperature has a strong influence on the developmental rate of *P. infestans* (Chapman, 2012). The growth rate / temperature profile generated from the detached leaf bioassay (Figure 2) will allow the probable rates of pathogen development to be included in the final decision aid model. This will be crucial because slower *P. infestans* growth at lower temperatures has been shown to improve the performance of curative fungicides (Genet *et al*., 2001). Formulation of the model for the decision aid is ongoing, with the data presented here acting as the basis for parameters and also as justification for which modifying factors to include. Additional results from field trials will be used to assess the predictive power of the aid at different sites and under different conditions.

ACKNOWLEDGEMENTS

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THE ORIGINS AND IMPLICATIONS OF A NOVEL POPULATION OF *PHYTOPHTHORA INFESTANS* ON POTATO CROPS IN SCOTLAND

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Summary: Understanding the source of *Phytophthora infestans* primary inoculum is helpful in mitigating and managing the emergence of late blight disease at the start of each season. Genetic fingerprinting of the UK pathogen population is providing insights into the proportion of late blight outbreaks occurring from either clonal or presumed sexual inoculum across the UK. Almost 90% of samples were of clones present in the preceding year. Pathogen genotypes not seen previously make up the remaining 10% and are grouped into a category termed 'Other'. A regional pattern was observed in samples of *P. infestans* isolates from Scotland with the proportion of 'Other' genotypes occurring in northeast Scotland being higher than in other potato growing regions. In this paper we examine the population of *P. infestans* in Scotland to help understand primary inoculum sources with a focus on the practical messages to the potato industry in northern Britain.

INTRODUCTION

Management of late blight disease, caused by *Phytophthora infestans*, remains a great challenge to the potato industry. Growers are generally constrained in their use of blight resistant cultivars and thus, at the start of each season, need to focus on managing sources of primary inoculum and optimising the timing of fungicide applications for effective crop protection. The emergence and rapid spread of aggressive clones that overcome host resistance (Lees *et al*., 2012) or have reduced sensitivity to fungicides (Cooke *et al*., 2012) continues to have an impact on the industry in Britain and continental Europe (Cooke *et al*., 2011; Li *et al*., 2012). The pathogen can, however, also reproduce via sexual recombination generating the next round of potentially aggressive clones. Sexual recombination occurs when isolates of opposing mating types co-infect a plant to form oospores. As well as generating diversity, these long-lived, soil-borne oospores are an additional source of inoculum that can emerge early in the season (Yuen & Andersson, 2013). Although oospores have not been reported directly in British crops, evidence of sexually reproducing populations in *P. infestans* in northeast Scotland is accumulating (Cooke *et al*., 2016). Research at The James Hutton Institute continues to track the change in *P. infestans* populations via the AHDB Potatoes Fight Against Blight (FAB) campaign with scouts providing samples from potato blight outbreaks across the country. In this way we have been able to track new clones and seek evidence of oospore-borne inoculum. Data on British crops are being extended in collaboration with the European industry-sponsored Euroblight programme (www.euroblight.net). In this paper we describe the key messages from late blight monitoring research from 2012 to 2017 with emphasis on the elevated diversity of populations in parts of Scotland.

MATERIALS AND METHODS

Late blight scouts for the AHDB Potatoes' FAB campaign sampled foliar and stem lesions from late blight outbreaks each growing season from 2003 to 2017. The majority of infected plant material was sent to Fera Science Ltd (Sand Hutton, York, UK) and forwarded to Dundee in potato tubers. Isolates, mycelium from the colonised potato tubers or material pressed onto Whatman FTA cards were genotyped using a previously described 12-plex simple sequence repeat (SSR) assay (Li *et al*., 2013). Data was processed in Excel spreadsheets to define the common clonal genotypes and uploaded, along with latitude and longitude data, to the Euroblight database (www.euroblight.net). Isolates that do not match those of the dominant clonal lineages are grouped in a 'catch all' category (termed 'Other'). Once recorded in the database, the location and genotype data was mapped and the genetic diversity data analysed using the R-based population genetics tool *poppr* 2.0 (Kamvar *et al*., 2015).

RESULTS

Analysis of the 2718 genotyped *P. infestans* samples collected from GB crops from 2012 to 2017 indicated that 87.4% of samples were of 10 clones with the majority of these belonging to just three lineages; 6_A1, 13_A2 and 8_A1 (Table 1). The remaining 12.6% of samples comprised a pool of genetically diverse isolates termed 'Other'. A breakdown of the data by country showed geographical differences in the frequency of the clones and, in particular, the frequency of 'Other' types which was 27.7% of samples in Scotland but 4.9% and 5.4% in England and Wales, respectively. Within Scotland, a consistent pattern was observed with the 'Other' samples being almost exclusively recorded from the Aberdeenshire, Moray and Highland regions whereas other potato growing regions such as Angus and Fife were dominated by clones such as 6_A1 (Figure 1).

Table 1 Percentage of *P. infestans* samples of each genotype collected between 2012 and 2017 from potato late blight outbreaks in England, Wales, Scotland and the whole of GB. The total number of samples is shown in each case.

Genotype	England	Wales	Scotland	GB
'Other'	4.9	5.4	27.7	12.6
1_A1	0.5	0.0	0.0	0.2
13_A2	28.5	30.6	10.1	23.1
6_A1	55.7	52.1	56.4	54.7
23_A1	0.5	0.3	0.4	0.4
2_A1	0.0	0.3	0.0	0.1
8_A1	1.4	11.4	4.8	5.8
33_A2	0.2	0.0	0.0	0.1
37_A2	7.9	0.0	0.0	2.6
36_A2	0.2	0.0	0.0	0.1
39_A2	0.3	0.0	0.5	0.3
Total	**1669**	**317**	**732**	**2718**

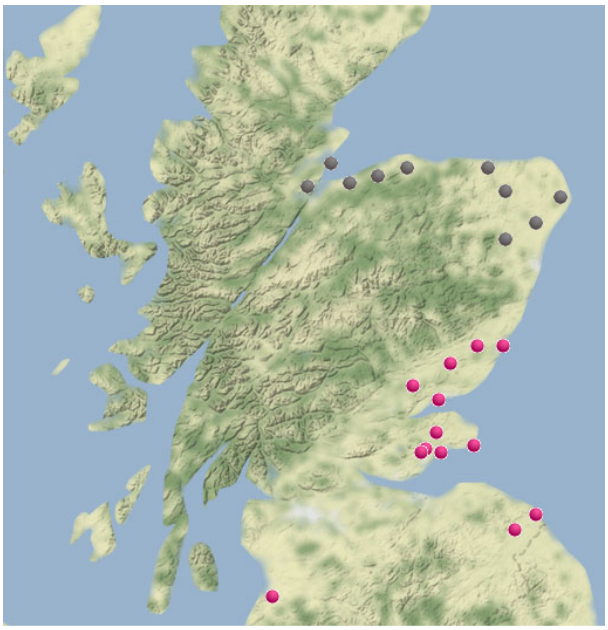

Figure 1. Location of late blight outbreaks sampled in Scotland in 2016 displayed according to the pathogen genotype. Pink indicates outbreaks caused by the 6_A1 clonal genotype and grey those caused by genetically diverse 'Other' genotypes of *P. infestans.* Data presented via the Euroblight web interface.

Analysis of the genetic diversity of Scottish samples using *poppr* 2.0 discriminated 127 multilocus genotypes (MLGs) amongst the 481 samples (data not shown). As predicted for a clone, only 14 MLGs were found amongst 258 samples of the 6_A1 genotype. These represent minor variants of the clone at hyper-variable SSR markers. In contrast, 96 MLGs were present amongst 156 'Other' samples. Each of these 96 MLGs is shown as a node on the Minimum Spanning Network (MSN) with the nodes varying in size from 1 to 5 samples of that MLG (Figure 2). Unlike the clones, the identical 'Other' MLGs almost exclusively occurred in a single disease outbreak from only a single year.

DISCUSSION

Recent data indicates that populations of *P. infestans* in northwest Europe, for example, Northern Ireland (Cooke, 2015), France (Mariette *et al*., 2015) and Britain (Cooke *et al*., 2012) are dominated by relatively few clonal lineages. In contrast, populations in the Nordic (Sjöholm *et al*., 2013) and Baltic States (Runno-Paurson *et al*., 2010) are highly diverse with almost every isolate being genetically unique. In the Netherlands and Poland, both clonal populations and highly diverse isolates are recorded (Brylińska *et al*., 2016; Li *et al*., 2012). The populations of *P. infestans* sampled in Britain from 2012 to 2017 show the dominance of three clones; 6_A1, first reported in the UK in 2004, 13_A2, reported in 2005 and 8_A1, reported as early as 1995 (Cooke *et al*., 2012). An additional clone, 37_A2, with reduced sensitivity to fluazinam is a more recent threat, to date limited to crops in England (Table 1). The pattern of clonal populations clearly indicates that the primary inoculum starting each year's epidemic originates from local sources such as volunteer potato tubers or outgrade piles. The contrast between dominant clone(s) across the more southern potato growing regions of Scotland and unique 'Other' genotypes in northern regions (Figure 2) is of scientific interest and has practical implications.

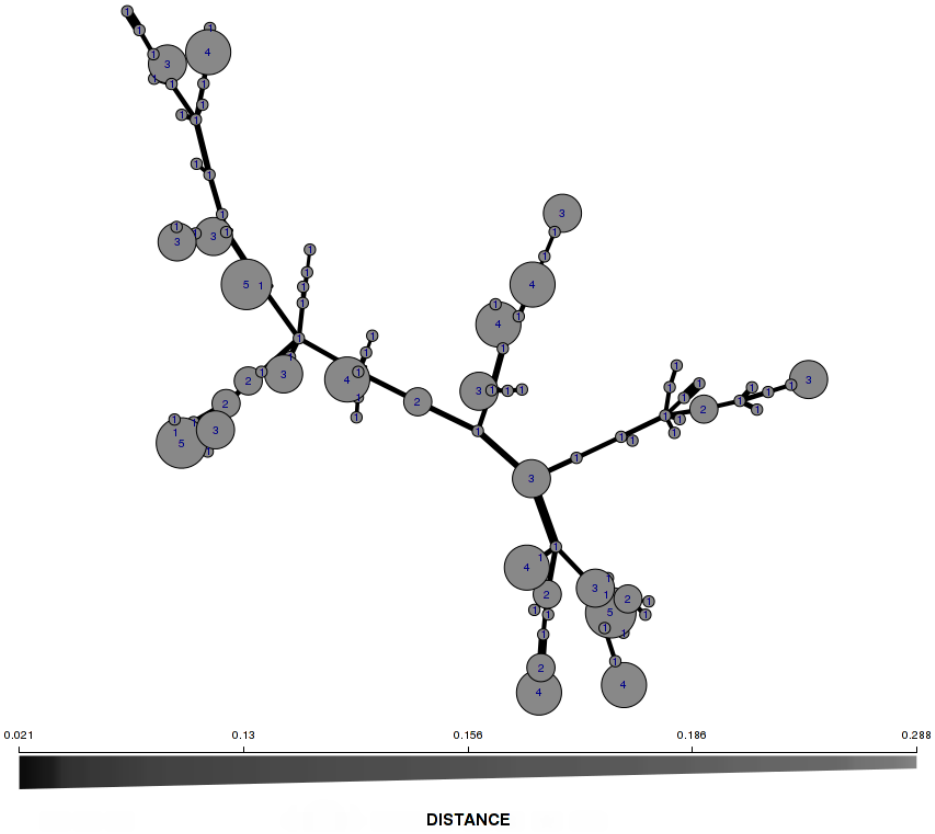

Figure 2 Minimum Spanning Network of SSR genetic diversity data from 156 samples of *P. infestans* sampled in Scotland from 2013 to 2016. Each of the 96 nodes is a unique pathogen genotype found from 1 to 5 times (small to larger node sizes).

The failure of clones such as 6_A1 and 13_A2 to become established in this region of Scotland may be due to its geographic isolation. A combination of the very narrow strip of cropped land around Stonehaven and the prevailing westerly winds are expected to limit inoculum spread from the potato crops in Angus and south Aberdeenshire. In addition, the predominance of seed potato production to the north establishes the region as a net exporter of potato seed that is also subject to plant health legislation that reduces the risk of importation of novel genotypes of *P. infestans*.

The pathogen diversity in this region is consistent with a sexually recombining population in which long-lived soil-borne oospores act as primary inoculum. Crop rotations are, however, between 5 and 7 years in this region resulting in a significant decline in oospore viability that will minimise their impact (Turkensteen *et al*., 2000; Andrivon, 1995). A more detailed assessment of the reported outbreaks is underway to understand the nature of the outbreaks in crops compared to those from volunteer potatoes, cull piles or gardens in which any effect of crop rotation is negated. The pathogen diversity data from these outbreaks (Figure 2) indicates that no MLG is found more than 5 times and rarely in consecutive seasons. It is thus clear that, unlike the dominant clonal populations, the outbreaks caused by 'Other' MLGs are ephemeral and geographically restricted. Furthermore, a wider analysis of GB data over more than a decade has revealed only a tiny proportion of this region's novel MLGs have been subsequently found elsewhere. This pattern is also seen in Baltic and Nordic regions (Runno-Paurson *et al*., 2010; Sjöholm *et al*., 2013) and further studies on the phenotype of these

'Other' MLGs and clonal populations are underway in the IPMBlight 2.0 project (http://euroblight.net/research-projects/ipmblight20/). One possible explanation lies in the pathogen's genetics. Many successful and aggressive clones have additional copies of their chromosomes that make up their genome and are triploid (Li *et al*., 2017) whereas the MLGs in the 'Other' category are predominantly diploid (data not shown).

The presence of oospores and increased pathogen diversity is thought to increase the risk of management failure due to the early emergence of primary inoculum and the unpredictability of novel genotypes. Growers in the regions affected by these 'Other' *P. infestans* should thus be aware of the potential threat and seek to minimise the opportunities for the formation and survival of pathogen oospores. Cull piles and volunteer potato plants should be destroyed to reduce their impact as a source of primary inoculum.

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OPTIMISING CIPC APPLICATION IN BOX POTATO STORES

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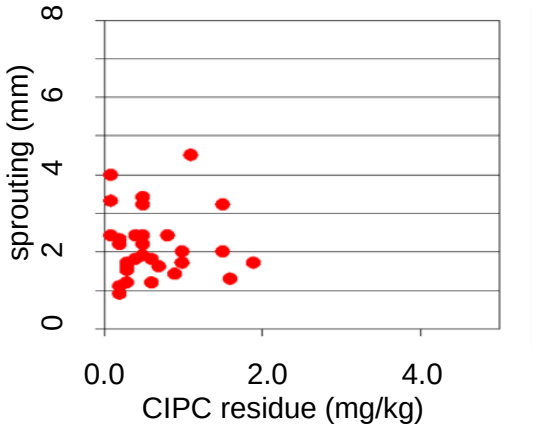
Summary: Chlorpropham (CIPC) is the most widely used sprout suppressant in potato storage and its use is currently subject to stewardship in the UK in order to reduce the risk of maximum residue level exceedance. Trials funded by AHDB were undertaken in commercial box potato stores to assess the effectiveness of adaptations to use recirculation of air/CIPC fog to improve the uniformity of application. Recommendations for the adoption of *'active recirculation'*, evaluated in these trials, have now been included on CIPC product labels for 2017/18.

INTRODUCTION

The aim of the work was to develop improved methods of CIPC application using fan assistance to recirculate the fog during application in order to achieve more uniform deposition and lower chlorpropham residues. Previous work conducted by SBCSR and the University of Glasgow (McGowan *et al.*, 2009) had successfully introduced the use of slow speed recirculation for this purpose to commercial bulk potato storage, resulting in both improved sprout control and lower chemical residues (Figure 1). Subsequent trials (Briddon *et al.,* 2013) to translate this technique to commercial box storage were only partially successful. In particular, application to passively ventilated 'overhead throw' box stores had yet to adequately address the risk of high residues. In statutory testing, MRL exceedances were still being detected in box stores. This series of trials was undertaken to take forward previous findings with the primary aim of developing solutions for these particular storage systems.

Figure 1. Use of recirculation results in a narrow range of residue concentrations and good sprout control (McGowan *et al.,* 2009)



MATERIALS AND METHODS

The trial work described here was conducted in commercial box potato stores. This was deemed necessary in order to adequately evaluate the impact of scale on the distribution of sprout suppressant. Store capacities were 900 tonnes and above. Due to the commercial nature of the stores, it was necessary to limit sample placement and removal to the times of store loading and unloading respectively.

Work was conducted over three seasons from 2013/14 to 2015/16 inclusive. At the outset of the trial, 'Be CIPC Compliant' best practice guidance recommended the use of a plenum for application of CIPC in overhead throw type stores (AHDB, 2014). The term *plenum* refers to a covered corridor (Figure 2) which bisects the main block of boxes into which the CIPC is applied. The cover largely prevents the hot-fog from rising directly into the store headspace reducing the risk of high residue levels on top boxes. This method was used in store MSF1 in 2013/14 as a benchmark for comparison. Due to the length of the store, two plenums were installed but, crucially, the store's fans were *not* used during application.

Figure 2. Mid-stack plenum design (AHDB, 2014).

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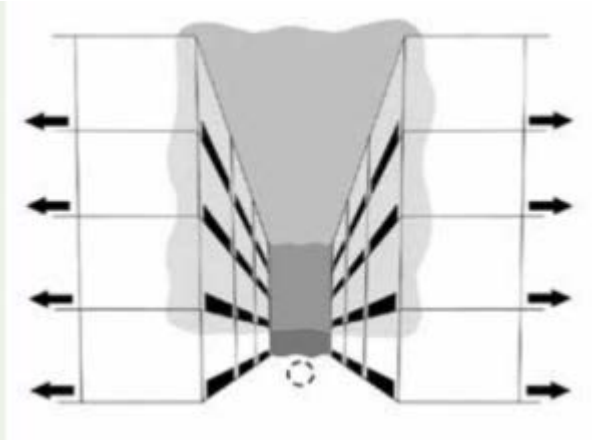

Additionally, across all three years, stores were assessed following modification in a way expected to influence CIPC residue distribution, principally through the introduction of some form of active recirculation of the air/fog mixture during the application process. This was achieved primarily by slowing the main ventilation fans down to air speeds around 20-30% of their normal flow. If this wasn't possible (e.g. because use of the main fans would pull CIPC through the fridge coils), then secondary fans were added, again to run at 20-30% of normal airflow (typically 0.004-0.006 $m^3/s/t$). In each store, residue distribution was determined using netted samples of the commercial crop, selected randomly, with each net placed systematically within boxes at store loading. A typical grid showing the store layout is shown in Figure 3, with sample locations marked 'X'.

At each sampling position within the store, nets were placed on three levels (top box and bottom box plus one box approximately halfway between). Each net contained c. 10kg of potatoes. Nets were located centrally in boxes, with the top of the net just visible at the surface, to give a representative split between surface and sub-surface tubers. Nets were recovered at the time of commercial unloading of the store; this meant that storage duration could not be specifically controlled, although storage duration was recorded. Three tubers from each net were then randomly selected and analysed individually for CIPC residue concentration by a GLP laboratory (ALS, Chatteris) and 25 tubers taken randomly from the net were assessed for sprout growth (length).

Over the three seasons' work, a selection of modified box stores were assessed with the aim of achieving improved CIPC residue distribution. These ranged from simple, low-cost modifications to overhead throw box stores to more fundamental changes, requiring complete refurbishment of the store. A range of varieties were used and stores covered both the fresh and processing potato sectors; the store types assessed in this work are shown in Table 1.

Figure 3. Typical plan of store sampling grid (nets place on 3 levels at each position shown X)

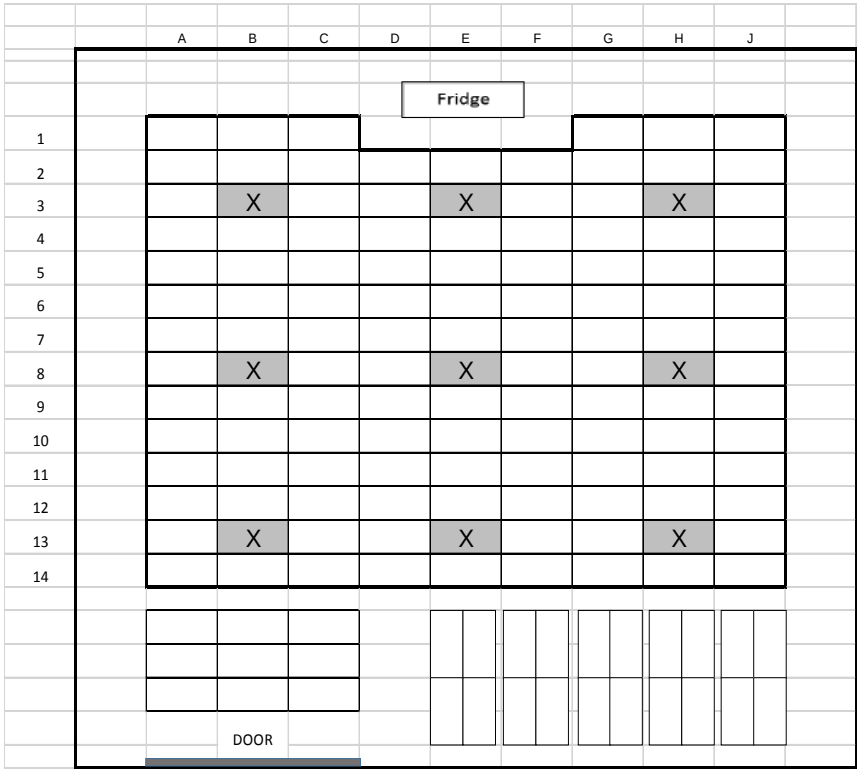

Table 1: Store types assessed in each season across the three year trial series

Store type	Code	Temp	Active recirc-ulation?	Year 1 2013/14	Year 2 2014/15	Year 3 2015/16
Passive mid-stack plenum	MSF1	Fresh	N	✓	☐	☐
Positive mid-stack plenum	MSP1	Process	Y	✓		
Positive lateral flow	PLF1	Fresh	Y	✓	✓	
Positive using foam blocks	PFB2	Fresh	Y^	✓	✓	
Open suction	OSP1	Process	Y	✓		
Open suction	OSP2	Process	Y	☐	✓	✓+ mesh
Open suction	OSP3	Process	Y	☐		✓
Open suction	OSF1	Fresh	Y		✓	
Open suction	OSF2	Fresh	Y			✓
Open suction*	OSF3	Fresh	Y	☐	☐	✓

Fresh stores held at 2-3°C; process at 7-9°C; *fitted with a half-height air separator curtain; ^see text.

In store OSP2, following measurement of local airflow in the store in 2014/15, it was found that the open suction systems remained prone to biased and disproportionately high airflow in front of the refrigeration units during normal operation. A 60% mesh restrictor was added extending 25% of the full store width either side of the fridge unit to try to reduce this. Airflows with the restrictor in place, and after its removal, were made to assess the impact.

RESULTS

Earlier work (McGowan *et al.,* 2009*,* Briddon *et al.*, 2013) showed CIPC residues with a CV% value below 100 were generally more uniform than standard application practice where ventilation was not used.

Mid-stack plenum

Application to store MSF1 resulted in a mean CIPC residue concentration of 2.9 mg/kg (SD 1.67) from a single application of 12 g/tonne. The highest concentrations of CIPC were detected in samples from middle boxes, closest to the plenum and the lowest residues (a number of boxes had residue values below 1 mg/kg), generally occurred in boxes furthest from the plenum. This demonstrates that the addition of ventilation, as in store MSP1, may help to even out any gradient.

In store MSP1, the use of a plenum with additional fans for recirculation, resulted in CIPC residue values which were relatively even (mean 2.7 mg/kg, SD 1.30, CV% 48). However, after two applications with a total dose of 26 g/tonne, there were still some boxes which had residue values which were too low (<1 mg/kg).

Whilst central plenum systems can work successfully with adequate recirculation to apply CIPC, their use requires attention to detail to eliminate localised effects and maximise the evenness of application.

Open suction (using an air separator curtain)

In 2013/14, the use of an air separator curtain coupled with low-speed recirculation of fog through open pallet apertures (Store OSP1), termed ‘open suction’, resulted in low CIPC residue variability (mean 1.1 mg/kg, SD 0.53, CV% 47). Although this variability may have been slightly underestimated by sampling top samples from the fifth box in stacks of six (necessitated by a variable stack height throughout the store) and overall residue levels were low, sprout control was effective (mean 1.4 mm, SD 1.29).

In 2014/15, as in the previous season, the use of an air separator curtain, together with low-speed recirculation of fog through open pallet apertures resulted in good control of CIPC residue variability and effective sprout control. This approach, in a store for the fresh potato sector (store OSF1), resulted in a mean CIPC residue concentration of 3.1mg/kg, SD 1.14, CV% 36 and in the processing sector (store OSP2) a mean residue concentration of 0.8mg/kg, SD 0.61, CV% 72. In both stores, differences in residue concentration as a result of sample position in store, was limited. Results confirmed those of 2013/14 (store OSP1).

In 2015/16, the open suction system of active recirculation resulted in low CIPC residue variability in stores holding for the fresh market, with coefficients of variation between 46% (store OSF3) and 83% (store OSP2). Variability was lowest in store OSF3, which was fitted with a half-height air separator, but this does not necessarily indicate this type of separator to be more effective, as the main block dimensions in this store were different from elsewhere, so a direct comparison cannot be made. Sprout control efficacy was very good in both stores (mean maximum sprout length <1mm) but comparisons cannot be made as, in store OSF3, the crop was also treated with spearmint oil before unloading.

Active recirculation using open suction also resulted in a moderate level of CIPC residue variability in store OSP2 but residues were generally very low (overall mean 0.1mg/kg, SD 0.13) – perhaps due to the extended storage period (68 days' storage after final application) – and this was reflected in the efficacy results. With such low residues, the coefficient of variation was unsurprisingly higher than previously observed at 121%; variation had before been in the range 36%-92%.

The addition of a mesh restrictor in this store helped to give more even distribution of air but the 60% restriction was not adequate to remove the imbalance in flow entirely (data not shown). Further work is being undertaken to refine modifications to improve uniformity.

Nevertheless, open suction systems, created by the use of air separator curtains, gave very promising results across three years' trials, with improved uniformity of residues (CV% <100). The system also has the benefit of modest conversion costs, estimated at £3-£5 per tonne.

Positive ventilation using foam blocks

In 2013/14, the use of foam blocks, inserted in alternate pallet apertures in store PFB2 (in addition to an air separator and low-speed recirculation as used in store OSP1), resulted in slightly higher but acceptable residue variability (mean 3.2 mg/kg, SD 1.84, CV% 58). Residue values in the store were generally highest in top boxes, especially at the front of the store, closest to the point where CIPC was introduced. The maximum residue measured in PFB2 (7.7 mg/kg) was less than in the other low temperature stores (9.7 and 9.3 mg/kg respectively for PLF1 and MSF1) where the position of the application port also had a significant effect. Further work was required to assess whether the additional use of the foam blocks in any format is capable of offering a significant benefit over a simple air separator curtain in this type of store.

In 2014/15, inserting foam blocks in alternate pallet apertures, in addition to an air separator curtain and use of low speed recirculation (store PFB2), did not result in a satisfactory residue distribution, with samples in the range 0.2-11.1 mg/kg from a single application of CIPC at 14 g/tonne. Considerable variation in residue concentration was evident in relation to sample box height (bottom box mean 2.9 mg/kg (SD 1.86), middle box mean 4.4 mg/kg (SD 2.00) and a top box mean of 7.5 mg/kg (SD 2.23). A similar effect of box height, with top boxes receiving a disproportionately large proportion of the total dose, although, in 2014/15, a greater application efficiency was achieved overall (mean residue 4.9 mg/kg, SD 2.78, compared with 3.2 mg/kg, SD 1.84 in 2013/14, both from an application rate at 14 g/t). Consequently, some tubers had a residue value of 10 mg/kg or greater. The use of foam blocks, in this type of store (with an air separator curtain) was not beneficial. It is considered that the backpressure on airflow, posed by the blocks, was too great and fan pressure, under low speed conditions, was insufficient to overcome this resistance and full, active recirculation did not take place. Short-circuiting of air is also a factor as this limits the amount of air reaching the end of the store furthest from the fans; observations suggest this resulted in a very low, almost undetectable, airflow at the point where CIPC fog was introduced into the store. As a consequence, fog was not drawn directly into the block of boxes and, instead, tended to rise directly up into the store headspace increasing the risk of high levels of deposition on the top surface of the stack. The use of foam blocks to retrospectively create positive ventilation in these types of store was therefore discontinued after 2014/15.

Positive lateral flow ventilation

Store PLF1 was converted to lateral suction (positive) ventilation, using the Pirie *Aspire™* system, in 2013. Previous AHDB research (Project R414) had shown this type of store results

in effective application of CIPC fog, with low residue variability, using low-speed fans for recirculation (Briddon, 2013). In this trial, the lateral suction system again performed well with a mean residue of 3.0 mg/kg (SD 1.63). Residue variability was lowest (CV% 54) of the three low temperature stores in the trial. However, it was noted that there was a marked effect of the location of the CIPC port, with particularly high values recorded in samples close to the application point.

To counteract the localised effect of the position of the fogging port observed previously, for 2014/15 the fog was introduced more centrally and the back-fill block was also connected to the main block, so that these boxes were also subject to recirculation of air and fog. These additional modifications were deemed successful as more even control of sprouting was observed. The maximum CIPC residue value in this store reduced from 9.7 mg/kg in 2013/14 to 8.4 mg/kg in 2014/15 whilst overall residue values were similar in both seasons (2013/14 mean 3.0 mg/kg, SD1.63, CV% 54; 2014/15 mean 3.1 mg/kg, SD 1.71, CV% 56). The lateral suction ventilation principle was also assessed in 2014/15 in a processing store but, due to excessive bacterial soft-rotting, results were not considered representative and are not presented. Overall a very good, even distribution of CIPC was achieved using the lateral suction system.

Proposals for the adoption of slow speed *'active recirculation'* as a standard practice for the treatment of box potato stores with CIPC were put forward by the Potato Industry CIPC Stewardship Group in March 2017 (PICSG, 2017) and have been incorporated into label recommendations for the 2017/18 season, as the industry made its final move to a pan-European maximum dose rate of 36 g/t (reduced over 5 years from 63.75g/t). No MRL exceedances occurred since February 2014 (Defra, 2017).

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FACTORS AFFECTING THE POPULATION DYNAMICS AND EPIDEMIOLOGY OF VIRUSES INFECTING POTATO

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Summary: While virus incidence is low in Scottish seed crops, aphid-transmitted viruses, as opposed to fungi-, nematode- and contact-transmitted viruses, represent about 80% of virus cases in symptomatic plants. Amongst the aphid-transmitted viruses, potyviruses and in particular *Potato virus Y* (PVY) have become the most prevalent species. Understanding their population dynamics and epidemiology is essential to manage them efficiently. Characterisation of a range of PVY isolates representing the main strains and phylogenetic groups suggest that PVY can overcome the hypersensitive response (HR)-mediated resistance. Contrastingly, genes mediating extreme resistance (Ry_{adg}, Ry_{sto}) still provide effective resistance to PVY. Mature plant resistance trials might suggest a higher capacity of $PVY^{EU\text{-}NTN}$ to infect older plants in comparison to PVY^{O}. PVY infection of re-growth foliage after flailing did not result in significant tuber infection. Our results suggest a differential ability of $PVY^{EU\text{-}NTN}$ in out-competing other PVY strains and overcoming plant resistance mechanisms, potentially explaining their prevalence.

INTRODUCTION

Viruses of the genus Potyvirus such PVA and PVY are the most prevalent virus species in cultivated potatoes worldwide. They are spread by vegetative propagation of potato tubers and by a wide range of aphid species in a non-persistent manner, whereby aphids acquire viruses 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 is the most damaging virus species infecting potatoes worldwide and exists as a complex of strains or variants. The earliest characterisation 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^{N} and PVY^{O} or PVY^{C} serotypes. Molecular typing by genome sequencing of isolates of the PVY^{N} serotype 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 characterisation of PVY field isolates by assessing their serology, genome structure and pathogenicity. 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 prevalence. We investigated the ability of PVY strains to overcome endogenous plant resistance mechanisms.

MATERIALS AND METHODS

Characterisation of field isolates of potato-infecting viruses

Symptomatic leaves from field grown seed potato crops from various locations in Scotland were collected as part of the yearly survey for the seed potato certification scheme. The dataset spanned a period of 24 years from 1993 to 2017. Data are presented as relative proportion of virus cases, where a virus case is defined as a crop in which a virus species was detected by ELISA. PVY ELISA-positive samples (PVY^{N} and $PVY^{O/C}$) intercepted at crop inspection during the 2009-2016 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.*, 2017). The ability of PVY variants representing various strain or molecular groups of PVY to infect potato carrying different resistance genes was undertaken by monitoring virus titres in upper leaves of the plant as previously described (Davie *et al.*, 2017). Five potato cultivars and lines were used, each carrying different resistance genes to PVY: Pentland Crown (P. Crown) (Ny_{tbr}), Pentland Ivory (P. Ivory) (Ny_{tbr}-*Nc*-*Nz*), Tacna (Ry_{adg}), Sante and G8866 (Ry_{sto}). Cultivars were obtained from SASA (UK) with the exception of cvs Tacna and G8866 (obtained from The James Hutton Institute, Dundee, UK).

Mature plant resistance and re-growth infectivity trials

Potato cultivars with comparable level of susceptibility to PVY (cvs Estima, Maris Piper and vales Sovereign) were assessed for their ability to develop mature plant resistance in field trials over a 3-year period. Plants emerged by 4 weeks after planting and were mechanically inoculated at different times after emergence (weekly between 1 to 10 weeks) with infectious sap of either $PVY^{EU\text{-}NTN}$ 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 (Davie *et al*., 2017). 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. The role of new growth of foliage (following flailing) on PVY infectivity in tubers was assessed on glasshouse grown plants (cv Slaney). Six plants per treatment (flailed, unflailed) were infected at 1, 2 or 3 weeks post flailing (respectively 9, 10 or 11 weeks post planting-WPP) by $PVY^{EU\text{-}NTN}$ infectious sap. Tubers were harvested 3 weeks after infection and PVY infection monitored for each individual tuber as previously described (Davie *et al*., 2017).

RESULTS

Dynamics and population structure of PVY strains

The relative proportion of PVY serotypes in symptomatic leaves from seed potato crops has been monitored since 1993, as part of the statutory annual survey of virus incidence in support of the Scottish seed potato certification scheme (SASA, UK). From the data presented in Figure 1, two distinct periods could be discerned: the first period before 1997 when $PVY^{O/C}$ prevalence was generally higher or comparable to PVY^{N}, followed by a second period from 1997 onwards when the relative proportion of PVY^{N} was consistently and increasingly higher than that of $PVY^{O/C}$ over the period. PVY^{N} now represents more than 90% of PVY cases (Figure 1 lower panel). Data from a previous survey on the molecular nature of PVY^{N} isolates by sequencing of a recombinant junction of the PVY genome (Davie *et al*., 2017), 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 through 2016 confirmed this trend with PVY$^{EU\text{-}NTN}$ and PVY$^{NA\text{-}NTN}$ accounting for respectively 92% and 8% of the PVY^{N} population.

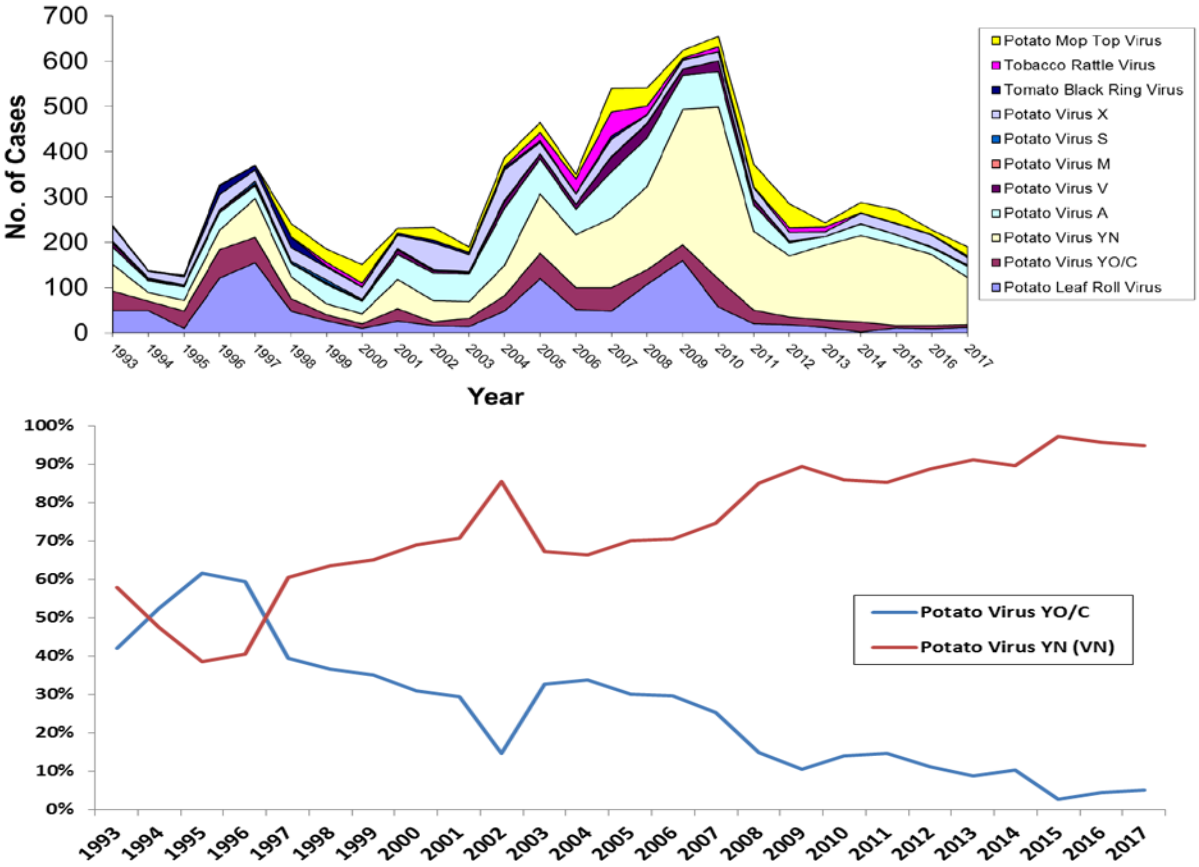

Figure 1. Upper Panel: Number of cases of virus-infecting potatoes species in symptomatic plants in the Scottish Seed Potato Certification Scheme (1993-2017). Lower panel: Nature and dynamic of PVY serotypes in the Scottish Seed Potato Certification Scheme (1993–2017). The relative proportion of PVY^{N} and PVY$^{O/C}$ strains ELISA-positive to PVY$^{O/C}$ and PVY^{N} antibodies for each year is presented.

Biological typing of PVY$^{O/C}$ isolates indicates that the majority belong to the PVY$^{N\text{-}Wilga}$ biotype (69% of PVY$^{O/C}$ cases in 2013) as opposed to PVY^{O} or PVY^{C} strains (31% of PVY$^{O/C}$ cases) (data not shown).

PVY variants overcome resistance mediated by *Ny$_{tbr}$*, *Nc* and *Nz* genes but do not break extreme resistance mediated by *Ry$_{adg}$* or *Ry$_{sto}$* genes.

Accumulation levels for a range of PVY strains were monitored by real-time RT-PCR in upper-non-inoculated leaves as an indicator of systemic movement and resistance-breaking trait (Figure 2). The highest accumulation levels for all PVY isolates tested were observed on cv Pentland Crown (*Ny$_{tbr}$*) with the exception of PVY^{EU-NTN} isolates 10766 and 10088 which accumulated to comparable levels in cvs Pentland Crown and Pentland Ivory. All other PVY isolates representing strain groups O, N-Wilga, and NTN (molecular subgroup NA-NTN) accumulated to a significantly lower level in Pentland Ivory (*Ny$_{tbr}$-Nc-Nz*) than in Pentland Crown (*Ny$_{tbr}$*). The PVY^{EU-NTN} isolate DV76 accumulation levels were significantly lower in P.

Ivory as opposed to the two others PVY^{EU-NTN} isolates previously mentioned, suggesting that *in planta* accumulation can differ significantly between isolates belonging to the same molecular subgroup clade. All PVY isolates tested could not be detected in upper non-inoculated leaves of cvs Tacna, Sante and G8866 harbouring Ry_{adg} and Ry_{sto} genes, suggesting that PVY does not accumulate or accumulate to very low levels below the limit of detection in cultivars harbouring *Ry* genes. Tuber transmission of PVY was assessed for most of the combinations of cvs and PVY isolates tested. None of the PVY isolates tested were detected in progeny tubers of cvs Tacna (Ry_{adg}) G8866 and Sante (Ry_{sto}), while PVY could be detected in cv P. Crown and P. Ivory.

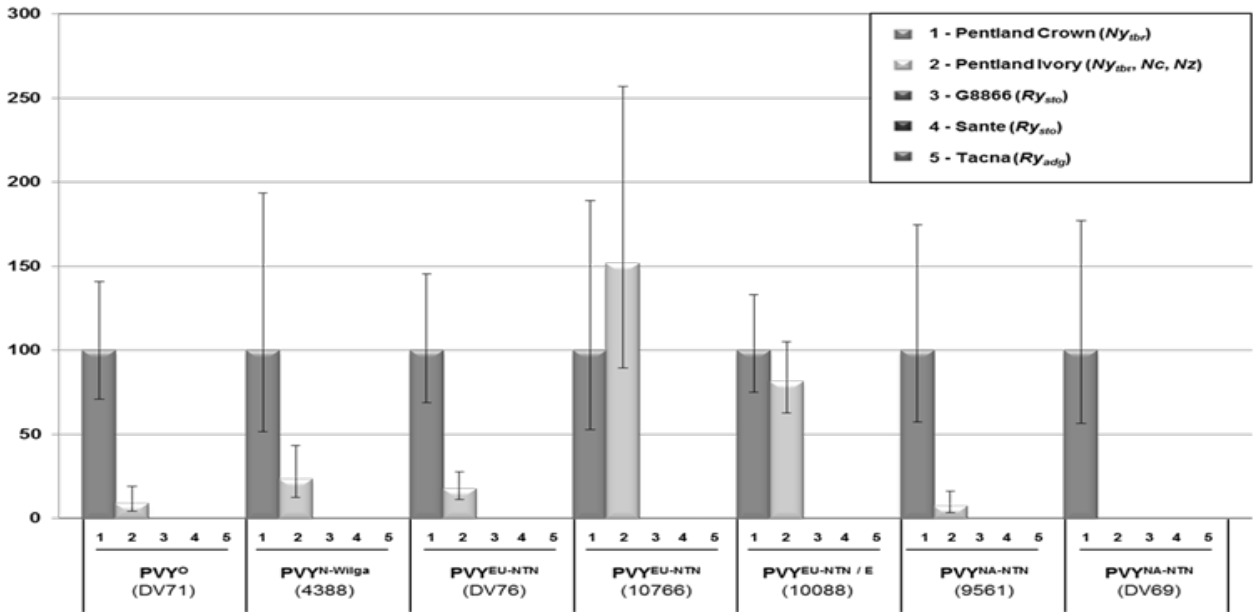

Figure 2. Relative PVY accumulation levels in potato cvs harbouring different resistance genes. PVY accumulation levels for each virus isolate tested on each of the five potato cvs are expressed as percentage of relative expression levels to Pentland Crown (Mean ± SD).

Impact of mature plant resistance and of foliage re-growth on PVY infection

We further assessed the effect of mature plant resistance (MPR) on the ability of PVY^{EU-NTN} and PVY^{O} isolates to infect potato plants at different developmental stages (Figure 4). MPR is a broad spectrum resistance mechanism where potato plants inoculated late in their development display increased resistance to PVY infection (Gibson, 1991). PVY^{O} and PVY^{EU-NTN} incidence was comparable while declining for both PVY^{O} and PVY^{EU-NTN} in plants inoculated during the 7 weeks post-emergence. While a comparable incidence in tuber progeny was found between 1 to 8 weeks post-emergence for both PVY isolates, only PVY^{EU-NTN} was found to successfully colonize their potato host at 9 weeks post-emergence (Figure 4). PVY^{EU-NTN} infection to progeny tubers was detected when plants were infected up to 9 weeks post emergence.

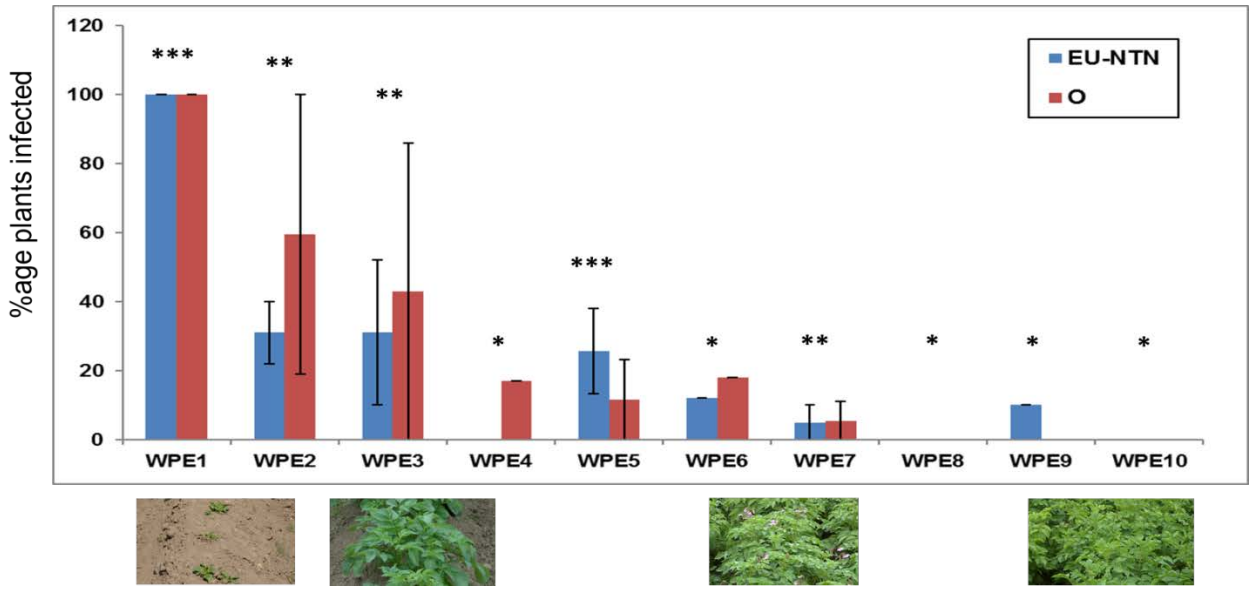

Figure 3. Relative accumulation levels of PVY isolates in infected potato cvs at different inoculation periods after emergence (Week Post Emergence – WPE). Data are expressed as percentage of relative expression levels to the earliest time of infection (WPE1) (Mean ± SD). The trial was run over a three-year period with for each inoculation period replicated over one (*), two (**) or three (***) years.

Further, the infectivity of PVY on newly grown leaves (re-growth) following flailing was investigated, as newly grown leaves at their most susceptible state could present a potential means of virus infection. Re-growth foliage from flailed plants and unflailed potato plants were inoculated with PVY^{EU-NTN} and the incidence of infected plants was assessed through post-harvest testing of tubers. 75% of plants infected at 3 weeks post planting had infected tubers, while 32% of unflailed plants infected at a later stage (9 to 11 WPP) had infected tubers. Contrastingly, only 2% of flailed plants in which re-growth foliage was inoculated were infected by PVY (Figure 4).

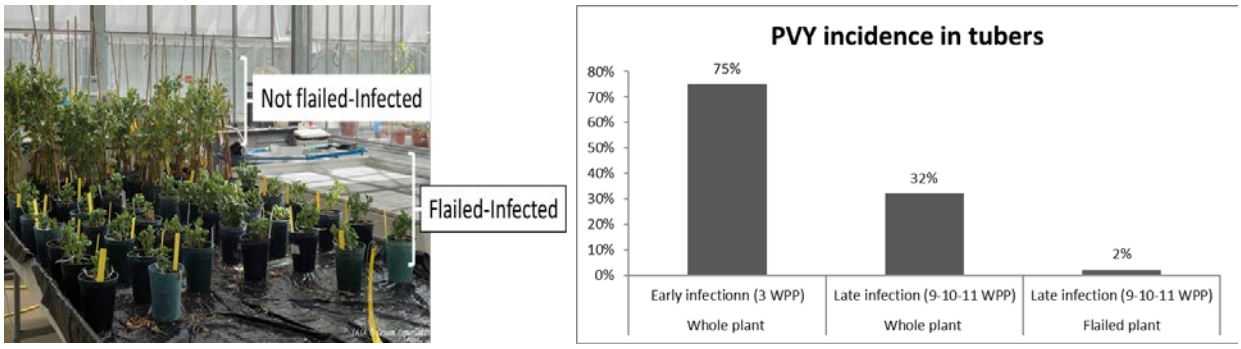

Figure 4. Incidence of PVY^{EU-NTN} in tubers from plants infected at different time after flailing (1-2-3 weeks post flailing respectively 9-10-11 weeks post planting-WPP). The incidence of PVY in infected unflailed control plants is presented, together with the incidence of PVY in tubers for plants infected at an earlier development stage (3 weeks post planting-WPP)

DISCUSSION

The survey of virus species in symptomatic leaves over a 24-year period revealed that aphid-transmitted viruses are the most prevalent species. Our results indicate that the prevalence of PVY serotype groups shifted towards the PVY^{N} serotype group 20 years ago and this has remained the most abundant serotype. Within the PVY^{N} serotype, isolates of the phylogenetic group EU-NTN are the most prevalent group as opposed to NA-NTN and EU-N groups; a trend observed worldwide as recombinant PVY^{NTN} strains and related PVY variants are the most prevalent in potato-growing areas and are displacing non-recombinant PVY^{O} and PVY^{N} strains. In contrast to other potato-growing areas (such as EU, North America) (Gray *et al*., 2010; Rigotti *et al*., 2011), isolates of the PVY^{N-Wi} strain, while accounting for the majority of $PVY^{O/C}$ cases, are not prevalent in our environmental conditions (Davie *et al.*, 2017).

The aim of our study was to examine the parameters that are driving PVY variant prevalence in Scottish seed crops. Our results suggests that while *Ry* genes provides a strong resistance status against all PVY strains tested, *N* resistance genes (Ny_{tbr} in this case study) does not provide resistance for all PVY strains/variants tested even for the field isolate of the PVY^{O} strain group. In addition, the ability of PVY^{EU-NTN} to infect potato plants at a relatively late developmental stage (*i.e.* up to 9 weeks post emergence) might suggest that PVY^{EU-NTN} could counteract mature plant resistance mechanisms more efficiently than PVY^{O}. Our data suggests that infection of newly grown foliage after flailing might not result in a higher PVY incidence in tubers in comparison to unflailed plants. The dynamics of PVY populations are likely to be dependent on complex interactions between PVY with its aphid vectors, the plant, environmental conditions, its ability to overcome host resistance mechanisms, and as observed for PVY^{EU-NTN}, in out-competing others PVY variants (Davie *et al,* 2017). This illustrates the complex nature of PVY dynamics and helps to explain the prevalence of PVY^{EU-NTN} over other PVY variants in Scottish field conditions.

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THE IMPLICATIONS OF "*CANDIDATUS* LIBERIBACTER SOLANACEARUM" IN *TRIOZA* ANTHRISCI FOR THE UNITED KINGDOM

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Summary: "*Candidatus* Liberibacter solanacearum" (Lso) is a psyllid-vectored pathogen that affects solanaceous and apiaceous crops. In the Americas and New Zealand, Lso is transmitted to potato by the psyllid *Bactericera cockerelli* which causes Zebra Chip disease in potato. Although *B. cockerelli* is absent from the EPPO region, Lso has been found in Europe where it is transmitted mainly to apiaceous crops by native European psyllid vectors. In 2017, Lso was reported for the first time in the United Kingdom. Lso was found in five specimens of *Trioza anthrisci* collected over a two year period (2015-16) in the northeast of Scotland. *Trioza anthrisci* has not previously been reported as a vector or carrier of Lso, although is closely related to *T. apicalis,* the main vector of Lso in carrots in northern Europe. We discuss the implications of this finding for the UK agricultural industry.

INTRODUCTION

The α-proteobacterium *"Candidatus* Liberibacter solanacearum" (Lso) is well-known as the causal agent of Zebra Chip in potato (Secor *et al*., 2009). Lso colonises the vascular system and infection in potato is characterised by necrosis of this vascular tissue. Infected plants usually die and any remaining potatoes produce dark stripes when fried rendering them unmarketable. Seed potatoes are also affected as infected tubers either cease to sprout at all or produce weak plants. Typical above ground symptoms include leaf curling and discoloration (Munyaneza *et al.*, 2007).

Lso is transmitted by phloem-feeding insects called psyllids, also known as jumping plant lice. There are currently three known psyllid species that transmit Lso; *Bactericera cockerelli*, *Bactericera trigonica* and *Trioza apicalis*. However, this list may increase as other psyllid species have been found to carry Lso but have yet to be tested in transmission studies (e.g. *Bactericera nigricornis* and *Trioza anthrisci*), whilst *Bactericera tremblayi* has been confirmed as a carrier but an inefficient vector between potato and carrot (Antolinez *et al.*, 2017).

There are distinct geographic distributional patterns seen in this pathosystem with spatial correlations seen between vector, plant host, and haplotype of Lso. Lso in potato and Zebra Chip disease has been reported in the Americas and New Zealand where it is associated with *B. cockerelli,* the tomato potato psyllid, and with two haplotypes of Lso - A and B (America only). This vector and both haplotypes are not present in Europe and are regulated as A1 quarantine pests in the EPPO region. In Eurasia and Africa, haplotypes C, D, and E are associated with disease in carrot, with E also infecting other apiaceous crops including celery, chervil, parsley, fennel, and parsnip (Munyaneza *et al,.* 2010; Nelson *et al.,* 2013; Teresani 2014; Munyaneza *et al,.* 2015; Tahzima 2016). Symptoms in carrot include leaf curling, shortening of taproot, and proliferation of secondary roots (Bertolini et al. 2014). Haplotype D & E have been associated with *B. trigonica*, with D reported in France, Spain, Greece, Morocco, and Israel, and E in Spain and Morocco (Nelson *et al.,* 2013; Teresani *et al.,* 2014;

Tahzima *et al.,* 2016; Holeva *et al.*, 2017). Lso haplotype C has been reported in growing crops in Finland, Sweden, Norway, and Germany and is associated with *T. apicalis,* the carrot psyllid (Nissinen *et al.,* 2014; Munyaneza *et al.,* 2015).

LSO IN THE UNITED KINGDOM

Lso has been reported in parsley seed sold in local shops in 2016 (Monger & Jeffries *et al*., 2016), although it has not been found in growing plants in the UK. Lso has since been found in historical collections of Apiaceae seed from the UK and several other countries worldwide (Monger & Jeffries *et al*., 2017). Whether this implied Lso was also in growing plants in the UK remains unknown as seed transmission studies, based on carrot, have shown conflicting results (Bertolini *et al.*, 2015; Loiseau *et al.*, 2017).

A finding of Lso in a psyllid in the UK was the first indication that the bacterium could be present in growing crops in the UK. Five specimens of *T. anthrisci* collected over a two year period (2015-16) from a single location in the north east of Scotland were found to be positive for Lso (Sjölund *et al.,* 2017). The psyllids were infected with Lso haplotype C which is commonly found in northern Europe.

Since psyllids require host plants to complete their lifecycle, this finding strongly suggests that Lso is present in growing plants in the UK. Although Lso has not been found in growing plants, it should be noted that there have not been any large-scale surveys for Lso. A small-scale survey on symptomatic carrots in the UK found no cases of Lso during 2012-2013 (Pearson *et al.,* 2014). The Lso positive *T. anthrisci* specimens were caught in a suction trap and therefore no information on plant hosts were available for the specimens. Psyllids require specific plants to complete their lifecycle (i.e. host plant), to feed on as adults (i.e. food plant), to overwinter (i.e. shelter plant), or land on without feeding (i.e. casual plant) (Burckhardt *et al.,* 2014). Food plants of *T. anthrisci* include apiaceous species, *Anthriscus sylvestris* (cow parsley) *Angelica sylvestris* (wild angelica), *Heracleum sphondylium* (hogweed), and *Chaerophyllum hirsutum* (hairy chervil) (Hodkinson 2009; Ouvrard 2017). Overwintering species include conifer species (Hodkinson 2009). It should be noted this was the first time that *T. anthrisci* has been implicated in the vectoring of Lso. Therefore, there has yet to be transmission studies to confirm its ability to vector the bacterium and to confirm which plants it is a suitable vector for.

IMPLICATIONS FOR PLANT HEALTH

Although the UK is likely to have Lso in growing plants, we do not have high numbers of the only vector that has been confirmed present in the UK, *T. apicalis*. Lso may be present in apiaceous weed hosts. If *T. anthrisci* were a competent vector for Lso, there could be low level transmission to other apiaceous crops if adventitious feeding/probing on related plant species were to occur. However, transmission is likely to remain at a low level as *T. anthrisci* does not thrive on apiaceous crop species and the only vector that has the ability to increase the spread of the pathogen, *T. apicalis*, is not a significant pest species in the UK as it occurs at low numbers, having only been recorded four times (NBN Atlas 2017; Sjölund *et al.,* 2017). In contrast, *T. apicalis* has been a significant pest on carrots in neighbouring Scandinavian countries for years, and has been recorded as early as 1896 in Denmark (Láska 2011), long before the first report of Lso (Hansen *et al.,* 2008). The reason for this disparity remains unknown. It may be due to the differences in plant communities between the UK and Scandinavia, especially in the density of overwinter conifer host species, or genetic differences between the UK and Scandinavian pest populations. Due to these differences, the current presence of Lso in growing plants poses little threat to the UK agricultural industry. However, if *T. apicalis* were to experience a population explosion, we may find that Lso may

become an issue, especially in apiaceous crops. This highlights the need for research into the factors which regulate *T. apicalis* population dynamics and the potential effects of land-use or climate change on their populations.

The global geographic distribution of Lso haplotypes and vectors reveals a complex pathosystem. There appears to be an association between haplotypes A/B and solanaceous plants, and C/D/E and apiaceous plants, although whether this association is a result of the physiological difference between haplotypes, a reflection of the plant host of the local vector species, or both, has yet to be distinguished. It is likely that the vector plant host preference is a significant factor shaping the associations we see globally. Further research is required on the transmission of C/D/E by efficient vectors in solanaceous crops, such as *B. cockerelli*. This would enable us to assess the risk of *B. cockerelli* introduction in the UK, with the knowledge that Lso haplotype C is probably present in certain plant species, albeit non-crop species. In 2016, haplotype E was found in two potato stores in Spain (MAPAMA 2017). Recent studies suggest that *B. trigonica* can transmit Lso to potato and tomato. However, transmission levels are low as potato and tomato are host species for *B. trigonica* (Antolinez *et al.,* 2017; Teresani *et al.,* 2017). Similar low transmission rates have been obtained for haplotype B from potato to carrot (Munyaneza *et al.,* 2016).

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INFLUENCE OF FOLIAR STABILISED NITROGEN ON POTATO TUBER YIELD

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Summary: This study demonstrates that form and stability of foliar applied nitrogen has an influence on yield. Foliar applications of stabilised NH_2 gave significant yield increases of 4.7%, on potato cv. Sassy, when tested on a farm in Yorkshire, northern UK. Conventional foliar nitrogen treatments had no significant effect on marketable yield. The positive effects of the foliar NH_2 treatment used in this study reflect similar yield improvements to those that have been reported in efficacy trials in France, Ireland and the Netherlands (Headland Agrochemicals Ltd., personal communication).

INTRODUCTION

The form of nitrogen taken up by plants has an influence not only on total growth, but also on resource partitioning. This has been suggested to be linked to the findings that exposure to different forms of nitrogen (nitrate, amine and ammonium) has differing effects on the rate of production, transport and relative abundance of the growth hormones cytokinin and auxin (Kiba *et al*., 2011). Nitrate promotes or stimulates leafy growth and apical dominance rather than lateral root production (Chamizo-Ampudia, 2017).

Nevertheless, little work has been done previously to establish the effect of form of N on crop production. From a practical perspective this is not easy as conventional nitrogen fertiliser is not environmentally stable, with typical losses through processes such as surface runoff, leaching and formation of volatiles being over 65% of that applied (Raun and Johnson, 1999). Furthermore, nitrogen is largely taken up as nitrate regardless of the form in which it is supplied.

When growing potatoes commercially, nitrogen uptake and use efficiency is dependent on how, when and where it is applied. For example, late applications of foliar N can be counterproductive as they have a tendency to produce leaves rather than tubers. Potato varieties vary considerably in their ability to take up applied nitrogen and convert it into tuber growth (nutrient use efficiency), and all varieties fail to take up significant amounts of the nitrogen that is applied to them (Zebarth *et a*l., 2003). Previous research has given rise to the much improved efficiency with which potato crops use nitrogen in the 21st century, however N form is still a relatively overlooked avenue of exploration.

It has been demonstrated in other crops that exposure to small bursts of NH_2 can have an effect on plant architecture disproportionate to the quantity applied (Bergmann and Eckert 1990). This study looks at field results from Yorkshire (UK) using foliar applications of Lono (a stabilised NH_2 formulation from Levity Crop Science) and the effects on yield of potato. The study compares this with the effects of two non-stabilised foliar nitrogen formulations. Also discussed are data from studies in France, Ireland and the Netherlands using foliar applications of the stabilised NH_2 formulation on different varieties.

MATERIALS AND METHODS

Yorkshire Efficacy Trial

This trial was carried out in 2016 in the North of England at Stamford Bridge, East Yorkshire, UK. The study was a randomised block design with 4-10 replicates of each of four treatments, using the indeterminate potato variety Sassy. Standard soil fertilisation at the site was 200 kg/ha nitrogen. The control treatment contained no additional foliar nitrogen (treatment 1, 4 replicates). Treatments 2-4 consisted of supplemental N applied to the developing crop as liquid foliar nitrogen treatments. The second and third treatments used two standard, commercially available liquid foliar nitrogen treatments (standard A and standard B, 4 replicates), and treatment 4 consisted of a liquid foliar stabilised NH_2 treatment (10 replicates):

Standard A (treatment 2) contained 340 g/l N (37 g/l NO_3, 140 g/l NH_4 and 163 g/l NH_2), applied in 3 litres/ha.

Standard B (treatment 3) contained 330 g/l N (49 g/l NO_3, 67 g/l NH_4 and 214 g/l NH_2), applied in 3.3 litres/ha.

Stabilised N (treatment 4) contained 195 g/l N (65 g/l NO_3 and 130 g/l stabilised NH_2), applied in 5 litres/ha

Knapsack-applications of foliar N treatments were supplied to the 22 plots as detailed in Tables 1 and 2.

Table 4. Foliar Nitrogen Application Rates

Nitrogen Treatment Designation	**Application 1 (17.06.16)**	**Application 2 (07.07.16)**	**Application 3 (21.07.16)**	**Application 4 (04.08.16)**
1	Untreated control	Untreated control	Untreated control	Untreated control
2	Standard A 3 litres/ha (1.02 kg N)	Standard A 3 litres/ha (1.02 kg N)	Standard A 3 litres/ha (1.02 kg N)	Standard A 3 litres/ha (1.02 kg N)
3	Standard B 3.3 litres/ha (1.09 kg N)	Standard B 3.3 litres/ha (1.09 kg N)	Standard B 3.3 litres/ha (1.09 kg N)	Standard B 3.3 litres/ha (1.09 kg N)
4	Stabilised N 5 litres/ha (0.98 kg N)	Stabilised N 5 litres/ha (0.98 kg N)	Stabilised N 5 litres/ha (0.98 kg N)	Untreated

Table 5. Plant Growth Stage* Schedule and Soil Moisture and Compaction Status for the Four Foliar Nitrogen Application Occasions

	Application 1 (17.06.16)	**Application 2 (07.07.16)**	**Application 3 (21.07.16)**	**Application 4 (04.08.16)**
Growth Stage*	40	61	69	74
Soil Status	Moist, settled	Moist, settled	Slightly dry, settled	Moist, settled
Crop Phenology and Height (cm)	Main stem elongation, tuber initiation; 38-44	Flowering (main stem); 70-75	Flowering (2nd inflorescence); 70-80	Fructification; 75-80

*according to BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie).

RESULTS

The crop was harvested on the 31st of October 2016, and all tubers (inclusive of green or cracked) from each plot were weighed (to achieve a total yield per plot – see Table 3) and graded for size. After discarding the damaged tubers and those under 45mm diameter, the weight of the marketable yield was measured. Each treatment was compared to the others using a simple *t*-test, and this showed that only treatment 4 (stabilised N) had a marketable yield that was significantly larger than that of the control ($p<0.1$). When unmarketable tubers were also included in the analysis, treatment 4 still had a significantly greater total yield than controls ($p<0.1$), and, additionally, so did treatment 3 (standard B).

Foliar application of stabilised NH_2 as used in this study, but not that of the two standard foliar N fertilisers, had a significant effect on marketable yield, with a 1.713 T/ha (4.7%) yield increase compared to the control.

Table 3. Yield data at harvest (31.10.16). *Significance levels ($p<0.1$) are denoted as x^{a-b}, and differing letters pertain to significant differences between treatments (within columns).

Treatment	Total Yield T/ha	Total Yield (% of control)	Marketable Yield T/ha	Marketable Yield (% of control)
1	42.104^{a*}	(100)	36.104^{a}	(100)
2	43.132^{ab}	102.44	35.444^{a}	98.17
3	45.472^{b}	108	38.146^{ab}	105.65
4	45.001^{b}	106.9	37.817^{b}	104.74

DISCUSSION

This study shows that form and stability of applied foliar nitrogen influences its efficacy as a nutritional fertiliser for the production of potato tubers. Three formulations were used, which supply similar levels of nitrogen to the plants (in fact the stabilised NH_2 supplied at least 110 g/ha less N to the crop, furthermore this was only applied on 3 rather than 4 occasions). On this occasion two conventional formulations had no significant effect on marketable yield. By contrast, the stabilised NH_2 formulation significantly increased marketable yield in this field study by 1.713 T/ha (4.7%).

It has not previously been demonstrated that stabilised NH_2 can increase the tuber yield of crop plants, although grain formation and basal internode growth in pot-grown rye and barley has been shown to increase when monoethanolamine was applied to the foliage of the plants (Bergmann and Eckert 1990, Bergmann *et al*. 1991).

Given that the three kilos of nitrogen applied in the study is less than the nitrogen content of the 1.7 ton extra yield achieved per hectare using the stabilised NH_2 treatment, it can be concluded that this growth increase is not due to higher access to nitrogen alone. A possible explanation for this is that exposure to foliar stabilised NH_2 has an effect on growth partitioning of potato crops, with the treated plants disproportionately increasing tuber growth and development. It has long been known that N nutrition affects plant growth hormone levels and transport in potato (Sattelmacher and Marschner 1978). One hypothesis is that stabilised N affects plant hormone synthesis and/or transport in a manner that is preferential for tuber formation as opposed to vegetative top growth (Ewing 1995). In contrast, Bergmann and Eckert (1990) found that nitrogen levels were preferentially increased in the basal internodes of cereal tillers under monoethanolamine nutrition, and proposed that this was the reason for the increased growth in this region.

The trial data described here, originating from a farm in Northern Britain, is consistent with data from other similar trials across Europe. Tuber yield increases were also seen in stabilised NH_2 treated plots on farms in France, the Netherlands and Ireland. These findings are summarised in Table 4 (from Headland AgroChemicals Ltd., personal communication). Thus

we can conclude that stabilised NH_2 application is a novel method for increasing potato harvests from our fields through improvements in crop nitrogen use efficiency.

Table 4. Supplementary data summary of European field trial results using the stabilised NH_2 formulation in addition to standard farm fertiliser regimes.

Country	Variety	Number of 5 litres/ha applications	Marketable Yield Increase T/ha	% Response compared to control plots
Ireland Co Meath 2016	Rooster	3	5.35	16.8
France Brittany 2017	Annabelle	4	5.88*	13.64
Netherlands N Friesland 2016	Innovator	5	6.10*	16.4

*p<0.1 (where the yield increase was calculated via analysis of variance and Fishers LSD to be significantly higher than that in the control plots treated under farmer standard conditions [level of N dependent on variety requirement and season length]).

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GETTING DOWN TO THE PLANT LEVEL: POTATO TRIALS ANALYSIS USING A UAV EQUIPPED WITH UN-MODIFIED AND MODIFIED COMMERCIAL OFF-THE-SHELF DIGITAL CAMERAS

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Summary: In recent years unmanned aerial vehicles (UAV) have become much easier to obtain and operate, and due to the aerial viewpoint they provide can be useful tools to enhance agricultural research. This paper investigates the use of UAVs equipped with commercial off the shelf (COTS) digital cameras, to determine if aerial imagery can give detailed information at the plot and plant level that is comparable to traditional ground based methods. A potato (*Solanum tuberosum L.*) trial was monitored over the 2016 growing season, with the performance of the trial being recorded using traditional ground based methods, and from the air using automatic analysis methods. The results indicate that very early emergence cannot be detected from the air, but that later counts of individual plants show correlation between methods. In contrast, ground cover estimates between methods correlate strongly and may prove to be a more robust monitoring method.

INTRODUCTION

Unmanned aerial vehicles are increasingly being used to monitor a large variety of crops (Zhang & Kovacs, 2012) including potatoes, where they have been used to assess damage (Zhou *et al.* 2016) and disease (Suigiura *et al.* 2016). Recently some studies have targeted emergence and establishment of both field crops (Gnädinger & Schmidhalter, 2017) and row crops (Sankaran *et al.*, 2017), indicating that it may be possible to monitor at the plant rather than plot level. For commercial growers of potato seed and ware crops, being able to accurately identify when plants have emerged and track their individual growth throughout their lifecycle could lead to more informed yield prediction, as well allowing identification of the source and spread of disease (Gibson-Poole *et al.*, 2017). For potato trials, being able to add more detail to existing recording methods will likely enhance the quality and efficiency of monitoring and may reveal new measures that could be used to make predictions. The aim of this paper is to investigate methods of collecting and analysing aerial data to enable the condition of individual plants to be monitored throughout their growing cycle, and compare these results with existing ground based methods.

MATERIALS AND METHODS

The trial plots used for this experiment were located to the east of Dundee, Scotland and were part of a series of trials investigating the effects of different treatments in a field system containing a high egg load of potato cyst nematodes. As this paper is investigating the differences between ground based and aerial based observations, the actual treatments and differences in their effectiveness are not directly reported on. The trial was composed of 48 plots, containing two beds (four rows in total, the outside two being guard rows) with 21 tubers

per row. All of the plots were planted on the 11/05/2016 and split into two varieties, 24 of Harmony and 24 of Maris Piper. Tubers were planted using a customised planter with an expected spacing of 25 cm and a drill width of 0.865 m.

Manual methodology

Two sets of manual data were acquired by an experienced observer to record potato development. Emergence counts were conducted at 19, 23, 30, 33 and 37 days after planting (DAP), with emerged plants being estimated by grouping closely located emerged shoots. Only the central two rows of each plot were counted (guard rows were ignored), added together and if equalling 21 or higher, then the 50% emergence DAP would be set for that plot. Ground cover assessments were conducted at 54, 61 and 89 DAP with percentage of potato leaf ground cover being estimated using a hand-held grid of 100 equal sized squares to view the central two rows (aligned to the trough-centres on outside of the rows), whilst ignoring the row-end plants.

Aerial methodology

Aerial data was acquired using two different aircraft and two different sets of sensors, with data acquired at 16, 22, 27, 33, 41, 46, 54, 61, 69 and 79 DAP. Nine sets of data were collected using a custom multi multi-rotor UAV (UAV1) equipped with a dual camera system capturing raw imagery, with one un-modified camera acquiring true colour imagery (RGB) and one modified to detect near infra-red (NIR) wavelengths (Gibson-Poole *et al.* 2017). The data acquired at 54 DAP was collected using a 3D Robotics Solo (3D Robotics, Berkeley, CA, USA) quadcopter UAV (UAV2) equipped with a single Canon ELPH 115 IS (Canon, Tokyo, Japan) capturing RGB imagery in JPEG (joint photographic experts group) format. Both UAVs used pre-programmed automatic flights at 35 m above ground level to capture imagery at ~1 cm per pixel ground sample distance, with an expected image overlap of 60% and side overlap of 87% for UAV1 but only ~60% total overlap for UAV2 as its camera was set to take a picture every 2 seconds. All datasets were captured with a photographic grey card placed within the scene surveyed to aid in image normalisation and 11 ground control points (GCP) to aid in georectification. The GCPs were surveyed using a Piksi (Swift Navigation, San Francisco, USA) real-time kinematic global navigation satellite system (GNSS), with an expected accuracy of ±13 cm.

Image Processing
The raw images from UAV1 were geotagged and processed into 16 bit linear TIFFs (tagged image file format) using the same method as Gibson-Poole *et al.* (2017), whereas the images from UAV2 were simply geotagged with no extra processing. All of the datasets were then processed using Agisoft Photoscan (v1.2.5; Agisoft LLC, St. Petersburg, Russia), using high settings with mild depth filtering to produce a georeferenced orthomosaic for each dataset (RGB and NIR) plus a digital surface model (DSM). The method indicated by Troscianko & Stevens (2015) was used to normalise the RGB and NIR orthomsaics, by using the average pixel values of the photographic grey cards placed within the scene of each survey.

Emergence analysis
Two automatic methods were employed, with the first (AUTO1) following that of Gibson-Poole *et al.* (2017), and the second (AUTO2) being a modification to make it more robust with regards to the spacing of tubers within each row and the high level of weeds present. Both methods required manual thresholding of the data in order to separate soil from vegetation for each survey date. This was achieved by using the normalised difference vegetation index (NDVI; Rouse *et al.* 1974), however as UAV2 was not equipped to capture NIR data the vegetation threshold was manually set by using the excess green minus excess red index in a similar manner to Meyer & Neto (2008). Five plots from each variety were also randomly

selected for direct visual analysis (VIS) of the aerial imagery, with the same field observer stepping through each survey date counting what they believed were emerged plants per date (they could look backwards but not forwards in time from the date they were currently assessing).

Ground cover analysis

The processed data for each survey date was classified using the object based image analysis (OBIA) software eCognition Developer (v9.2.1, Trimble, Munich, Germany) into five classes, potato, potato flowers, weeds, soil and shadow following a similar method to Gibson-Poole *et al.* (2017) but with modifications using fuzzy logic to separate vegetation into weeds or potatoes.

RESULTS

Statistical analysis was carried out using Microsoft Excel (Microsoft, Redmond, WA, USA) to calculate the Pearson correlation coefficients (*r*) and probability values (*p*).

Emergence

The manual data revealed that all Maris Piper plots had reached 50% emergence by 23 DAP and all Harmony plots by 30 DAP. AUTO1 and AUTO2 reached the same level by 27 DAP and 41 DAP respectively, indicating that the automatic methods may not be as sensitive as the manual method in detecting emergence. In general, the manual method detects more emerged plants earlier than any of the other methods. Both ground and aerial surveys were conducted at 33 DAP so direct comparisons could be made (Table 1). For the Maris Piper plots, the AUTO1 and AUTO2 methods showed a significant moderate correlation whilst the VIS method showed a significant strong correlation with the manually acquired data. However, for all methods, no significant correlation was achieved for the later emerging Harmony plots. The automatic and visual methods indicated that all plants had emerged by 54 DAP, however final plant counts were not recorded manually so this could not be directly compared.

Table 1. Correlation analysis between manual emerged plant counts and the three analysis methods at 33 DAP (*r* correlation coefficient, *i* intercept, *s* slope, *p* probability, *n* number of pairs, *Not significant at α = 0.05).

Method	Variety	*r*	*s*	*i*	*n*	*p*
AUTO1	Maris Piper	0.43	0.18 ± 0.08	34.86 ± 3.02	24	0.0373
	Harmony	0.29*	0.09 ± 0.07	37.67 ± 1.56	24	0.1673
	Combined	0.52	0.11 ± 0.02	37.31 ± 0.85	48	0.0002
AUTO2	Maris Piper	0.47	0.27 ± 0.11	30.72 ± 4.38	24	0.0215
	Harmony	0.29*	0.09 ± 0.06	37.70 ± 1.53	24	0.1621
	Combined	0.52	0.10 ± 0.02	37.41 ± 0.83	48	0.0002
VIS	Maris Piper	0.94	0.63 ± 0.13	16.11 ± 5.16	5	0.0156
	Harmony	0.07*	-0.04 ± 0.33	39.31 ± 11.37	5	0.9147
	Combined	0.50*	0.29 ± 0.18	28.95 ± 6.80	10	0.1436

Ground cover

Direct comparison of potato leaf ground cover could be made for 54 and 61 DAP, with the manual method reporting a larger percentage of potato leaf ground cover in general however both dates showed a strong positive correlation for both varieties (Table 2) that were also highly significant.

Table 2. Correlation analysis results between the manual and automatic analysis of potato leaf ground cover (*r* correlation coefficient, *i* intercept, *s* slope, *p* probability, *n* number of pairs.

DAP	Variety	*r*	*i*	*s*	*n*	*p*
54	Maris Piper	0.81	12.64 ± 7.09	0.99 ± 0.15	24	< 0.0001
	Harmony	0.75	12.59 ± 7.39	1.32 ± 0.24	24	< 0.0001
	Combined	0.73	23.71 ± 4.44	0.82 ± 0.11	48	< 0.0001
61	Maris Piper	0.82	14.09 ± 9.12	0.90 ± 0.14	24	< 0.0001
	Harmony	0.66	27.15 ± 7.60	0.69 ± 0.16	24	0.0004
	Combined	0.80	22.60 ± 4.90	0.78 ± 0.09	48	< 0.0001

DISCUSSION

From the emergence results it is clear that the resolution of the aerial imagery is not sufficient to be able to detect emerging shoots until they had started to develop some leaves (i.e. a leaf area > 1 cm^2), hence why the Maris Piper plots showed significant correlation compared to the Harmony plots, as the Maris Piper emerged earlier and were therefore large enough to be detected by the automatic methods. As the 50% emergence measure can be used to allow prediction of tuber initiation (O'Brien *et al.*, 1998), being able to detect this from the air would be advantageous, so increasing resolution by flying lower or using a different sensor could help solve this, although this could lead to increased flight times and the time taken to post process the imagery produced.

The resolution should have been sufficient to get accurate plant counts but was hampered by irregular tuber spacing due to tubers rolling during the planting operation, resulting in a reduction of plants counted due to the closer proximity of emerging shoots and early merging of canopies, which in turn lead to irregular sized plant growth spaces (Figure 1d). AUTO1 consistently produced lower plant counts as it was not robust enough to handle the irregularity and although this improved with AUTO2 the final plant counts per plot was still generally lower than the expected amount. Including plant sizing as another parameter in the automatic method as Sankaran *et al.* (2017) have shown, or making more use of height data could improve this, as would ensuring that the planting itself was more regular in the first place as tuber spacing (and weed control) are factors important to the development of the crop (Bussan *et al.*, 2007). The emergence of weeds (Figure 1a) to a very high level by the end of the trial (100% weed coverage for some plots towards the end of the growing season), resulted in an increase in false positive results (mainly for the later emerging Harmony plots), an issue that Gnädinger & Schmidhalter (2017) also commented on when trying to count maize plants. Even the direct visual analysis of some plots resulted in weeds being misinterpreted as emerged plants.

Aerial ground cover analysis initially looked poor when the raw numbers were compared as the manual method reported much higher ground cover in general. However, the two methods

correlated well and further investigation into the manual method revealed why the raw numbers may have differed so much. Due to the perspective that the observer has when looking at a plot on the ground using a grid, a considerably smaller area of the plot is in fact viewed. This resulted in only ~1.65 m wide and ~1.2 m long area of the plot being measured for ground cover with more of it being obscured if some of the plants are tall. The manual method is a fast and efficient measure however the aerial approach could give a more representative measure as it is observing the entire plot and the spaces between the rows. The very high level of weeds within the rows and canopy of the potato plants made classification more difficult (Figure 1c), likely resulting in error being introduced. However, the accuracy of the classification was not directly verified in this paper, it was only compared to the ground truth results.

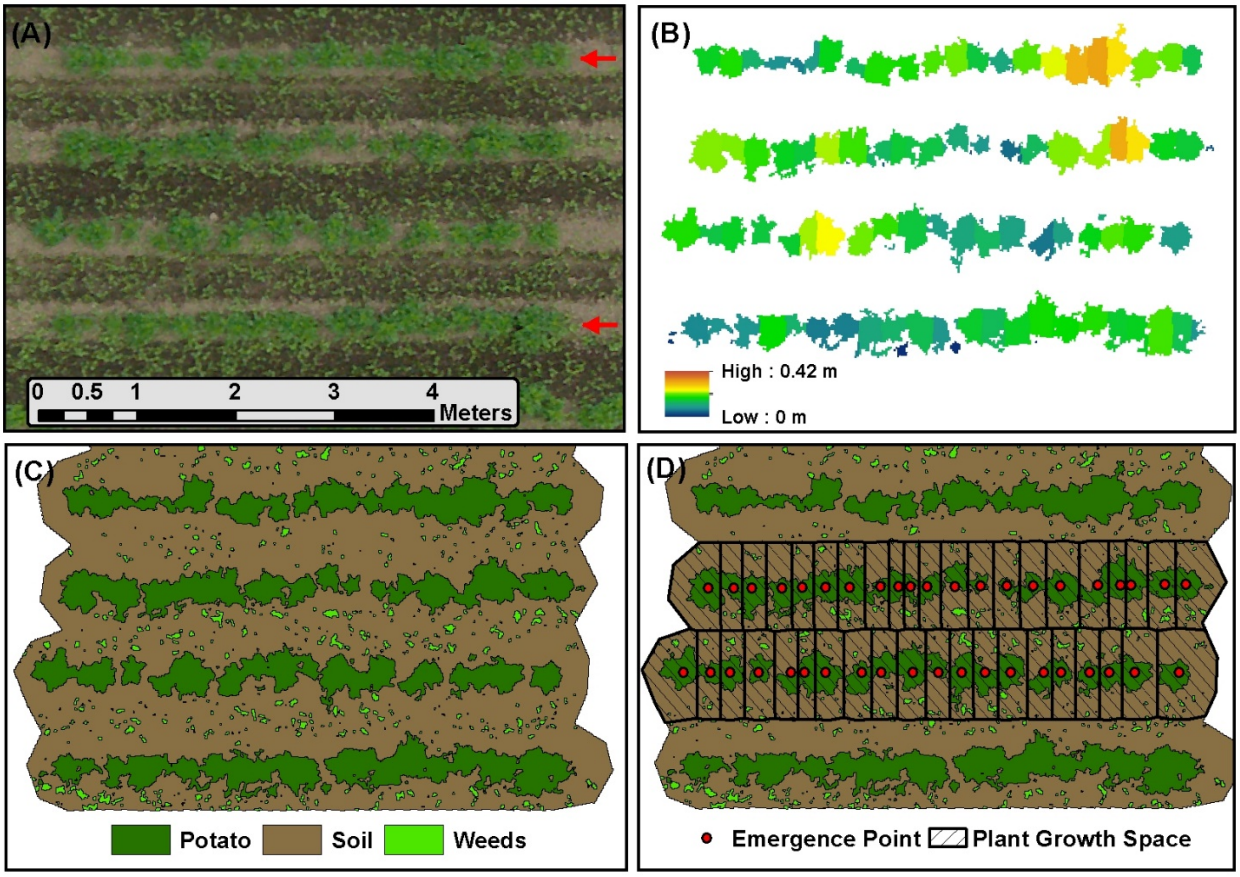

Figure 1. Example plot results at 41 DAP; (A) RGB where weeds can already be seen between rows, showing guard rows (red arrows); (B) Mean height of individual plants; (C) Classification of plot; (D) Detected emerged plant points and growth spaces allocated to each plant.

Using aerial data allows measurements of all aspects of the trial from the same survey effort. Being able to view and analyse the trial as individual plants rather than just plots or rows could allow more detailed analysis of trial development and issues. As the photogrammetry process produces high resolution DSM as well as orthomosiac data, the ability to measure the height of the plants surveyed is also possible (Figure 1b) and would further add to trial analysis as plant height has input in predicting yield (Arslan, 2007). Producing accurate height data can be an issue once the crop reaches a stage of complete canopy closure as the ground level cannot be seen. The use of highly accurate GNSS systems onboard the UAV could help with this respect, would negate the need to use GCPs and likely help with other issues

encountered in this trial with regards to slight shifts in the georeferenced position of orthomosaic data between survey dates and possibly reduce misalignment when co-registering the RGB and NIR layers.

To conclude, as small UAVs can only really be used in good weather conditions (i.e. not raining and wind speeds < 8 m/s to ensure safe operation), they are unlikely to replace traditional ground based methods completely, but the results of this study indicate that aerial data would complement existing methods and give a wider coverage of measures that would benefit trials monitoring and analysis in the future.

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THE IMPACT OF THE REGULATION OF POTATO CYST NEMATODES ACROSS SCOTLAND – ARE WE RUNNING OUT OF LAND FOR SEED PRODUCTION?

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Summary: SASA has recently completed the collation of historic potato cyst nematode (PCN) results dating back to 1973 when the UK entered the EEC and PCN became regulated under the 1969 PCN Directive. Since then, the total area of land in Scotland recorded as infested with PCN has increased to 19,108 ha. Consequently, restrictions have been placed on the growing of potatoes and especially the production of seed potatoes. In recent years the incidence of *Globodera pallida* has increased markedly, almost certainly as a result of a lack of marketable varieties resistant to this species. Over the last 7 years, the area of land infested with *G. pallida* has increased by 94% compared with just 4% for *G. rostochiensis*. Although PCN are more prevalent in certain parts of the country: Angus, Fife, Perthshire, Kincardineshire and Aberdeenshire; the evidence suggests that the rate of increase of both species is not markedly dissimilar across the country. At present it is estimated that 12.8% of the land that should be available for potato production in Scotland is recorded as infested with PCN. The undoubted presence of PCN in ware land that is not subject to official testing under the EU Directive makes this figure an underestimate. It is clear that significant changes are required to the current approaches to the management of PCN across the Scottish potato industry to limit the rate at which PCN, particularly *G. pallida*, is increasing.

INTRODUCTION

Since the UK entered the EU in 1973, all land intended for seed potato production has required a soil test to indicate freedom from the presence of the potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis*. The introduction of the 2007 EU PCN Directive (EC, 2007), which came into effect in 2010, introduced several significant changes, primarily specifying the rate at which fields should be sampled, but also stated that EU member states are required to maintain a register of infested land (see Pickup *et al*., 2012 for further details of 2007/33/EEC). Until 2010, SASA maintained paper records of PCN tests based on paper maps, together with a database recording soil tests by farm code. Since 2010, all PCN tests applied for under the EU PCN Directive have been managed using SPUDS (Seed Potato Universal Data System) based on digital maps showing field boundaries and the subdivision of fields into sampling units. In recent years, the paper records showing infested land have been transferred onto the SPUDS database, a process completed in 2016. We are now able to carry out an analysis of the total area of land currently recorded as infested with PCN.

MATERIALS AND METHODS

From 1973 to 2010, sampling of fields to be used for the production of seed potatoes was based on units of a maximum size of 4 ha. A single sample of 600ml of soil comprising a minimum of 100 cores was taken from each sampling unit. The presence or absence of PCN

was determined by extracting any cysts using a Fenwick Can process, followed by visual examination of the resulting extract (the 'float'). In 2010, two EU sampling rates were introduced: a 'standard' rate of 1500 ml/ha, and a 'reduced' rate of 400 ml/ha. The 'reduced' rate can be used where the previous history of testing and cropping of the land indicates a low probability of finding PCN, e.g. if the land has not grown potatoes in the previous six years, or if records of tests show a history of PCN freedom. Over 95% of land in Scotland is sampled at the reduced rate. Since 2010, cysts have been extracted using an automated carousel system with the vast majority of diagnoses carried out using an automated high throughput polymerase chain reaction (PCR) diagnostic technique developed at SASA (Reid *et al*., 2010). If PCN are found in the sample, the sampled unit is 'recorded' as infested. No potatoes intended for further planting may be grown in infested land, although the 2007 Directive now permits the cultivation of ware potatoes, but only if an official control programme is in place. The restrictions imposed on land which has been found infested with PCN can only be lifted after an official 'derecording' soil test has been completed and found free from live cysts. All derecording tests are carried out at the standard rate of 1500ml/ha. If a derecording test is found clear of PCN, the recording notice is revoked and no further restrictions are applied to the field. Potatoes may be grown for ware, farm saved seed or classified seed**.**

RESULTS

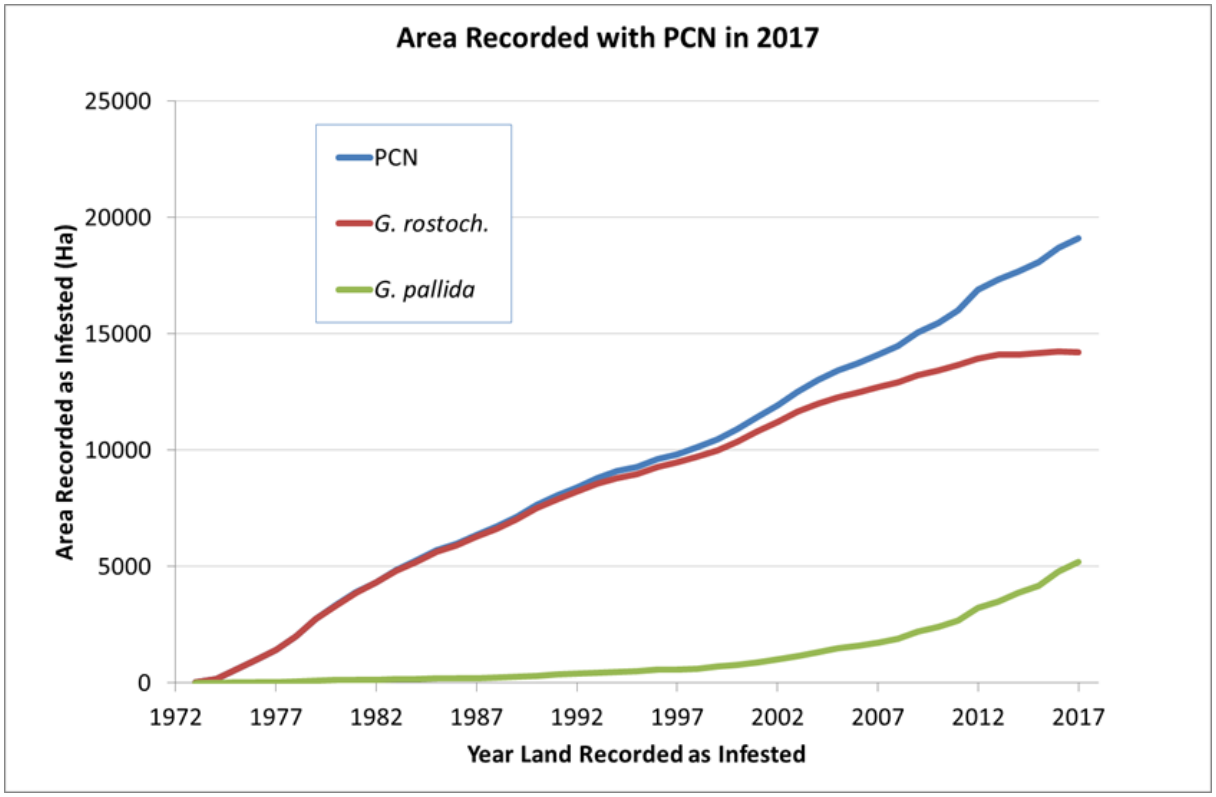

Figure 1. The area of land recorded as infested with PCN, *Globodera rostochiensis* and *Globodera pallida* as of July 2017. The data are presented by the year in which the land was originally recorded as infested.

As of July 2017, the area of land officially recorded as infested with PCN is 19,108 ha. Of this total, 14,217 ha (74%) is recorded as infested with *G. rostochiensis* and 5,214 ha with *G. pallida* (27%), including 322 ha (1.7%) with both species (Figure 1). Until the 1990s, nearly

all infestations were of *G. rostochiensis*. In 1990 only 4% of the currently infested land was infested with *G. pallida*. In 2017 the comparable figure is 27%.

Table 1. Area of land recorded by SASA as infested with potato cyst nematodes in 2017 in relation to the estimated area of potato production in the main potato growing counties.

County	**Area with PCN (Ha)**	**Area with *G. pallida*** (Ha)	**Area with *G. rostoch.*** (Ha)	**Potato Area 2011-16 (Ha)**	**Estimated Incidence of PCN (%)**	**Estimated Incidence of *G. pallida*** (%)	**Estimated Incidence of *G. rostoch.*** (%)
Angus	8394	3489	5132	49655	16.9%	7.0%	10.3%
Perth	3535	545	3032	23392	15.1%	2.3%	13.0%
Fife	3394	250	3165	13542	25.1%	1.8%	23.4%
Aberdeen	789	210	585	12527	6.3%	1.7%	4.7%
Kincardine	1050	287	770	9487	11.1%	3.0%	8.1%
East Lothian	250	54	199	7665	3.3%	0.7%	2.6%
Berwick	129	31	98	6615	2.0%	0.5%	1.5%
Ross	83	2	81	5706	1.5%	0.0%	1.4%
Moray	386	93	302	4678	8.2%	2.0%	6.5%
Roxburgh	124	52	72	4252	2.9%	1.2%	1.7%
Banff	291	87	204	3777	7.7%	2.3%	5.4%
Others	683	115	575	8281	8.2%	1.4%	6.9%
Total	19108	5214	14217	149577	12.8%	3.5%	9.5%

Table 1 shows the breakdown by county of the 19,108 ha of land infested with PCN, with the 11 counties with the greatest area of potato production over the six years 2011-16 highlighted (based on SASA's records of the area of seed and ware potatoes planted). Based on this estimate of the area of potato production, the area recorded as infested with PCN equates to 12.8% of the Scottish potato production area, with 9.5% infested with *G. rostochiensis* and 3.5% infested with *G. pallida*. The county with the greatest infested area is Angus with 44% of PCN infested land in Scotland, comprising 67% of the land infested with *G. pallida* and 36% of the land infested with *G. rostochiensis*. Fife is the county with the highest incidence of land infested with PCN in relation to its area of estimated potato production (25%), largely due to historical infestations of *G. rostochiensis*. Ross-shire has the lowest incidence of PCN, at 1.5% of its estimated area of potato production.

Since 2011, the area recorded as infested with *G. rostochiensis* has increased from 13,661 ha to 14,217 ha, an increase of 4% (Figure 2). Over the same period, the area recorded as infested with *G. pallida* has increased from 2,685 ha to 5,213 ha, an increase of 94% (Figure 3). Figure 2 also shows the nett change in the incidence of *G. rostochiensis* broken down by the area of new recordings and the area of previous infestations that have been cleared ('derecordings'). Over this seven year period, new infestations of *G. rostochiensis* have been found on an average of 345 ha each year, whilst an average of 233 ha has been derecorded, resulting in a nett increase in *G. rostochiensis* infested land of 112 ha p.a. Over the same period, new infestations of *G. pallida* have been found on an average of 448 ha each year, whilst an average of just 48 ha has been derecorded, resulting in a nett increase in *G. pallida* infested land of 400 ha p.a. The difference between the two species in the area derecorded is

largely driven by the much higher submission rate for land infested with *G. rostochiensis* (average of 303 ha p.a.) compared with *G. pallida* (average of 82 ha p.a.). The relative success rate for derecording tests is also higher for *G. rostochiensis* at 77%, compared with 59% for *G. pallida*.

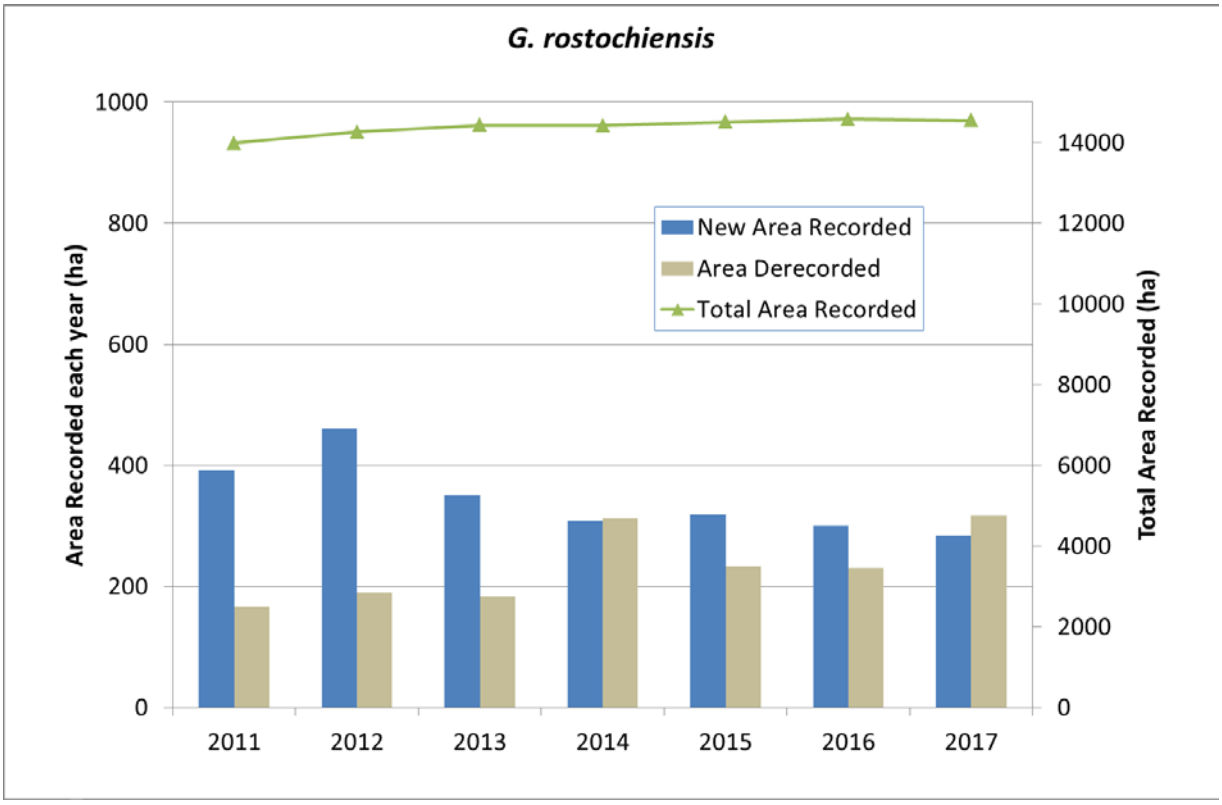

Figure 2. Area of new land testing positive for *Globodera rostochiensis*, and the area of land that previously contained this species but has been derecorded. Data from 2011 to 2017. The total area of land in Scotland recorded as infested with this species is also included.

Table 2. Change in the area of land recorded by SASA as infested with the two species of potato cyst nematodes between 2011 and 2017

County	Area with *G. pallida* 2011 (Ha)	Area with *G. pallida* 2017 (Ha)	Increase in Incidence of *G. pallida*	Area with *G. rostoch.* 2011 (Ha)	Area with *G. rostoch.* 2017 (Ha)	Increase in Incidence of *G. rostoch.*
Angus	1789	3489	95%	4782	5132	7%
Perth	260	545	110%	3013	3032	1%
Fife	150	250	66%	3213	3165	-1%
Aberdeen	137	210	54%	560	585	4%
Kincardine	118	287	143%	724	770	6%
Others	231	433	88%	1369	1532	12%
Total	**2685**	**5214**	**94%**	**13661**	**14217**	**4%**

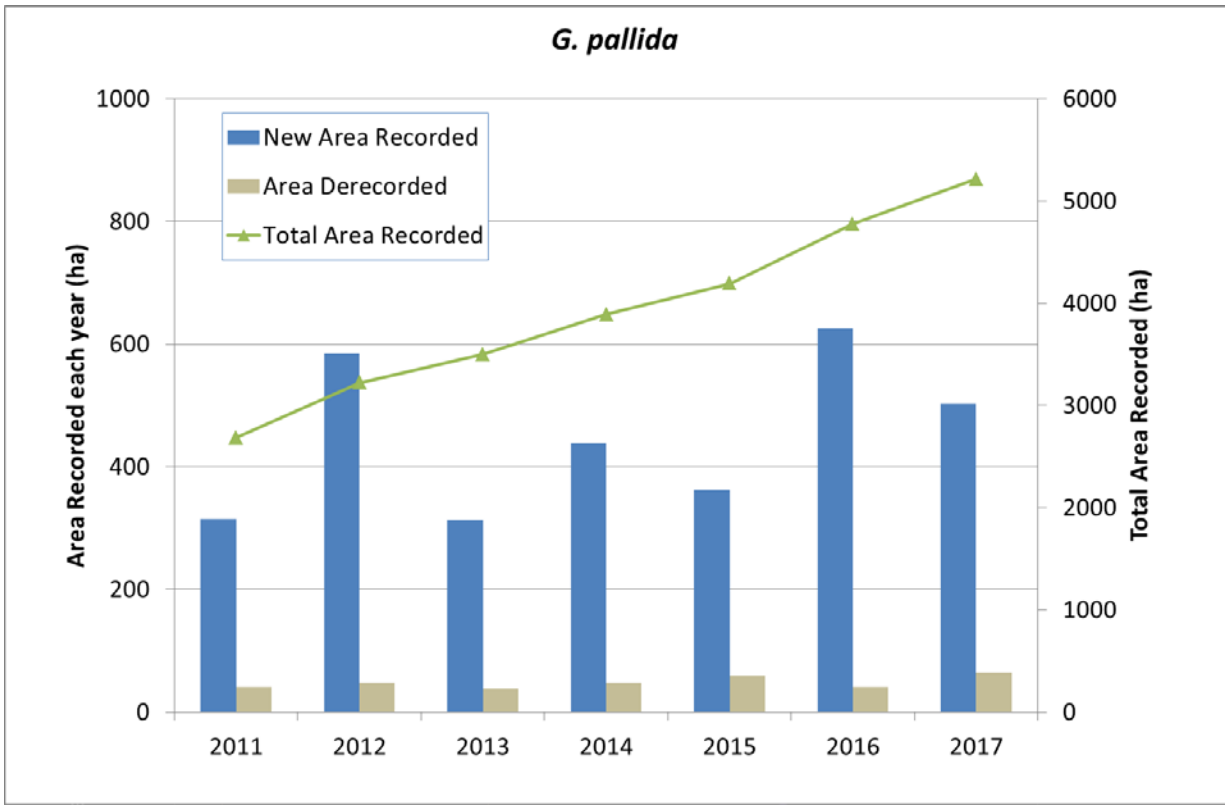

Figure 3. Area of new land testing positive for *Globodera pallida*, and the area of land that previously contained this species but has been derecorded. Data from 2011 to 2017. The total area of land in Scotland recorded as infested with this species is also included.

Table 2 provides the relative change in incidence of both species of PCN over the period 2011 to 2017, highlighting the changes in the 5 counties with the highest incidence of *G. pallida* in 2011. For each of these counties, there has been a marked increase in the incidence of *G. pallida*, ranging from 54% in Aberdeenshire to 143% in Kincardineshire. The increase of 95% in Angus is similar to the increase of 94% across the whole of Scotland. In contrast, the increase in the area of land recorded with *G. rostochiensis* over the same period is much lower, and the change in each county is very similar to the national average of 4%.

DISCUSSION

The overall area of land recorded as infested has increased markedly since the introduction of the higher sampling rates in 2010 stipulated within the new EU Directive. However the change is markedly different when the two species of PCN are compared. Since 2011, land recorded as infested with *G. pallida* increased by 94% over the period to 2017, i.e. the area of land infested with *G. pallida* has nearly doubled within 7 years. In contrast, the area of new land recorded with an infestation of *G. rostochiensis* has only increased by 4% over the same period. It is difficult to come to a precise figure for the land used for potato production in Scotland: the Scottish Government's Agricultural Census indicates that c. 27,500 ha of seed and ware potatoes were planted in 2016 (Scottish Government, 2016) and SASA's SPUDS database indicates a total area of nearly 150,000 ha were planted with potatoes between 2011 and 2016, assuming an average rotation of 6 years. Therefore, the area of land recorded as infested with PCN is currently estimated as equivalent to 12.8% of the total, with

G. rostochiensis found on 9.5% and *G. pallida* on 3.5%. Although in recent years recordings of *G. pallida* have started to be more frequent than those of *G. rostochiensis,* the extent of historic recordings of *G. rostochiensis* has resulted in infestations this species currently outnumbering those of *G. pallida* by nearly 3:1.

Current control measures practised across the Scottish potato industry are having much greater success in controlling *G. rostochiensis* than *G. pallida*. The most likely explanation for this is that the widespread cultivation of varieties highly resistant to *G. rostochiensis* (c. 50% of the Scottish potato crop) is keeping this species under control. For *G. pallida*, only a very small percentage of varieties have any resistance to this species. Over 90% of the Scottish potato crop has no resistance to *G. pallida*. Furthermore, the most widely available varieties with high levels of resistance to *G. pallida* are processing varieties, but the Scottish environment is generally not conducive to achieving the levels of dry matter that are demanded by potato processors. If increasing the area of *G. pallida* resistant varieties grown in Scotland is currently not an achievable goal, then the industry will need to explore other options to improve on the current levels of control. With a typical rotation of 6 years, it is already probably too late to intervene to prevent the area of land infested with *G. pallida* from increasing nearly two-fold by 2023.

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PHENOTYPIC CHARACTERISATION OF POTATO CYST NEMATODE (PCN) POPULATIONS IN SCOTTISH FIELDS

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Summary: The potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* cause significant economic losses each year in the UK. At the James Hutton Institute (JHI), populations of *G. pallida*, representing the pathotypes Pa1 and Pa2/Pa3, have been collected from different sites in the UK for more than 50 years. When comparing recent field populations of *G. pallida* with some historic populations, a change in the composition in populations collected from the same field was observed using molecular markers, with a more complex composition in the recently collected population, indicating a potential for hybrids between pathotypes. Single cyst lines from recently sampled field populations from a site in Scotland were generated in order to phenotypically characterise the composition of *G. pallida* populations using a pot test with six different potato genotypes with different levels of resistance to this species of PCN.

INTRODUCTION

Potato is the third most important global food crop for human consumption, with more than 5 million tons of tubers produced per year in the UK. Seed potato production is also important economically in the UK, especially in Scotland. Controlling pests and diseases of this crop is challenging and requires ongoing monitoring to ensure appropriate control strategies are employed. The potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* cause annual losses in the UK estimated at £50M/year (DEFRA, 2010). Genetic studies have shown that there have been three distinct introductions of *G. pallida* from South America into Europe (Plantard *et al*., 2008, Hockland *et al*., 2012); the distribution of these introductions has recently been examined in Scotland using the cytochrome B mitochondrial marker (van den Akker *et al.*, 2015) and indicated that the Pa1 pathotype appears to be more widely distributed that previously reported.

Populations of PCN in the JHI collection were collected from different sites in the UK during a period of more than 50 years and include populations that represent *G. pallida* pathotypes Pa1, Pa2/Pa3 (Phillips & Trudgill, 1998). Several populations from this collection and recently collected field populations have been compared in order to determine the composition of the introductions within these populations and to investigate if there are any novel introductions, by both molecular and phenotypic characterisation. A Terminal Restriction Fragment Length Polymorphism (T-RFLP) assay was used with individual PCN cysts to determine their mitotype. This T-RFLP assay is based on mitochondrial DNA, which is maternally inherited (Gibson *et al.,* 2007) and can distinguish distinct lineages or introductions. T-RFLPs are based on either size polymorphisms of the amplified product or sequence polymorphisms in the restriction sites which result in different size polymorphisms of the digestion fragments. For further phenotypic characterisation, single cyst lines were generated from some recent field samples and *G. pallida* populations from the JHI collection and a pot test was performed with six potato cultivars with different levels of resistance to *G. pallida* (Kort *et.al.*, 1977) to determine their relative levels of multiplication.

MATERIAL AND METHODS

PCN Populations:

Table 1. PCN populations investigated in this study.

Source	Population	Geographic origin
JHI collection	Lindley	England, West Yorkshire
	Luffness field 1	Scotland, East Lothian
	Pa1	Scotland, Duddingston
Recent field samples	Luffness field 1 2010, 2014	Scotland, East Lothian
	Luffness field 3 2010	Scotland, East Lothian

Terminal Restriction Fragment Length Polymorphism (T-RFLP):

T-RFLP allows the molecular composition of PCN populations to be examined. It is based on PCR amplification with fluorescent dye-labelled primers of variants of mitochondrial DNA isolated from nematodes or cysts. The PCR product is digested with a restriction enzyme and the products separated electrophoretically and their sizes determined. With this assay, three mitotypes of *G. pallida* in the UK can be distinguished.

DNA Extraction:
Individual cysts were selected under a low power microscope, picked with tweezers and placed into a 1.5ml Eppendorf tube. The cyst was crushed with a plastic pestle in 30µl of MicroLYSIS®-Plus buffer (Microzone) for 2-3 min, centrifuged at 13,000 rpm for 90 sec, then the supernatant was transferred to a 0.2ml PCR tube and heated to 65ºC for 15 min, 96ºC for 2 min, 65ºC for 4 min, 96ºC for 1 min, 65ºC for 1 min, 96ºC for 30 sec, 20ºC hold, then stored at -20ºC.

PCR:
The PCR reactions contained 1.5µl 10x HF buffer (Invitrogen), 0.6µl of each of the primers F3 mtDNA-222(FAM) (5'-ATT AGA CCG ATA AGT TTA CAC CTT G-3)' and SCMT4-8(HEX) (5'-GAC TAG GTC CAT CAA TCT GAA CC-3') (10µM), 0.6µl $MgSO_4$ (50mM), 1.0µl dNTPs (2mM), 0.6µl BSA (10mg/ml), 0.2µl Platinum Taq Polymerase (Invitrogen), 8.9µl H_2O and 1.0 µl DNA. These were heated to 94°C 2 min, 94° 30 sec, 55°C 30 sec, 68°C 60 sec for 40 cycles, 68°C 10 min.

Digestion with Restriction Enzyme:
A 1µl aliquot of master mix comprised of 0.1µl MULTI-CORE®buffer, 0.8µl H_2O, 0.1 ul restriction enzyme Taq1 (Promega) per reaction was transferred to each well of a 96 well PCR plate, then 5µl fluorescent PCR product was added to each well, mixed, and briefly centrifuged. Reactions were digested for 4 h at 65°C, then frozen at -20°C or processed to the next step.

T-RFLP Electrophoresis and Evaluation:
A 9µl aliquot of master mix (895µl formamide (Sigma) and 5µl ROX1000 marker (GeneScan™ 401098)) was added to each well of a 96 well plate (AB600); then 1µl of the T-RFLP digestion product was added to each well. The plate was transferred to the JHI sequencing facility and run on an ABI micro-capillary gel (Applied Biosystems) with laser detection. The fluorescent reads were analysed by Genemapper software v3.7.

Generation of Single Cyst Lines:

To limit the genetic variation in a PCN population, single cyst lines were generated. For this one cyst was put in a pot filled with 1:1 sand/loam mixture and a tuber piece of the susceptible *S. tuberosum* cultivar Desirée was cut with a melon scoop so that it had a single sprout. The plant was grown for 3 months, and then the cysts were collected by floatation using a MEKU nematode carousel (MEKU Pollähne) on 24 cm round filter papers (Macherey-Nagel MN 751) (Reid *et al.* 2015). In the first multiplication up to 50 cysts were obtained. Subsequent multiplication used 10 cysts/ pot to amplify each line as above. This was repeated until enough cysts (~400) for the experiment were obtained.

Pot Test for Phenotypic Analysis:

Clay pots were filled with 400g 1:1 sterilized sand/loam mixture and sunk into a sand bed in an unheated glass house. The single cyst lines used and the year of the experiment are shown in Table 2. 15 cysts per line were sealed in a nylon mesh (125 nm), in order to retrieve the initial cysts and separate them from the new cysts at the end of the experiment. Six potato cultivars or breeding clones (Table 3) with different levels of resistance to *G. pallida* were used to characterise 7 single cyst lines.

Table 2.: Single cyst lines used in this study.

Single cyst lines from field populations	Year tested 2016	2017	Population pool (year multiplied)	Year tested 2016	2017
Luff 2014 1-4	X		Luffness JHI (2014)	X	
Luff 2010 1-12	X	X	Luffness JHI (2016)		X
Luff 2014 1-19		X			
Luff 2014 1-30		X			
Luff 2010 3-8		X			
Luff 2010 3-17(b)	X	X			
Luff 2010 3-18(a)	X	X			

Table 3. Potato cultivars used and their resistance to *G. pallida*.

Potato cultivars	Source of Resistance
Desirée	susceptible
Maris Piper	susceptible
P55/7	H2, *Solanum multidissectum*
Vales Everest	H3, *S. tuber*osum spp *andigena* CPC2802
62.33.3	Gpa5, *S. vernei* acc V24/20
Innovator	Gpa5 *S. vernei* acc LGU8

Each cyst line was tested in 4 replicates. After 3 months, the cyst bag and plant material was removed from each pot and the soil was dried. The cysts were washed out from the soil at SASA (Scientific Advice for Scottish Agriculture) as described above. The cyst number for each sample was determined using a low power microscope.

RESULTS

Mitotyping of *G. pallida* Populations

T-RFLP patterns from 106 single cysts from 3 PCN populations from the JHI collection, and 3 recently collected field samples from Scotland were examined. Cysts from the 3 populations from the JHI collection had T-RFLP patterns with one type that predominated in 68, 71 and 96% of the cysts compared to 58, 60, and 88% of recently collected field cysts, see Table 4. A change in the composition in the Luffness population from the collection and that collected recently from the same field was observed, with all three mitotypes occurring in the Luffness field 1 2010 sample including the T-RFLP pattern associated with the Pa1 population.

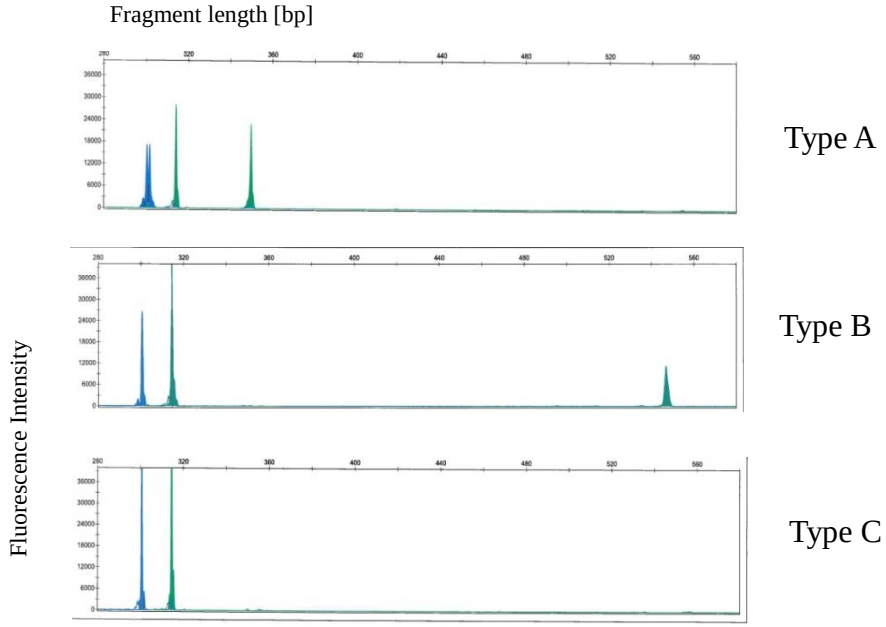


Figure 1. T-RFLP patterns which represent the three *G. pallida* mitotypes.

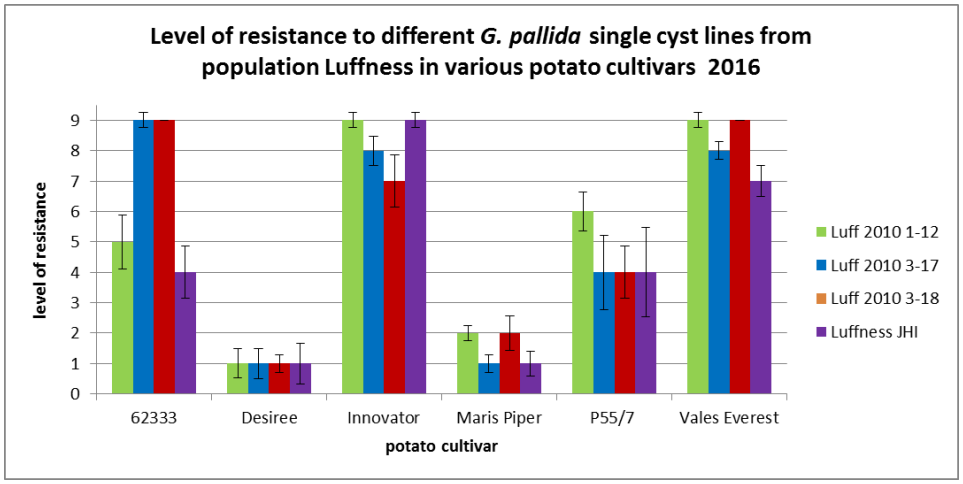
Table 4. Results from T-RFLP analysis for different PCN populations from single cysts.

Samples	Population	Type A	Type B	Type C	No. cysts analysed
JHI collection	Lindley	25 (96%)	1 (4%)		26
	Luffness field 1	11 (32%)	23 (68%)		34
	Pa1	4 (19%)	2 (10%)	15 (71%)	21
Field	Luffness field 1 2010	3 (25%)	7 (58%)	2 (17%)	12
	Luffness field 1 2014	3 (60%)		2 (40%)	5
	Luffness field 3 2010	1 (13%)	7 (88%)		8

Phenotyping Phenotyping Experiment:

3 single cyst lines from the Luffness population were tested and evaluated in a pot test in 2016. In 2017 these lines and an additional 4 single cyst lines were tested. The Luffness JHI (pool) was also tested in both years. To assess resistance of potato cultivars, the scoring system described in ((2006), EPPO Bulletin) was applied. This scoring system is not linear.

A



B

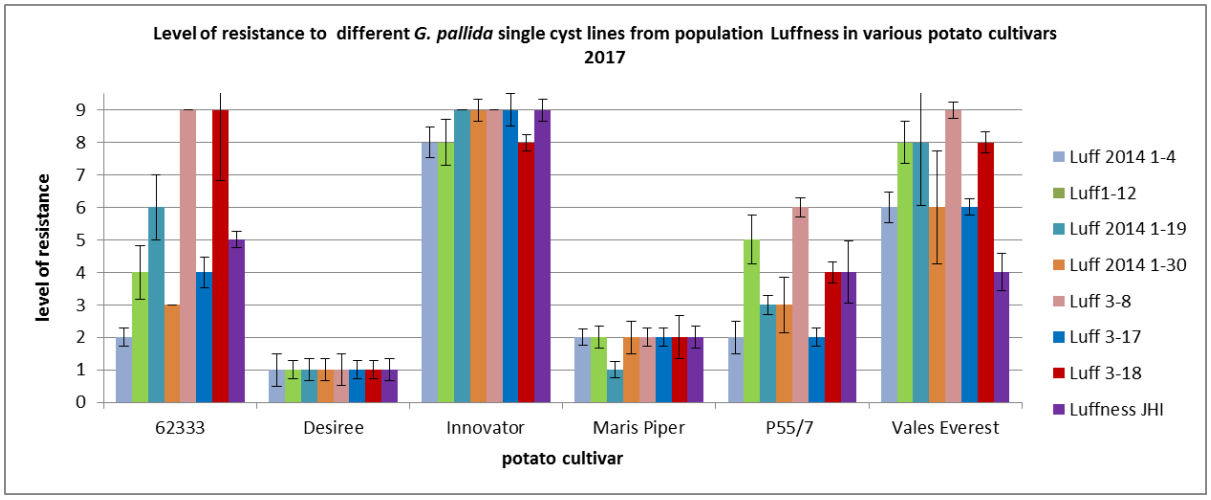


Figure 2.: Assessment of the resistance to *G. pallida* Luffness single cyst lines and JHI pool on six potato cultivars. The scoring is as described in ((2006), EPPO Bulletin, 36). Scoring ≤3 is full susceptibility, between 3 and 8 partial resistance, ≥8 full resistance. Error bars are standard error. Assessment of single cyst lines and JHI pool in 2016 (A) and 2017 (B).

DISCUSSION

Our results with field populations from Scotland using T-RFLP mitotype assay, indicate that current field populations of *G. pallida* can be composed of up to 3 mitotypes which confirms the report of van den Akker *et al.*, 2015. Thus, these fields are likely to comprise mixtures of the different historical introductions from South America. Our comparison of the 3 JHI PCN collection samples with recently collected field populations suggests that the populations' composition is becoming more complex over time and that the Pa1 introduction is likely to be more widespread than previously reported. This increasing complexity in field samples raises the possibility of hybridisation between the different genotypes of *G. pallida* that coexist in the same field and the potential for the generation of novel hybrid genotypes with new

phenotypes. It cannot be determined how this occurred, as we do not have consecutive historical samples from the same fields in the UK.

Maris Piper and Desirée were fully susceptible to all of the *G. pallida* single cyst lines tested. Innovator provided the best level of resistance; however there was variation to levels below 8, which could mean that the resistance could be overcome. Generally 62.33.3, P55/7 and Vales Everest showed the greatest variability in nematode multiplication and is consistent with their having partial resistance to *G. pallida*. It will be interesting to see if mitotyping of the single cyst lines reveals any correlation with the phenotypic behaviour. Monitoring the composition of current field populations is needed to ensure that resistance used in potato breeding programs is suitable and that it will provide broad-spectrum and durable resistance to *G. pallida*.

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THE DEVELOPMENT OF BCS-AR83685 FOR PCN REDUCTION ON POTATO CROPS IN THE UK

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Summary: AR83685, a novel nematicide containing the active ingredient fluopyram, was tested in 12 potato field trials across Great Britain in 2015 and 2016. AR83685 was applied by either over-all spray then incorporated before planting or as in-furrow sprays at planting at a rate of 250g a.i./ha. Oxamyl granules applied at 5500g a.i./ha were used as a commercial comparison. Mean yield results using susceptible varieties resulted in AR83685 increasing yields by 4.7- 6.8 t/ha above untreated controls and in resistant or tolerant varieties by 1.7-2.3 t/ha. In both cases, the yield increases from oxamyl were greater. Further tests using AR83685 in sequence with half rate oxamyl or half rate fosthiazate granules resulted in potato yields that were equal to or greater than that given by the full rates of the granules alone.

INTRODUCTION

Fluopyram has been developed worldwide by Bayer as a fungicide on a wide range of crops for control of a range of plant pathogens. In 2009, during work on banana crops in Costa Rica, it was discovered that the active substance had a nematicidal effect which improved root growth. Subsequent tests revealed that the mode of action of fluopyram as a nematicide was biochemically the same as against fungal pathogens in that it disrupts Complex ll of the mitochondrial respiratory chain. This causes the inhibition of mitochondrial succinate dehydrogenase (SDH) enzyme which leads to a fast and severe depletion of the nematode´s cellular energy, ATP (adenosine triphosphate). When juvenile nematodes come into contact with fluopyram, they rapidly become paralysed and die thereafter (Fürsch et al., 2015). Since 2012, trials have been conducted in northern Europe, particularly in the UK and the Netherlands, for reduction in damage caused by potato cyst nematode (both *Globodera pallida* and *G. rostochiensis*) and this paper reports on the trials conducted in 2015 and 2016 in the UK by independent research organisations.

MATERIALS AND METHODS

A liquid formulation of fluopyram specifically for potato crops was coded AR83685 which will be used in this paper. AR83685 was applied at a rate of 250g a.i./ha using two application methods: as an overall spray (diluted in 200 litres/ha of water) applied pre-planting and immediately incorporated during bed formation or as an in-furrow spray (diluted in 100 litre/ha water) at planting. The randomised complete block trials consisted of plots of 4 rows x 6-8m long with 4 or 5 replicates. Pre-planting treatments were applied using plot sprayers with 2m booms for the liquid treatments whilst for the granules by hand spreading or modified plot granule applicators. In-furrow spray applications were applied by single nozzle hand lances or tractor mounted in-furrow sprayer. Bed formation was undertaken using standard farm equipment. All treatment rates are given in g a.i./ha.

Efficacy Trials

Twelve single variety trials were conducted in 2015 and 2016 on maincrop and second early varieties, with seven varieties classed as *G. pallida* susceptible and five as resistant or tolerant. All sites were predominantly populated with *G. pallida* in locations from central Scotland to East Anglia. All trials were placed within commercial crops and received the standard crop treatments with the exception of nematicides. Sampling of 400g soil for PCN egg numbers per gram of soil was undertaken in every plot at crop planting and again at harvest, using a standard method by a single laboratory. The final population (Pf) divided by the initial population (Pi) gave the resulting Pf/Pi count score for propagation rates for each individual plot in each trial. The Pf/Pi score for all replicates for individual treatments are meaned to give the overall score for each treatment.

RESULTS

The principal assessments reported in this paper are crop yield and the rate of nematode population increase or decrease following treatment application (Pf/Pi).
Analysis of variance was statistically tested (P=.05, Student-Newman-Keuls) and the LSD for assessments in individual trials has also been recorded.

Trials were conducted on both *G. pallida* susceptible and resistant or tolerant varieties and the resultant harvested yields are given in Tables 1 and 2.

Table 1. Mean gross yield in tonnes/ha in susceptible varieties: Maris Piper (3 sites); Maris Peer (3); Lady Claire (1)

Location (year)	Untreated	AR83685 250 g/ha In-furrow	AR83685 250 g/ha pre-plant	Oxamyl 5,500g/ha pre-plant	LSD
Yorks 1 (2015)	17.9	21.5	23.1	25.1	8.4
Fife 1 (2015)	59.9	56.6	57.6	63.9	8.2
Suffolk 1 (2015)	29.9	33.4	35.8	38.4	6.5
Fife 2 (2016)	17.2	31.1	36.9	40.2	10.0
Suffolk 2 (2016)	23.6	21.4	30.7	32.9	12.0
Lincs (2016)	32.6	39.1	39.5	37.9	5.2
Norfolk (2016)	12.1	23.2	17.0	29.0	6.1
MEAN	*27.6*	*32.3*	*34.4*	*38.2*	

The yield results in both tables 1 and 2 demonstrated few significant differences between treatments, however in all of the individual trials, some or all of the nematicide treatments numerically increased the crop yield compared to the untreated controls. The pre-planting then incorporated applications of AR83685 resulted in slightly higher yields than the in-furrow application of AR83685 in the majority of trials with both susceptible and resistant/tolerant varieties. In all but three of the 12 trials, oxamyl granules equalled or out yielded either or both of the AR83685 treatments.

Table 2. Mean gross yield in tonnes/ha in resistant or tolerant varieties: Arsenal (2 sites); Eurostar (2); Performer (1)

Location (year)	Untreated	AR83685 250 g/ha In-furrow	AR83685 250 g/ha pre-plant	Oxamyl 5,500g/ha pre-plant	LSD
Yorks 2 (2015)	16.9	20.8	23.1	35.6	6.9
Fife 3 (2015)	54.8	55.6	56.3	56.3	7.2
Suffolk 3 (2015)	30.3	30.7	33.4	39.3	5.9
Fife 4 (2016)	42.5	40.5	44.2	43.8	6.9
Suffolk 4 (2016)	31.8	37.4	30.9	41.0	11.1
MEAN	*35.3*	*37.0*	*37.6*	*43.2*	

As recorded in tables 3 and 4, the trials were planted into fields with a wide range of PCN egg counts in order to evaluate the performance of the treatments under the range of conditions found across the country. Tables 5 and 6 record the Pf/Pi scores from both sets of trials.

Table 3. Mean Pi egg counts and Pf/Pi scores at each trial site (per gram of soil) in untreated plots of susceptible varieties

Location	Year	Cultivar	Pi	Pf/Pi
Yorks 1	2015	M. Piper	90.0	2.1
Fife 1	2015	M. Piper	1.4	90.4
Suffolk 1	2015	M. Peer	8.8	7.9
Lincs	2016	M. Peer	21.3	3.6
Norfolk	2016	Lady Claire	8.9	7.1
Fife 2	2016	M. Piper	31.3	7.1
Suffolk 2	2016	M. Peer	22.3	8.0

Table 4. Mean Pi egg counts and Pf/Pi scores at each trial site (per gram of soil) in untreated plots of resistant or tolerant varieties

Location	Year	Cultivar	Pi	Pf/Pi
Yorks 2	2015	Arsenal	64.8	0.5
Fife 3	2015	Eurostar	3.6	2.1
Suffolk 3	2015	Arsenal	5.3	0.2
Fife 4	2016	Eurostar	26.1	1.3
Suffolk 4	2016	Performer	15.3	0.9

Table 5. Mean Pf/Pi egg count score in susceptible varieties: Maris Piper (3 sites); Maris Peer (3); Lady Claire (1)

Location (year)	Untreated	AR83685 250 g/ha In-furrow	AR83685 250 g/ha pre-plant	Oxamyl 5,500g/ha pre-plant	LSD
Yorks 1 (2015)	2.1	2.8	2.2	0.4	1.0
Fife 1 (2015)	90.4	72.1	59.2	93.8	124.5
Suffolk 1 (2015)	7.9	3.8	3.0	5.3	10.7
Fife 2 (2016)	7.1	3.1	3.9	2.3	3.6
Suffolk 2 (2016)	8.0	2.7	5.4	6.0	8.1
Lincs (2016)	3.6	5.6	5.1	4.3	2.0
Norfolk (2016)	7.1	12.0	5.8	5.5	7.0
MEAN	*18.0*	*14.8*	*12.1*	*16.8*	

Table 6. Mean Pf/Pi egg count score in resistant or tolerant varieties: Arsenal (2 sites); Eurostar (2); Performer (1)

Location (year)	Untreated	AR83685 250 g/ha In-furrow	AR83685 250 g/ha pre-plant	Oxamyl 5,500g/ha pre-plant	LSD
Yorks 2 (2015)	0.5	0.5	0.4	0.4	0.4
Fife 3 (2015)	2.1	1.5	0.0	0.5	2.0
Suffolk 3 (2015)	0.2	0.2	0.5	0.1	0.3
Fife 4 (2016)	1.3	1.2	0.7	0.5	1.1
Suffolk 4 (2016)	0.9	0.5	0.7	0.7	0.7
MEAN	*1.0*	*0.8*	*0.5*	*0.4*	

The Pf/Pi egg count scores for untreated plots in the susceptible varieties (tables 3 and 5) ranged from 2 to 8 in all but one trial and whilst not significantly so, virtually all the treatments reduced the scores. One trial, Fife 1, having started with a very low egg count and then a relatively modest increase in egg numbers at Pf had as a consequence, an exceptionally high level of egg propagation as calculated by Pf/Pi.
The importance of the resistance properties of the varieties in tables 4 and 6 was clearly demonstrated but never the less, all the nematicides further reduced the Pf/Pi egg scores (but not significantly so).

As part of the development of AR83685, in 2016 treatments were included in trials with both susceptible and resistant or tolerant varieties which evaluated the efficacy of programme applications pre-planting with half rates of oxamyl or fosthiazate followed by AR83685 in-furrow (250 g/ha) at planting when compared to the full rates of the granule treatments applied alone (tables 7 and 8). The yield comparisons in table 7, demonstrated no significant differences between the full rate granule applications and the half rate granules followed by the AR83685. However, the results demonstrated that in every trial, the half rate oxamyl followed by AR83685 increased yield compared to the full rate oxamyl alone (but not significantly).

Table 7. Mean gross yield in tonnes/ha (2016: programme treatments)

Granular nematicide	Oxamyl 5,500g/ha pre-plant	Oxamyl 2,250 g/ha pre-plant	Fosthiazate 3,000g/ha pre-plant	Fosthiazate 1,500g/ha pre-plant	LSD
followed by	-	AR83685 250 g/ha In-furrow	-	AR83685 250 g/ha In-furrow	
Fife 2 (2016)	40.2	42.5	40.6	33.7	10.0
Fife 4 (2016)	43.8	52.6	51.4	44.6	6.9
Suffolk 2 (2016)	32.9	33.7	38.1	42.1	12.0
Suffolk 4 (2016)	41.0	45.3	36.5	49.2	11.1
Norfolk (2016)	29.0	32.3	23.6	20.9	6.1
MEAN	*37.4*	*41.3*	*38.0*	*38.1*	

Table 8. Mean Pf/Pi egg counts scores (2016: programme treatments)

Granular nematicide	Oxamyl 5,500g/ha pre-plant	Oxamyl 2,250 g/ha pre-plant	Fosthiazate 3,000g/ha pre-plant	Fosthiazate 1,500g/ha pre-plant	LSD
followed by	-	AR83685 250 g/ha In-furrow	-	AR83685 250 g/ha In-furrow	
Fife 2 (2016)	2.3	3.6	4.1	4.3	3.6
Fife 4 (2016)	0.5	1.3	0.9	0.8	1.1
Suffolk 2 (2016)	6.0	3.9	9.9	11.0	8.1
Suffolk 4 (2016)	0.7	1.2	0.4	0.5	0.7
Norfolk (2016)	5.5	4.8	9.4	7.9	7.0
MEAN	*3.0*	*3.0*	*4.9*	*4.9*	

With fosthiazate, the full rate granules gave higher yields than the half rate followed by AR83685 in three trials but lower yields in two trials but overall there was no significant difference between either of the treatments.
The Pf/Pi egg count scores in table 8 also had no significant differences between any of the treatments with the half rate of each granule followed by AR83685 being as effective as the full rate of each granule alone.

DISCUSSION

The results from the independent field trials conducted across Great Britain clearly demonstrated that AR83685 applied either as an overall spray then incorporated prior to planting or as an in-furrow spray application at planting, both had a substantial if not significant increases in crop yield compared to the untreated controls, but did not equal that of

the commercial granular treatment oxamyl. However the reduction in PCN egg propagation from especially the pre-planting applications of AR83685 was similar to that given by oxamyl. Under conditions of high PCN infestations, growers will want to maximise their potential yields and the trials initiated in 2016 showed that combining the applications of AR83685 with half rates of the granular nematicides can results in yields as high as or even greater than that given by full commercial rates of the granular nematicides alone.

In an increasingly stringent regulatory environment, it is important to maintain and increase the different modes of action available to give protection against PCN. Fluopyram, the active ingredient in AR83685, met this criterion in a number of different ways. Firstly, the mode of action being an SDHI is markedly different from the acetylcholinesterase (AChE) inhibition in the carbamate and organophosphate granular nematicides (IRAC, 2017). Fluopyram differs from five other chemicals with SDHI modes of action in that it demonstrated negative effect on the motility of free living nematode species *Meloidogyne incognita* and *Rotylenchulus reniformis* unlike the other chemicals tested (Faske & Hurt, 2015). The rate of active ingredient in AR83685 (250g /ha) required to reduce PCN activity is substantially lower than that in the approved rates of the granular nematicides. The European Food Standard Agency (EFSA) have evaluated fluopyram for the effects in soil micro-organisms and concluded that there is no negative effect on both the nitrogen and carbon cycles. In addition they assessed the risk against earthworms and other non-target soil organisms (meso- and macrofauna) and again came to the conclusion that there were no negative effects to any of the representative species (Anon, 2013).

Finally, because of the relatively benign profile of AR83685 in terms of mammalian toxicity, it is possible for it to be formulated as a flowable suspension concentrate (SC) which has practical advantages for the users. Application does not require the use of dedicated machinery as most potato growers will already have access to overall or in-furrow spraying equipment. If growers already have granule applicators, the simultaneous or sequential application of AR83685 is relatively straight forward. If and when AR83685 receives regulatory approval, it will provide potato growers with an additional tool to combat PCN that will contribute well with integrated control measures involving some or all of cultural (rotational) measures, resistant varieties, bio-fumigation and established chemicals.

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DETERMINING THE EFFECTS OF ISOTHIOCYANATES AND BIOFUMIGATION ON *GLOBODERA PALLIDA*

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Summary: Management of potato cyst nematodes has become more challenging due to increased restrictions on nematicide use. Biofumigation is an alternative pest control method in which Brassicaceae cultivars containing glucosinolates are broken down and incorporated into the soil to produce toxic isothiocyanates (ITCs). ITCs are thought to have nematicidal activity but due to a lack of broad range control their effectiveness depends on the type and concentration of ITC released as well as the target species. The aim of this study was to determine if biofumigation has the potential to be used to control *G. pallida*. Allyl ITC (AITC) was effective at reducing juvenile hatch and the viability of encysted juveniles in soil. Of several potential biofumigant cultivars screened, two were able to reduce hatch under glasshouse conditions, both of which were *Brassica juncea* and contained high concentrations of the AITC parent glucosinolate, sinigrin. This research will contribute towards the development of an effective biofumigation strategy desirable to both growers and environmentalists.

INTRODUCTION

In the UK around six million tonnes of potatoes are produced annually. Potato cyst nematodes (PCN) are major potato pests and have been identified in 64% of potato fields in England and Wales with 67% of these fields infested with *Globodera pallida*, 8% infested with *G. rostochiensis* and 25% infested with both (Minnis *et al.*, 2002). Control of these species traditionally relies on crop rotation, host resistance and nematicide use. Due to the European Council regulation (EC) No 1107/2009 under Directive 91/414/EEC, which changed risk-based assessment of plant protection products to hazard-based criteria, increasing emphasis is being placed on the reduction of nematicide use as there are concerns regarding the possible negative impact on the environment. In response, a considerable amount of interest is being shown in the development of environmentally-friendly, economically-viable and effective pest management strategies (Matthiessen & Kirkegaard, 2006).

One such strategy is biofumigation. Biofumigation is the suppression of soil pests and diseases by volatile hydrolysis products released into the soil after the incorporation of glucosinolate-containing plant tissue, from Brassicaceae spp. (Angus *et al.*, 1994). This hydrolysis has the potential to release breakdown products such as isothiocyanates (ITCs), nitriles and thiocyanates (Fenwick *et al.*, 1983) of which ITCs are considered the toxic product required to suppress pathogens (Lazzeri *et al.*, 1993). Biofumigation and ITCs appear to have nematicidal activity against a range of nematode species and previous *in vitro* research has indicated that certain glucosinolate (GSL) hydrolysis products can cause *G. rostochiensis* and *G. pallida* mortality. More recent research on the effects of Brassicaceae material and ITCs on encysted second-stage juveniles (J2) have shown that different cultivars have varying effects on the viability and hatch of *G. pallida* (Wood *et al.* 2017). Most studies are focussed on the

effect of a select few Brassicaceae species and GSL breakdown products, namely *Brassica juncea* and its major GSL, sinigrin. As different species contain a number of different GSLs able to release various concentrations of ITCs, there is a need to screen a wide range of ITCs and Brassicaceae against *G. pallida* in order to determine which cultivars will be most effective as a PCN biofumigant.

MATERIALS AND METHODS

Hatching assays

Hatching assays were performed with *G. pallida* cysts in six-well suspension plates. Plates were stored in the dark at 18°C with the exception of during counting when the cysts were exposed to light. Batches of cysts were soaked in dH_2O for three days prior to exposure to transfer to 2 ml potato root diffusate (PRD) which was refreshed weekly throughout each assay. Hatched J2 were counted at regular intervals, using a Wilovert HF microscope, for four weeks or until the rate of hatch decreased.

Meldola's blue dye viability assays

Meldola's Blue Dye (MB) stain was applied to cysts after a hatching assay. When used in the absence of a hatching assay, cysts were soaked in dH_2O for a week at room temperature in the dark to hydrate cysts. When used directly after a hatching assay, pre-soaking in dH_2O was excluded from the protocol. Cysts were exposed to 0.05% (w/v) MB (Avonchem Ltd., UK) for seven days. In order to remove excess stain, cysts were soaked in dH_2O for 24hrs before viability determination. Cysts were crushed with a micropestle and eggs were rinsed into a 15 ml tube, topped up with dH_2O (1 mL for each cyst in the sample) and gently mixed. A 1 mL aliquot was transferred into wells of a six-well suspension plate and both unstained (viable) and stained (non-viable) unhatched J2 within eggs were counted using a Wilovert HF microscope.

Potato cyst nematode collection

Cysts were collected from soil using a Fenwick can extraction method. Soil was placed in an 85µM sieve and washed with H_2O in order to separate the soil into organic material retained on the sieve, sand which collected at the bottom of the Fenwick can and debris containing cysts that floated to the top and was collected in a 25µM sieve. The collected cyst-containing debris was transferred to a filter paper and dried. Cysts were separated from the debris by hand under a SWF10X S-4400 microscope (Euromex, Netherlands). Cysts were stored at 4°C in the dark for a minimum of four weeks before use.

The influence of soil composition on AITC efficiency and encysted *G. pallida* viability and multiplication

Two muslin bags containing ten cysts each were placed at a depth of 10cm in 2 L pots filled with sandy silt loam, clay loam or sandy loam soil. AITC treatments (0, 100, 500, 1000 and 1500ppm) were incorporated into each soil type and pots were sealed using plastic film. Six replicates of each treatment were included and pots were set up in a randomised block design layout. After four weeks one cyst bag was removed and a hatching assay and MB stain was carried out. Potato tubers of cv. Desiree were planted in each pot containing the remaining cyst bag and grown under glasshouse conditions and incubated at day/night temperatures of 20 ± 2°C/18 ± 2°C under a 16hr photoperiod for 16 weeks. Newly formed cysts were counted and a hatching assay and MB stain was completed on a subsample of ten cysts to determine

total number of new eggs and viability; when less than ten cysts were present the entire sample was analysed.

The effect of five biofumigant cultivars / species on *G. pallida* cyst viability, hatch and multiplication

A muslin bag containing ten cysts was placed in 5 L pots at a depth of 10cm. Seeds of five potential biofumigant cultivars (Bento, Ida Gold, ISCI 99, Nemat & Scala) were sown and left to grow for eight weeks. In addition to the five cultivars of interest two control treatments were included, a low-GSL cultivar (Temple) green manure control and non-GSL bristle oats (*Avena strigosa)* catch crop control. Four replicates of each treatment were included and pots were arranged in a randomised block design layout. After eight weeks the cyst bag was removed and the ten cysts were subjected to a hatching assay. Plant samples were collected, freeze-dried and stored in preparation for LC-MS analysis and biomass incorporation studies.

To determine the biomass of material that would be incorporated for each plant treatment the above-ground plant material from the four replicates of each cultivar were combined and the total fresh weight was recorded and material distributed equally between the six replicates. Using calculated dry weights the soil moisture level was equalised at 40%.

A muslin bag containing ten cysts each were placed in 2 L pots. The biofumigant material was macerated in a blender with H_2O before being incorporated into pots and sealed for four weeks. In addition to the seven plant treatments, a Fallow negative control was included where 342 mL H_2O was mixed into soil. Six replicates of each treatment were included and pots were arranged in a randomised block design layout. After four weeks under the conditions described above: pots were unsealed, the cyst bag removed, and the cysts subjected to a hatching assay and MB stain.

RESULTS

The influence of soil composition on AITC efficiency and encysted *G. pallida* viability and multiplication

As AITC concentration increased, cyst hatch decreased with a corresponding increase in mortality (Table 1). Soil type had no overall effect on AITC efficiency and there was no effect of the interaction between concentration and soil type. Several AITC treatments increased the percentage of dead J2; 500ppm AITC treatments in sandy and clay loam soil led to significantly higher mortality compared to the controls. After exposure to 1000ppm and 1500ppm AITC in all soil types, J2 mortality increased significantly. Cysts exposed to 1000ppm AITC in sandy loam soil contained a higher percentage of dead J2 compared to 100ppm AITC treated cysts. 1500ppm AITC in clay loam soil led to significantly higher mortality than cysts exposed to 100ppm AITC in clay loam soil. Soil type had no effect on the percentage of dead J2 within concentration. There was no significant increase in mortality for concentrations above 500 ppm. Hatch was significantly reduced after treatment with 500-1500ppm AITC in all three soil types compared to the water control. In addition, AITC treatments between 500-1500ppm significantly reduced hatch compared to 100ppm AITC in sandy silt and sandy loam type soil. In clay loam soil, 500ppm and 1000ppm AITC treatments reduced hatch compared to 100ppm AITC treatments however 1500ppm AITC did not. Soil type had no effect on AITC-related hatch suppression within concentration. The percentage of unhatched viable J2 in each sample was unaffected by both soil type and AITC concentration.

Table 1. Percentage of *G. pallida* J2 that were; dead, hatched and unhatched viable after exposure to AITC for four weeks in different soil type. Within columns, means followed by the same letter are not significantly different.

Concentration (ppm)	Soil Type	Percentage Total J2 (%)		
		Dead	Hatched	Unhatched
0	Sandy Silt Loam	63.44^{ab}	21.90^{a}	14.66
	Sandy Loam	52.79^{a}	33.6^{a}	13.60
	Clay Loam	56.23^{a}	27.67^{a}	16.11
100	Sandy Silt Loam	75.96^{abcde}	14.88^{a}	10.14
	Sandy Loam	61.42^{abc}	22.72^{a}	15.86
	Clay Loam	70.6^{abcd}	13.62^{ab}	15.72
500	Sandy Silt Loam	79.34^{bcde}	3.95^{bc}	16.72
	Sandy Loam	83.67^{bcde}	0.02^{bc}	16.32
	Clay Loam	88.01^{de}	0.00^{c}	11.99
1000	Sandy Silt Loam	86.86^{cde}	0.00^{c}	13.14
	Sandy Loam	86.53^{de}	0.01^{c}	13.46
	Clay Loam	82.26^{bcde}	0.00^{c}	17.74
1500	Sandy Silt Loam	89.49^{e}	0.00^{c}	10.51
	Sandy Loam	84.03^{bcde}	1.50^{bc}	15.34
	Clay Loam	90.32^{e}	0.02^{bc}	9.65

High concentrations of AITC significantly reduced the number of newly formed cysts and egg content post-multiplication (Table 2). Concentration was the only factor to affect the number of cysts. For all soil types, treatment with 500ppm and 1500ppm AITC led to a significant reduction in new cysts compared to the control. When treated with 1000ppm AITC, cyst numbers were significantly lower than the controls in the clay loam soil but not the sandy silt loam soil type. 500ppm and 1000ppm AITC in sandy loam soil completely inhibited the formation of new cysts. The number of cysts collected was significantly lower after treatment with 1500ppm AITC than after treatment with 100ppm AITC in sandy silt loam soil. There was an effect of concentration and the interaction between concentration and soil type on egg number and an effect of concentration on viable egg number in each sample and cyst. All cysts collected after exposure to 500-1500ppm AITC, with the exception of 500ppm AITC in clay loam soil and 1000ppm AITC in sandy soil loam soil, were empty. 500ppm AITC in clay loam soil significantly reduced the number of eggs, eggs per cyst, viable eggs and viable eggs per cyst compared to the controls. Cysts treated with 1000ppm AITC in sandy silt loam soil contained a lower egg content than the 100ppm AITC treated cysts in this soil type.

Table 2. The effect of exposure to AITC in three different soil types on the number and viability of new *G. pallida* cysts post-multiplication. Within columns, means followed by the same letter are not significantly different.

Concentration (ppm)	Soil Type	Cysts	Eggs	Eggs/ Cyst	Viable Eggs	Viable Eggs/ Cyst
0	Sandy Silt Loam	52.0^{a}	7837.2^{ab}	129.9^{ab}	5424.4^{ab}	92.7^{ab}
	Sandy Loam	73.7^{a}	11178.8^{a}	130.2^{a}	8083.1^{a}	87.8^{a}
	Clay Loam	65.3^{a}	13995.2^{a}	174.1^{a}	11157.8^{a}	141.8^{a}
100	Sandy Silt Loam	10.7^{ab}	1605.0^{a}	147.18^{a}	1223.4^{a}	104.2^{a}
	Sandy Loam	23.5^{abc}	4327.2^{ab}	125.9^{ab}	3573.5^{ab}	101.5^{ab}
	Clay Loam	4.7^{abc}	780.0^{abc}	70.0^{abc}	597.3^{abc}	47.0^{abc}
500	Sandy Silt Loam	0.5^{bc}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}
	Sandy Loam	0.0^{c}	-	-	-	-
	Clay Loam	0.2^{c}	7.3^{bc}	7.3^{bc}	2.7^{bc}	2.7^{bc}
1000	Sandy Silt Loam	0.7^{abc}	28^{bc}	28^{bc}	25^{bc}	25^{bc}
	Sandy Loam	0.0^{c}	-	-	-	-
	Clay Loam	0.3^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}
1500	Sandy Silt Loam	0.3^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}
	Sandy Loam	0.2^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}
	Clay Loam	0.5^{bc}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}

Biofumigation and encysted *G. pallida* viability and multiplication under glasshouse conditions

There was an effect of treatment, sampling point and the interaction between the two on *G. pallida* hatch (Table 3). During plant growth there were no significant differences between biofumigant cultivars / species and plant controls. Post-incorporation, ISCI 99 and Scala treatments significantly reduced hatch compared to all other treatments during growth and post-incorporation. None of the other treatments had an effect on hatch compared to the controls and, with the exception of the *B. juncea* treatments (ISCI 99 & Scala), there were no differences before and after plant incorporation for each individual treatment.

Post-incorporation, the percentage of dead and hatched J2 were significantly affected by treatment ($P<.001$ for both; Figure 1). Both ISCI 99 and Scala increased mortality with a corresponding decrease in hatched J2 compared to the other treatments. There was not an overall effect of treatment on unhatched viable J2 ($P=0.096$).

Table 3. Sinigrin concentrations (mg g-1 DW) and hatch of *G. pallida* J2s. For all data, means followed by the same letter are not significantly different. ND is not detected.

Treatment	Sinigrin	*G. pallida* hatch	
	mg g-1 DW	During Growth	Post-incorporation
Fallow	-	-	826.3b
Bento (*Raphanus sativus*)	ND	786.0b	870.0b
Ida Gold (*Sinapis alba*)	ND	1034.0b	846.0b
ISCI 99 (*Brassica juncea*)	32.3	858.0b	97.0a
Nemat (*Eruca sativa*)	ND	755.8b	1013.7b
Scala (*B. juncea*)	41.2	1149.5b	39.3a
Temple (*B. napus*)	ND	955.0b	888.7b
Bristle Oats (*A. strigosa*)	-	539.0b	1143.0b

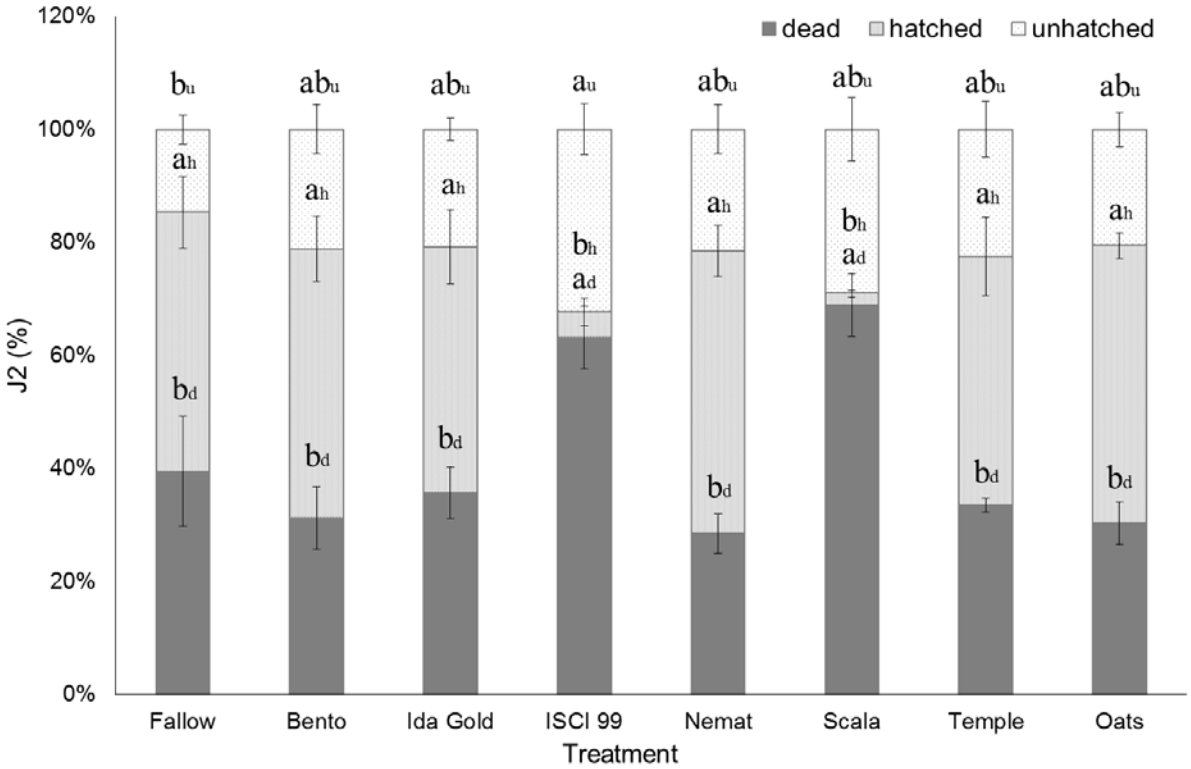

Figure 1. Percentage of *G. pallida* J2 that were: dead, hatched and unhatched viable four weeks post-incorporation in the second pot trial. Error bars represent the standard errors. Significant differences are shown by different letters; the categories to which these relate are indicated by one of the subscript letters: d (dead) h (hatched) u (unhatched viable).

DISCUSSION

Previous studies have not examined the effect of pure AITC in soil on *G. pallida*. These studies provide valuable information with respect to the effect of AITC in soil and the potential influence of various factors on AITC efficiency, specifically: the presence of soil, ITC combination, AITC concentration, soil composition, and temperature. The presence of soil decreased the efficiency of AITC as a higher AITC concentration was required for complete hatch suppression and an increase in mortality compared to *in vitro* experiments (Wood *et al.*, 2017). *In vitro*, 100ppm AITC suppressed *G. pallida* whereas in the pot trials, 100ppm AITC had no effect on PCN hatch or multiplication. A concentration of 500ppm AITC applied to soil was the lowest able to completely inhibit hatch and increase mortality. It should be noted that concentrations between 100ppm and 500ppm were not included in the soil trials so there is the potential that AITC release within this range would be effective. The decrease in AITC efficiency in soil compared to *in vitro* is likely due to several factors: increased surface area, increased headspace, and contact interference. AITC would volatize into the environment quicker, reducing the direct contact of AITC to cysts. In addition, AITC would be interacting with the organic material portion of soil (Borek *et al*., 1995; Brown &Hampton, 2011). Due to the decreased efficiency noted, biofumigant cultivars containing a high concentration of sinigrin would be required for successful *G. pallida* control in soil.

The *B. juncea* (ISCI 99 and Scala) green manures effectively suppressed *G. pallida* populations. A reduction in hatch was noted with a corresponding increase in encysted J2 mortality providing evidence to support that these cultivars release nematotoxic compounds. These results are consistent with a soil microcosm study where three sinigrin-containing *B. juncea* green manures applied to *G. pallida* cysts in soil caused over 95% mortality of encysted J2 (Lord *et al*., 2011). In contrast, a previous study found no effect of *B. juncea* incorporation on *G. pallida* hatch in a pot trial (Brolsma *et al*., 2014). This is most likely due to lower sinigrin concentrations where, assuming a 1% GSL to ITC conversion (Morra and Kirkegaard, 2002), the plants released <50ppm AITC which as this study has shown is too low to control *G. pallida* in soil. In the current study, the ISCI 99 and Scala cultivars reduced the number of newly formed cysts compared to a fallow control but had no effect on the egg content of cysts. This suggests that the released ITCs are toxic to the encysted J2 present but have no effect on the ability of the *G. pallida* J2 to reproduce once hatched. Encysted *G. pallida* suppression by ISCI 99 and Scala compared to other treatments cannot be attributed to differing soil moisture content, due to standardisation, or to the amount of fresh material incorporated, as high and low biomass treatments affected encysted *G. pallida* similarly, rather there appears to be a clear biofumigant effect of the *B. juncea* cultivars. This is most likely due to the major GSL found in ISCI 99 and Scala, sinigrin. Sinigrin concentration was high in both cultivars at time of incorporation and able to release a minimum of 323ppm and 412ppm AITC for ISCI 99 and Scala, respectively, if assuming a minimum 1% GSL to ITC conversion during hydrolysis. This provides evidence that cultivars containing high concentrations of sinigrin (above 30mg g-1 DW) can be effective at suppressing *G. pallida* in soil.

In contrast to the *B. juncea* cultivars, none of the other treatments had a suppressive effect on encysted *G. pallida*. A lack of effect with *S. alba* and *R. sativus* incorporation on *G. rostochiensis* hatch has been shown in a previous glasshouse trial (Valdes *et al*., 2011). In a later study *S. alba* and *R. sativus* green manures reduced *G. rostochiensis* multiplication on potato in a pot trial (Fatemy & Sepideh, 2016). Fatemy and Sepideh, (2016) did not analyse the GSL content of incorporated material and therefore it is not known if inconsistencies are due to the type and concentration of ITCs released; experimental conditions did differ from the present study as they used a different soil type (40% sandy loam) and did not track the moisture content of soil during biofumigation. Differences between biofumigant cultivars highlight the importance of the major type of GSL produced in each cultivar with respect to targeted pest control. The differences between cultivars on *G. pallida* suppression under

controlled conditions demonstrates the necessity to study the effect of potential biofumigants on pathogens prior to use, as different biofumigants can exert a variety of effects depending on their GSL content.

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DIMETHYL DISULFIDE FOR CONTROLLING THE POTATO CYST NEMATODE *GLOBODERA* SPP. IN POTATO IN NORTHERN EUROPE

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Summary: Dimethyl Disulfide (DMDS), as a shank formulation (99.1%), was tested to control Potato Cyst Nematode (PCN) *Globodera pallida* in potato. Five trials were carried out in the UK (2 trials in 2015) and the Netherlands (2 in 2014 and 1 in 2015). DMDS was applied at rates of 300 and 400 kg a.i./ha (respectively 280 and 380 litres/ha) and compared with Metham Sodium (MS) at 153 kg a.i./ha (300 L product/ha) and untreated control. PCN numbers were recorded in all trials. All fumigant treatments increased yields when compared to the untreated control. An application rate of 400 kg a.i./ha DMDS controlled the nematode by as much as 84% over untreated (standard MS was 82%). The relative yield was 157% for DMDS 400 kg a.i./ha (untreated considered 100%) and 148% for the standard MS.

INTRODUCTION

Potato is an important crop in Europe, particularly in Northern Europe, where 121,000 ha and 162,600 ha were cultivated in the UK and the Netherlands respectively in 2017.

Potato crops in the UK and the Netherlands are vulnerable to potato cyst nematode (PCN) attack from *Globodera* species (*G. pallida* and *G. rostochiensis*). These nematodes are included in EPPO A2 Pest Quarantine List (present in Europe but under regulated control measures). In the UK, PCN is estimated to cost the potato industry (mainly processing and fresh market sectors) £26M with current control options (AHDB Potatoes R415); however, if current control measures were removed, this figure would increase to £55M (based on recent AHDB Potatoes estimates).

Dimethyl Disulfide (DMDS) will be available from Arkema with Certis providing market support once registered in the Netherlands and the UK. At present the active ingredient is under registration in the EU. DMDS has been shown to be effective in several open field situations in different host crops against the most important plant-parasitic nematodes, such as *Meloidogyne* spp., *Heterodera carotae* and *Pratylenchus* spp. (Curto *et al*., 2014; Fritsch *et al*., 2014). *Globodera* spp. are reported to be controlled by DMDS in Southern Europe (Fritsch *et al*., 2014). Five trials were conducted in 2014/15 to assess the effectiveness of DMDS to control PCN in potato crops in Northern Europe, namely in the Netherlands (3 trials, 2 in 2014 and 1 in 2015) and the UK (2 trials in 2015).

MATERIALS AND METHODS

Trial Design

All 5 trials were conducted according to a randomized complete block design with four

replicates per trial. DMDS was applied at the rates of 300 and 400 kg a.i./ha as shank fumigation (broadcast) and compared with the standard fumigant metam sodium (MS) (brands Metham in UK and Monam in The Netherlands). Gas tight films (Virtually Impermeable Films = VIF) were used to cover all plots fumigated with DMDS but not those treated with MS or non-treated controls. Note that when the trials were carried out the MS label did not stipulate the use of film in either country.

Soil temperatures ranged from 8 to 11 °C at application step, but generally they increased in the period of plastic covering after the applications. All trial sites were infested with *Globodera pallida*. Details of the trial sites are shown in Table 1. Sampling for PCN was done from the net area of each plot. In NL, 24 cores were sampled from each plot, providing 1 litre soil per plot. Sampling depth was 20cm. In the UK 20 cores were taken providing 2 litres of soil per plot and sampling depth was 15 cm.

Plot size gross area was minimum 12m in length and 3m width (36 m^2), while the net area was at least 3.6m long and 1.5 m width (5.4 m^2).

Table 1. Characteristics of the 5 trial sites including location of trial, date of application of fumigant, soil type, organic matter (OM%), temperature of soil on application of fumigant and initial (Pi) PCN populations as eggs/g soil.

Nr	Location	Date of application	Soil			Pi eggs/g. soil
			Type	OM (%)	Temp (°C)	
1	Erm, NL	15 April 2014	Sand	4.8	9	38
2	Nieuwe Pekela, NL	15 April 2014	Sand	9.4	8	24
3	Nieuwe Pekela, NL	22 April 2015	Sand	6.5	11	24
4	Tumby, UK	30 April 2015	Sand	6.5	11	25
5	Gosberton, UK	01 May 2015	Silt	n.a.	8	13

In the UK cultivars Melody and Victoria were used at Gosberton and Tumby respectively. In the Netherlands all trials used cv. Hansa. Cultivars Melody and Victoria are resistant to *G. rostochiensis* Ro1 and susceptible to *G. Pallida* Pa 2/3. Cultivar Hansa is susceptible for Ro1, Ro2, Ro3, Pa2 and Pa3. For the varieties, no resistance score against *G. Pallida* 2 and 3 is mentioned in the official Dutch variety list which is published on the website of the "Netherlands Food and Consumer Product Safety Authority". If there is no resistance then no scores are used.

Shank Applications

In all trials, applications were made by shank at 18-20 cm depth by experienced applicators. The fumigation was carried out by using equipment supplied and used by the contractor, C. Thijssen. The Forigo Deeper Ino 300 was used to apply DMDS. For MS, the Imants shank injector was used, in combination with a spading machine (standard practice) to mix the MS.

Film Tarping

In all trials, the plots fumigated with DMDS were covered with gas tight film before the shank application. Film removal occurred 22-28 days after treatment in the NL trials and 27 to 28 days in UK trials. Plastic tarps were cut the day before removal. Plots treated with the standard fumigant MS were not covered with gas tight film as per label at time of operation and as standard practice in the UK by the only contractor.

Observations and Statistical Analysis

Crop safety assessments were carried out on all trials by recording crop emergence and other crop stages associated with potato production.

To assess the initial population of PCN (Pi), soil samples were taken 1 day prior to application of treatments. The initial population is expressed as eggs/g soil, assuming that all eggs were viable (containing living larvae). To assess the effect of the fumigants on the nematode (Pf), cysts of the nematode were again extracted from the soil after sampling just before potato planting and the nematode population expressed as living larvae/g soil. The time between initial sampling and final sampling before planting was 35 days in the UK trials and 26 - 57 days in the NL trials. For the analysis of the number of cysts and content (eggs), the samples were dried, weighed, and washed using the Fenwick can wash method extract the cysts from the soil. For the Pi, the cysts were crushed so the number of eggs could be counted. For the Pf, a natural hatching agent was used to hatch the living larvae from the eggs. Only living larvae emerge from the cysts during the hatching test. Unhatched eggs were considered dead. Analysis of Pi samples (eggs/g) in the UK was carried out by Richard Austin associates. In NL, Pi sampling analysis was done by Hilbrands Laboratorium. All Pf samples were analysed by Hilbrands Laboratorium. Finally, for statistical analysis of the data, analysis of variance was used. If the statistical program recommended a transformation on the Pf results, a log transformation was carried out.

RESULTS

No crop safety issues were observed in any of the five trials carried out in 2014 and 2015.

Table 2 summarises the effect on the Pf of the different fumigant treatments for all trial sites. The overall average efficacies were 84% for DMDS 400, 73% for DMDS 300, and 82% for the standard MS. Due to high variation within the Pi and Pf on the Erm and Tumby sites, the efficacy results were not significant. But the dose response seen on these 2 sites were in line with the results of the other sites.

Table 2. Initial (Pi) and final (Pf) nematode population densities (per gram soil) recorded in the five trials of PCN control

	Erm 2014			Nieuwe Pekela 2014			Nieuwe Pekela 2015			Tumby 2015			Gosberton 2015		
	Pi[1]	Pf[2,5]	Eff[3]	Pi[1]	Pf[2,5]	Eff[3]	Pi[1]	Pf[2]	Eff[3]	Pi[1]	Pf[2]	Eff[3]	Pi[1]	Pf[2,5]	Eff[3]
Untreated	52a[4]	59a	-	31a	28a	-	19a	23a	-	18a	30a	-	17a	10a	-
DMDS 400	34a	0.1a	99	22a	3b	81	22a	7bc	73	29a	13a	73	13a	0.4bc	94
DMDS 300	32a	3a	92	27a	8ab	68	29a	17ab	52	27a	20a	56	6a	0.2c	95
MS 153	34a	11a	73	19a	5ab	66	21a	1c	96	32a	7a	87	12a	1.0b	87
LSD p=0.05	*73*	*12*		*17*	*22*		*20*	*13*		*20*	*21*		*16*	*4*	
F-Prop	*0.97*	*0.14*		*0.71*	*0.003*		*0.75*	*0.02*		*0.61*	*0.14*		*0.48*	*0.001*	

[1]Eggs/gram soil; [2]Living larvae/gram soil;
[3]Efficacy according Henderson &Tilton Formula:

$$100 * \left[1 - \frac{Ta - Cb}{Ca - Tb} \right]$$

Ta = number of nematodes after application in the treated object
Tb = number of nematodes before application in the treated object
Ca = number of nematodes after application in the untreated control
Cb = number of nematodes before application in the untreated control

[4] Means followed by the same letter do not significantly differ (P=0.05, LSD)
[5] Log transformation carried out

The average relative yield for each of the trail sites is shown in Table 3. The overall average relative yield for each of the treatments were 157% for DMDS 400, 155% for DMDS 300, and 148% for the standard MS. The yield effects on the Erm site were lower compared to the other 4 sites. This site did suffer from heavy rain fall in late May over a 3 day period. Yield results at this site were variable as some potato ridges were completely under water and very wet for a long period which affected the growth negatively.

Table 3 Effect of the treatments on potato yield (t/ha) and relative yield (%)

	Erm 2014		Nieuwe Pekela 2014		Nieuwe Pekela 2015		Tumby 2015		Gosberton 2015	
	Yield	Rel. Yield	Yield	Rel. Yield	Yield	Rel. Yield	Yield	Rel. Yield	Yield	Rel. Yield
Untreated	51.6 a	100%	34.7 a	100%	40.2 a	100%	15.6 a	100%	29.3 a	100%
DMDS 400	55.9 a	108%	47.1 b	136%	53.8 b	134%	31.6 b	202%	59.6 b	203%
DMDS 300	61.9 a	120%	49.6 b	143%	51.5 b	127%	29.9 b	192%	56.4 b	192%
MS 153	57.6 a	112%	52.4 b	151%	52.8 b	131%	26.5 b	170%	52.0 b	177%
LSD p=0.05	*10.8*		*5.1*		*6.2*		*6.8*		*8.8*	
F-Prop	*0.35*		*0.0002*		*0.004*		*0.003*		*0.0001*	

DISCUSSION

Even with applications in spring, as in these trials, there was no obvious adverse effect on crop emergence after the use of DMDS in any of the trials in 2014 or 2015 in the UK or the Netherlands. Normally potato growers would apply a soil sterilant (i.e. MS) in early autumn when there is still warmth in the soil and moisture levels have risen for more effective treatment.

All treatments effectively controlled the PCN and increased yield in all 5 trials indicating robustness of both the standard treatment MS and the potential new soil sterilant DMDS. On average the 5 trials indicated that DMDS at the 400 kg a.i./ha rate resulted in 84% efficacy compared to 82% efficacy of the standard MS treatment.

Although growers can use PCN resistant cultivars, there is also the need to control PCN with soil sterilants such as DMDS, especially in the presence of high initial PCN levels. The availability of various control measures is essential for maintaining a sustainable and stable production of potatoes. The results demonstrate that DMDS, after its registration, can be an effective tool in addition to existing fumigants.

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PREDICTIVE DIAGNOSTICS FOR SOIL-BORNE DISEASES OF POTATO: AN ESSENTIAL COMPONENT OF IPM

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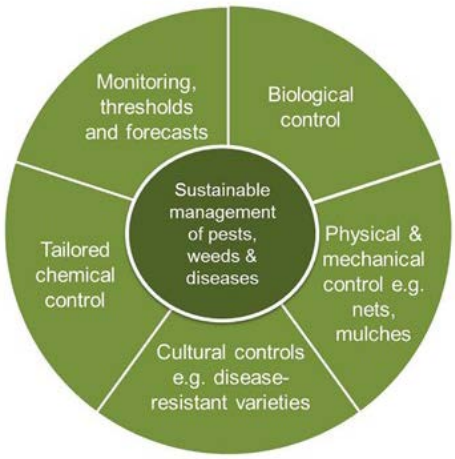
Summary: Molecular diagnostic methods to detect and quantify soil-borne potato pathogens have been developed. Establishing disease risk associated with soil-borne inoculum informs decisions on Integrated Pest Management (IPM). In this paper we review risk predictions for black dot and powdery scab, and outline the ongoing research into establishing disease risk thresholds for *Rhizoctonia solani* AG3 and pathogenic *Streptomyces* spp.

INTRODUCTION

Integrated Pest Management (IPM) is an environmentally and economically sustainable approach to managing the impact of crop pests, pathogens and weeds. IPM strategies combine available methods (IPM tools) for pathogen and disease monitoring, risk prediction and control of pest, pathogen and weed populations into programmes where the tools operate synergistically to reduce pests and disease with minimal environmental impact and economic risk. IPM as summarised by the Scottish Government are shown in Figure 1. http://www.gov.scot/Topics/farmingrural/Agriculture/Environment/Pesticides/IntegratedPestManagement



- Appropriate cultural controls such as crop rotations and the use of resistant varieties.
- Physical and mechanical controls including the use of nets, mulches and mechanical weeding.
- Enhancement of wildlife habitats to encourage biodiversity and beneficial organisms that provide biological control.
- Monitoring of crops for pests, weeds and diseases and the use of forecasts and thresholds for treatment.
- Tailored and efficient use of chemical inputs such as fertilisers and pesticides.

Figure 1. The main components of IPM as summarised by the Scottish Government.

Soil-borne pathogens of potato cause a number of serious blemish diseases. By employing appropriate soil sampling strategies in conjunction with a method for soil DNA extraction and real-time PCR assays to detect and quantify target pathogens, we can establish the relationship between soil-borne inoculum and disease risk. Knowing the risk of disease associated with soil-borne inoculum allows informed decisions on IPM to be made. Further

information on IPM research at The James Hutton Institute can be found at http://ipm.hutton.ac.uk/

PREDICTING DISEASE RISK FROM SOIL-BORNE PATHOGENS

Soil sampling and pathogen quantification

An appropriate soil sampling strategy underpins the validity of pathogen detection and disease risk assessments on a field scale. A soil sample should be taken pre-planting in a W shape across a field (<4 ha) consisting of 1kg bulked from soil taken using a mini auger, narrow trowel or grass plot sampler from 100 points (0-10cm depth). After thoroughly mixing the sample, a sub-sample (60g) is taken for processing using a Planetary Ball Mill PM400 (Retsch) prior to DNA extraction (Brierley *et al*., 2009). Inoculum levels of *Colletotrichum coccodes*, *Spongospora subterranea*, *Rhizoctonia solani* AG3 and pathogenic *Streptomyces* spp. can be determined using real-time PCR assays (Cullen *et al*., 2002; van de Graaf *et al*., 2003, Lees *et al*., 2002 and Qu *et al*., 2011, respectively). Before an estimate of disease risk can be ascribed to detectable pathogen levels, a process of validation is required.

Assessing disease risk

Through the monitoring of commercial potato crops and extensive glasshouse and field trials, the risk of black dot and powdery scab associated with levels of *C. coccodes* and *S. subterranea* respectively has been determined. Work is ongoing to establish disease risk associated with *R. solani* AG3 and pathogenic *Streptomyces* spp.

Black dot: Soil inoculum levels of *C. coccodes* can be reliably quantified and levels relate to risk of disease (Lees *et al*., 2010). It has been demonstrated that seed-borne inoculum is relatively less important than soil-borne inoculum in causing black dot on progeny tubers at harvest. The incidence and severity of black dot at harvest were low in trials where seed was the main source of inoculum, irrespective of the level of seed-borne inoculum visibly present at planting (Lees *et al*., 2010). The effect of soil inoculum on black dot development on tubers has been studied in conjunction with control options such as; cultivar resistance, azoxystrobin in-furrow treatment, irrigation and crop duration (time from planting to harvest) (Brierley *et al*., 2015). Therefore, by assessing disease risk associated with soil inoculum, growers can make informed decisions regarding cultivar selection and further reduce risk using a combination of reduced irrigation, shorter crop duration and in-furrow application of azoxystrobin where appropriate.

Powdery scab: Quantifying *S. subterranea* in soil prior to planting provides an assessment of disease risk (Brierley *et al.*, 2013). With no effective chemical control options, utilizing site selection (i.e. avoiding high risk fields) and host resistance remain the most effective strategies for controlling powdery scab. Reliable alternative crop protectants and bio-pesticides effective against powdery scab have yet to be identified, but could potentially provide a component to IPM in the future.

Black scurf: Detection of *R. solani* AG3 in field soil indicates an increased risk of black scurf developing in a crop (33% when inoculum detected compared to 11% when not); however, disease was found in crops even when no seed or soil-borne inoculum was detected (Brierley *et al.*, 2016). Ways to improve the robustness of detection are being explored, for example, increasing sampling intensity and extraction of the fungal propagules from soil preceding DNA extraction which may increase sensitivity of detection and optimise the value of testing for this pathogen.

Common scab: The causal pathogen of common scab is a group of saprophytic bacteria described here as pathogenic *Streptomyces* spp. Difficulty with identifying a diagnostic target specific to only pathogenic species and strains of *Streptomyces* has hindered research into establishing disease risk associated with seed- and soil-borne inoculum. Validation of the quantification of seed and soil inoculum using a real-time PCR assay (Qu *et al.*, 2011) which detects txtAB genes, widely thought to be associated with the majority, if not all, of the pathogenic *Streptomyces* spp. is underway.

To date three seed stocks, 24 washed tubers per stock, have been tested. One stock was disease free, a second had a 4% incidence, and the third a 29% incidence of common scab based on visual inspection. All tubers with common scab symptoms tested positive for pathogenic *Streptomyces* spp. when tested with real-time PCR. A number of symptomless tubers also tested positive (at relatively low levels). In the seed stock with no symptoms this might indicate symptomless infection, in the other two stocks, as symptoms were present on some tubers, it is more difficult to distinguish symptomless infection from surface contamination. The quantification of pathogenic *Streptomyces* spp. in artificially inoculated soil has been demonstrated (Figure 2). Artificially inoculated soils are currently being used to determine sensitivity of inoculum detection and establish the relationship between soil inoculum and disease development in glasshouse trials. Additionally, seed stocks and field soil are being tested to determine our ability to detect inoculum and determine its relation to disease development. This research is utilising the centre for sustainable cropping (CSC), a 6 field-rotation, at The James Hutton Institute.

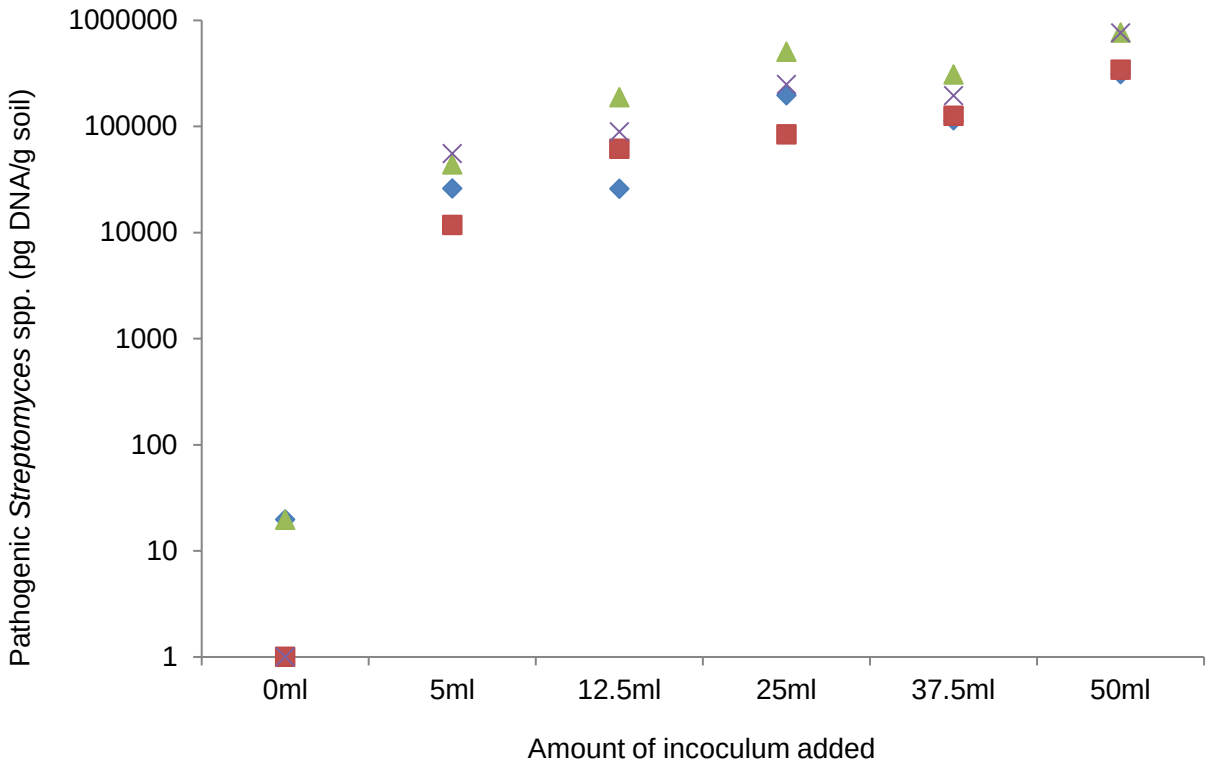


Figure 2. Detectable *Streptomyces* spp. (pg DNA/ g soil) in soil inoculated with increasing amounts of inoculum suspension (*Streptomyces* spp. isolated from common scab lesions on cultivar Maris Piper): 4 replicate soils per inoculum level.

CONCLUSIONS

Control options to reduce the risk of blemish diseases developing on potato crops can be employed based on knowledge of disease risk associated with individual fields. This development enables field selection to be a principal component of integrated disease control. Where a number of fields are tested, those posing a high risk to disease development can be avoided, or planted with a cultivar with some resistance. This is particularly useful on rented land where cropping history may not be known. On land which does not contain inoculum, or where there is a low risk associated with soil inoculum level, planting of infected seed should be avoided to prevent further soil contamination. By quantifying soil inoculum prior to planting, growers can exploit host resistance and target cultivars to fields. Utilizing host resistance remains the most effective strategy for controlling powdery scab, whilst for black dot other crop management options can be employed to further reduce risk; such as reduced irrigation, early harvest and where appropriate in-furrow application of azoxystrobin. Work is continuing to establish robust predictive risk assessments for black scurf and common scab.

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ABBREVIATIONS

The following abbreviations can be used without definition

acid equivalent	a.e.	milligrams per litre	mg/l
active ingredient	a.i.	milligrams per kg	mg/kg
approximately	c.	millilitres(s)	ml
body weight	b.w.	millimetre(s)	mm
boiling point	b.p.	Minimum	min
centimetre(s)	cm	minimum harvest interval	MHI.
coefficient of variation	CV	minute (time unit)	min
colony-forming unit(s)	cfu	moisture content	M.C.
compare	cf	molar concentration	M
concentration x time product	ct	no significant difference	NSD
concentration required to kill 50% of test organisms	LC_{50}	organic matter	o.m
		page	p.
correlation coefficient	*r*	pages	pp.
cultivar	cv.	parts per billion	ppb
cultivars	cvs.	parts per million	ppm
day(s)	d	parts per trillion	ppt
days after treatment	DAT	pascal	Pa
degrees Celsius (centigrade)	DC	percentage	%
degrees of freedom	df	polyacrylamide gel electrophoresis	PAGE
dose required to kill 50% of test organisms	LD_{50}	polymerase chain reaction	PCR
		post-emergence	post-em.
dry matter	d.m.	power take off	p.t.a.
emulsifiable concentrate	EC	pre-emergence	pre-em.
enzyme-linked immuno-sorbant assay	ELISA	pre-plant incorporated	ppi
fast-protein liquid chromatography	FPLC	probability (statistical)	*p*
for example	e.g.	relative humidity	r.h.
freezing point	f.p.	revolutions per minute	rev/min
gas chromatography-mass spectrometry	GC-MS	second (time unit)	S
		standard error	SE
genetically modified	GM	standard error of the difference	SED
genetically modified organism	GMO	standard error of the mean	SEM
gram(s)	g	soluble powder	SP
growth stage	GS	species (singular)	sp.
hectare(s)	ha	species (plural)	spp.
high performance (or pressure) liquid chromatography	HPLC	square metre	m^2
		subspecies	ssp.
high volume	HV	suspension concentrate	SC
hour	h	systemic acquired resistance	SAR
integrated crop management	ICM	tandem mass spectrometry	MS-MS
integrated pest management	IPM	technical grade	tech.
kilogram(s)	kg	temperature	temp.
kilogram(s) per hectare	kg/ha	thin-layer chromatography	TLC
kilometres per hour	km/h	time for 50% loss; half life	DT_{50}
least significant difference	LSD	tonne(s)	t
litre(s)	litre(s)	tonne(s) per hectare	t/ha
litres per hectare	litres/ha	ultralow volume	ULV

logarithm, common, base 10	log	vapour pressure	v.p.
logarithm, natural	ln	variety (wild plant use)	var.
low volume	LV	volume	**V**
maximum	max	water dispersible granule	WG
maximum residue level	MRL	weight	*wt*
metre(s)	m	weight by volume	*wt/v*
metres per second	m/s	weight by weight	*wt/wt*
milligram(s)	mg	wettable powder	WP
less than	<	mega (x 10^{6})	M
more than	>	kilo ($x10^{3}$)	k
not less than	|<	milli ($x10^{-3}$)	m
not more than	|>	micro ($x10^{-6}$)	*μ*
		nano ($x10^{-9}$)	n
		pico ($x10^{-12}$)	p