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SCOTLAND IN THE INTERNATIONAL YEAR OF PLANT HEATH

I K Toth[1] and G S Saddler[2]
[1]*The James Hutton Institute, Invergowrie, Dundee DD2 5DA*
[2]*SASA, Roddinglaw Road, Edinburgh EH12 9FJ*
E-mail: ian.toth@hutton.ac.uk

Plants play important roles in many aspects of our lives including the environment, biodiversity, our diet and wellbeing and were recently valued at £19.2 billion p.a. to the Scottish Economy. Plant health has always been an important consideration at the heart of this but, due in part to the outbreak of ash dieback in 2012 and a major UK government (Defra) report entitled ‘A Plant Biosecurity Strategy for Great Britain’ in 2014, a new momentum in plant health began with governments, funders, researchers, industry, public bodies, and others becoming increasingly aware of the dangers that pests and diseases pose to our plants. As part of this momentum Defra, in collaboration with the devolved administrations and the Forestry Commission, set up the UK Plant Health Risk Register to record plant health threats. There are now more than 1000 registered threats and the number is increasing (https://secure.fera.defra.gov.uk/phiw/riskRegister/), due in part to climate change and increases in trade and tourism.

In 2016, the Scottish Government produced ‘The Scottish Plant Health Strategy’ and soon afterwards appointed their first Chief Plant Health Officer for Scotland (CPHOS – Gerry Saddler), who has a role to provide strategic and tactical leadership across crops, forestry and the natural environment as well as to minimise the risk and impact of plant health threats in Scotland. The CPHOS works closely with the UK plant health service and is responsible for co-ordinating the Scottish Government’s plant health response in terms of policy, inspections and surveillance activities. It is also the role of the CPHOS to lead and coordinate the Scottish Government’s response in the event of a plant health outbreak and provide stakeholder guidance.

At the same time and arising directly from ‘The Scottish Plant Health Strategy’, the Scottish Government created Scotland’s Plant Health Centre (PHC) of expertise to provide rapid call down evidence to inform policy decisions. Working under the direction of the CPHOS, the PHC brings together the different plant sectors across forestry, horticulture, environment and agriculture to co-ordinate plant health knowledge, skills and activities across Scotland. The PHC is led by Ian Toth and managed by Sonia Humphris from the James Hutton Institute, and has a wider Directorate including SRUC (Fiona Burnett), Forest Research (Chris Quine) and the Royal Botanic Garden Edinburgh (Pete Hollingsworth). It also has a Science Advisory and Response Team (SART) made up of 11 other plant health experts from organisations across Scotland and the wider UK.

On the request of the CPHOS and stakeholders from the different sectors, the Centre has commissioned over 20 projects, since its launch in 2018, designed to fill important evidence gaps. Where possible, it has developed projects that are of direct relevance to all sectors, e.g. ‘Impacts of climate change on the spread of pests and diseases in Scotland’, and ‘Network analysis – where do people in Scotland get their plant health information?’ Further it has sought to map plant health expertise from across the UK as a vital step in creating a network of expertise able to inform policy or respond in an outbreak situation. However, several projects have been aimed fully or partly at the agricultural / horticultural sectors, including:

- Impact on Scottish crops if the molluscicide metaldehyde is withdrawn
- Potential impacts arising from pesticide withdrawals to Scotland's plant health
- The future threat of PCN in Scotland
- Monitoring for the Brown Marmorated Stink Bug (BMSB) *Halyomorpha halys* on soft fruit in Scotland
- Assessing the potential of the psyllid *Trioza anthrisci* to vector the zebra chip pathogen *Candidatus* Liberibacter solanacearum (Lso) in Scotland

As well as bringing together the different plant health sectors, the PHC aims to develop knowledge networks, examples of which include: bringing together experts from across the UK working on the devastating bacterial pathogen *Xylella fastidiosa*; facilitating the training of scientists to identify potential insect vectors for this pathogen.

The PHC has organised or taken part in over 50 events to engage with stakeholder groups in Scotland, raising awareness of plant health threats and identifying issues for further study. Working with colleagues in Scottish Government, the Horticultural Trades Association (HTA), Scottish National Heritage (SNH) and Scottish Forestry each year the PHC organises Scotland's Plant Health Conference (for information on the conference this year and to access content from previous years please visit www.planthealthcentre.scot/events). The PHC has also featured on BBC Scotland's Beechgrove Garden TV show, which has an audience of over a million people across the UK (500,000 in Scotland alone). Through the show, the PHC was able to promote messages directly to members of the public of the need to exercise care when purchasing plants and the risks associated with bringing back plants from trips abroad.

Bringing plants into the UK from abroad, whether on a commercial or personal basis, is a major route by which pests and pathogens can enter Scotland. The European Plant Protection Organisation (EPPO)-led campaign 'Don't Risk It' aims to raise public awareness about the risks of moving plants and their associated pests during international travel and to encourage responsible behaviour.

2020 is the International Year of Plant Health and so, more than ever, plant health is at the top of everyone's agenda, and this year the CPHOS and PHC have a plethora of engagements arranged to raise the profile of plant health to a wider audience, and to ensure that it remains a key consideration to all those working with plants wherever they are and whatever their use (to find out more visit: https://www.planthealthcentre.scot/international-year-plant-health).

For further information on the CPHOS visit:
Web: www.sasa.gov.uk/content/cphos Twitter: @plantchiefscot

For further information on the PHC visit:
Web: www.planthealthcentre.scot Twitter: @PlantHealthScot

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UPTAKE OF INTEGRATED PEST MANAGEMENT ACTIVITIES IN SCOTTISH AGRICULTURAL AND HORTICULTURAL CROP PRODUCTION

J Hughes, C Monie, G Reay and J Wardlaw
SASA, Scottish Government, Roddinglaw Road, Edinburgh, UK, EH12 9FJ
E-mail: Jackie.Hughes@sasa.gov.scot

Summary: This paper summarises a series of surveys describing the uptake of integrated pest management (IPM) activities by Scottish growers. The results demonstrate that, whilst pesticide use is integral to Scottish crop production, all sectors also adopt a range of IPM measures. This data collection series, in combination with statistics describing pesticide use, is designed to inform the Scottish Government about Scottish crop protection practices. This will help to describe and predict grower response to crop protection drivers such as changes in pesticide availability, advances in crop protection technologies and the impact of Government initiatives to encourage greater sustainability in the use of pesticides.

INTRODUCTION

The EU Sustainable Use of Pesticides Directive (2009/128/EC), which will be incorporated into UK domestic legislation post EU-exit, requires member states to promote low pesticide input crop management. The legislation stipulates that Governments must encourage professional users to control pests using methods with the lowest risk to human health and the environment; wherever possible prioritising non-chemical control and supporting the implementation of integrated pest management (IPM) as a central principle of crop production. Member States must demonstrate the measures they implement to achieve this in their national action plans.

It is a legislative requirement to conduct post-approval surveillance of pesticide use (EU Statistics Regulation 1185/2009/EC). In the UK, the Working Party on Pesticide Usage Surveys (WPPUS), a sub-group of the Expert Committee on Pesticides, coordinates collection of pesticide usage statistics. As a member of this group, SASA's pesticide survey unit have been monitoring and publishing pesticide usage statistics for Scottish crops for more than 50 years. However, there is no equivalent regulatory requirement to monitor uptake of IPM practices. Therefore, whilst trends in pesticide use are well documented, across crop sectors and over time, there are no corresponding data sets to describe trends in use of alternative pest control practices, or to monitor the effectiveness of initiatives encouraging the sustainable use of pesticides. To address this issue, this paper describes the introduction of a series of Scottish IPM surveys of the main agricultural and horticultural crop production sectors.

MATERIALS AND METHODS

The Voluntary Initiative IPM plan was used as the basis for developing the survey. This plan was adapted and expanded, with input from researchers at JHI and SRUC, to form a series of survey questionnaires relevant to Scottish growers in the target crop sectors. Questions were structured around three core categories: risk management (actions employed to reduce or prevent the risk of pest damage requiring control), pest monitoring (actions undertaken to ensure that control is economically justified and targeted) and pest control (actions to ensure evaluation and employment of optimal control methods in relation to efficacy and risk). Within these core categories, a series of detailed questions were developed to elicit a thorough description of the IPM measures implemented. Data were collected by one-to-one interview, either in person or by telephone. Interviews were conducted in a uniform and structured manner to ensure that they elicited a comprehensive and comparable response from growers, or their agronomists, with differing levels of familiarity with IPM practices.

The IPM surveys were conducted as a supplement to the Scottish Government's pesticide monitoring, and as such survey timing reflects the predetermined schedule of pesticide surveys, with different crop sectors surveyed in different years (Table 1). The IPM survey data collection was a voluntary addition and data were not collected from all respondents to the pesticide survey (Table 1 displays survey response rates). Results in this paper are expressed as the number of respondents in the sample stating that they implemented the described activity. This paper is a summary of the results collected from these surveys, full details of IPM activities, pesticide use and data collection methods, employed in each crop sector are published in the individual survey reports (Monie et al, 2016, 2017 & 2018, Reay et al 2017). IPM data were also collected for edible crops grown under permanent protection (Reay et al, 2016) but are not included in this summary paper due to the limited size of the Scottish crop area (ca 30 ha).

Table 1. IPM survey timing and sample size

Crop sector	Survey year	Total crop area (ha)	No. holdings surveyed (representing % total crop)
Vegetable	2015	16,672	84 (11%)
Soft fruit	2016	1,876	33 (14%)
Arable	2016	494,167	123 (4%)
Grass & fodder	2017	Fodder 16,304	119 (8%)
		Grass 4,363,985	(<1%)

RESULTS

Pesticide Use

Summary annual pesticide use data from each survey, indicating the relative contribution from each crop sector to total Scottish pesticide use, are presented in Table 2 to provide context for the following IPM data.

Table 2. Estimated use of pesticide active substances in each crop sector

Crop sector	Total use (tonnes)	% of crop area treated	Overall app^n rate (kg/ha)	Mean no. sprays
Vegetable (2015)	67	98	4.037	5.8
Soft Fruit (2016)	15	94	7.851	9.3
Arable (2016)	1,490	98	3.015	4.2
Fodder (2017)	8	63	0.497	1.4
Grass (2017)	84	4	0.019	1.0

Adoption of Integrated Pest Management Activities

Using an IPM plan helps growers to make the best possible, and most sustainable, use of all available methods for pest control. However, it is a voluntary activity and in these surveys the majority of Scottish growers did not complete a plan. Uptake was greater in those sectors where pest control, and pesticide use, is more prevalent (36%, 24% and 18% of growers in the vegetable, arable and soft fruit sectors respectively) and lower amongst those growing grass and fodder crops (5%), reflecting their significantly lower pesticide input and crop protection activity.

IPM programmes aim to prevent, or reduce, the risk from pests by minimising the likelihood of damage that necessitates control measures. Overall, almost all surveyed growers reported that they adopted at least one measure associated with an IPM risk management approach (100% of arable, soft fruit and vegetable growers and 97% of grass and fodder crop growers). Figure 1 displays a summary of the individual actions implemented in relation to this category.

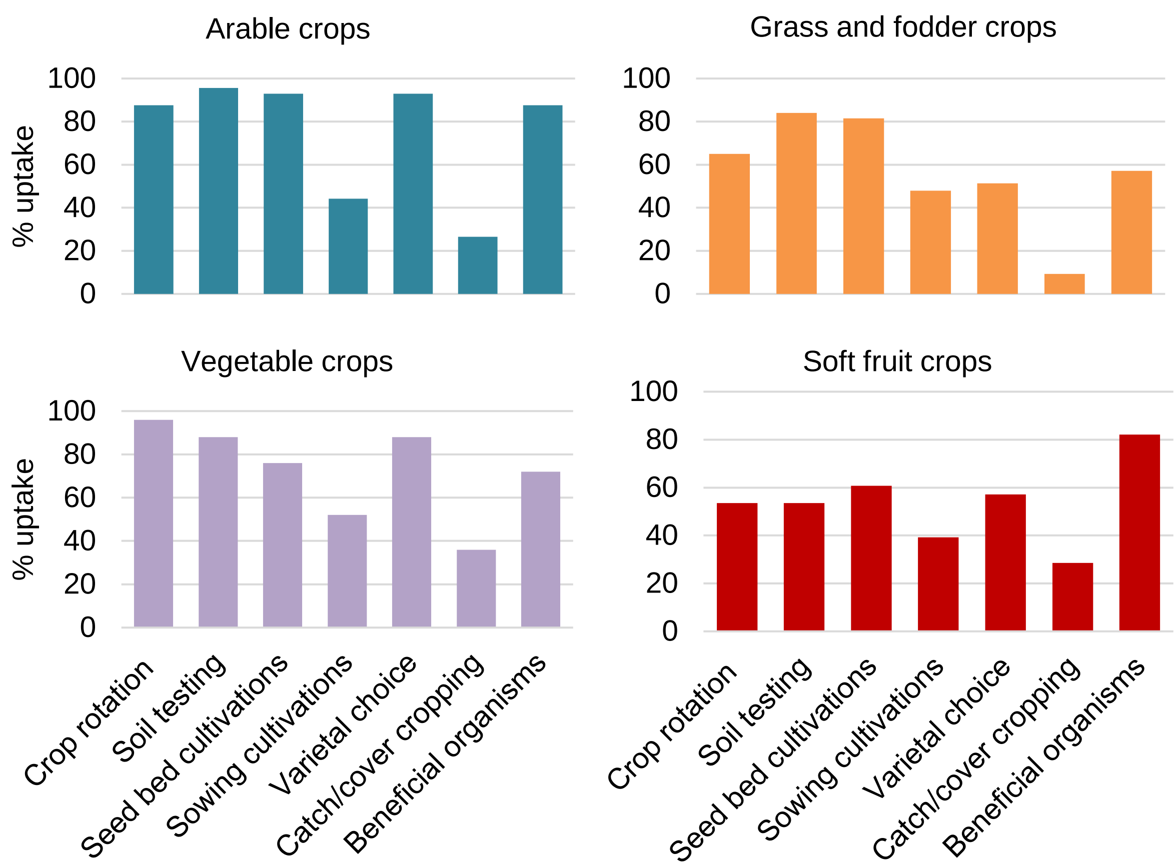

Figure 1. Adoption of risk management measures

Crop rotation, the basic tenet of farming, can reduce the risk of pest damage by breaking the link between pest and host, reducing pest populations and improving soil health and structure, and consequently crop health. Rotation was routinely adopted in the arable and vegetable sectors but less so in grass & fodder crops (much of area in permanent pasture) and soft fruit (limited growing area and range of growing media used).

The majority of growers reported testing their soil in order to tailor inputs; lower testing levels were encountered in soft fruit crops reflecting the use of non-soil growing media. In all sectors, soil testing was primarily related to investigation of nutritional status. However growers, also tested for a range of pests (nematodes in all crop sectors, wheat bulb fly & leatherjackets in cereal crops) and pathogens (such as club root and powdery scab). The vegetable sector reported the highest levels of testing for disease and nematodes (60 & 44% of respondents respectively).

Most growers (61 to 93% depending on sector), and particularly those growing arable and fodder crops, reported that they managed their seed bed agronomy to reduce pest risk. Seed bed adaptations primarily consisted of improving soil organic matter to increase crop vigour and adopting a range of different tillage and non-inversion techniques to reduce weed populations. To a lesser extent (39 to 52%) growers also adapted sowing cultivations to reduce pest risk. The most common actions were varying timing, density and occasionally depth of sowing, to reduce or mitigate damage from a range of pests (insects, birds and weeds).

Varietal choice was also a widely adopted strategy in pest risk reduction, particularly in arable and vegetable crops (93 and 88% respectively). The most common aspect was the selection of pest and pathogen resistant varieties, where available, to reduce damage. However, the use of certified seed, and testing of home saved seed, was also widely encountered. A lower proportion (around a fifth of arable, fodder and vegetable growers) reported that they employed varietal diversification (using a range of different varieties) to increase overall resistance to pests and environmental stresses.

Across all sectors, the use of catch and cover crops was low in comparison to other risk management strategies; influenced at least in part by Scotland's climate challenging the timing for establishment and growth. Approximately a third of vegetable, arable and soft fruit growers and less than a tenth of grass and fodder growers, reported routinely growing cover crops. These crops were primarily sown to improve soil quality by reducing erosion and providing a green manure. Growers also used cover crops to supress weeds and, in vegetable and soft fruit cultivation, to control pests by bio-fumigation.

The final risk management activity captured by the surveys was those actions employed to protect or enhance populations of beneficial organisms. At least one measure was adopted by 88, 82, 72 and 57% of arable, soft fruit, vegetable and grass and fodder farmers respectively. The most common was the creation and/or maintenance of uncultivated habitat areas, including grass margins, beetle banks, wild flower strips, hedging, ponds and wetlands. In the soft fruit sector more targeted enhancement techniques were encountered, such as growing mayweed at the end of tunnels to provide a host for natural predators of aphids.

The second core category of survey data collection is pest monitoring. In an IPM system, pests and crop growth stages are monitored to determine whether control is economically justified and to effectively target control decisions and options. The majority of the growers sampled (100% of arable and vegetable, 94% of grass and fodder and 89% of soft fruit growers) reported that they implemented at least one pest monitoring measure. Figure 2 displays a summary of the individual actions implemented in relation to this category.

High levels of pest and crop growth stage monitoring were reported across all sectors, particularly the field crops. In the arable, grassland and vegetable sectors this information was primarily collected by BASIS qualified agronomists (>75%) and, to a lesser extent, by self-inspection by growers. In the soft fruit sector, reflecting the more specialised nature of these crops, the majority of pest monitoring was conducted by growers (86%) rather than agronomists. Trapping to monitor pests was encountered more frequently in the vegetable and soft fruit sectors (40 and 25% respectively) than in arable and fodder crops (15 and 5% respectively, almost exclusively slug trapping). Other, less frequently used sources of pest monitoring information included press articles, technical bulletins and use of risk warnings.

Most vegetable and arable growers reported that they, or their agronomist, used action thresholds to determine whether control was necessary when monitoring pest populations (88 and 68% respectively). Lower use of action thresholds was reported in grass and fodder crops (18%, influenced by the very low pesticide input in this sector) and soft fruit (32%, perhaps reflecting a more prescriptive use of pesticides in this high value fresh market sector with a low tolerance for pest damage).

Growers also adopted the use of specialist diagnostics for identification of issues that were more problematic to identify or monitor, particularly in the arable and vegetable sectors (58 and 60% respectively). Specialist advice was sought for identifying nutritional deficiencies, for field and pest mapping services and for clinic services for identification of pests and pathogens.

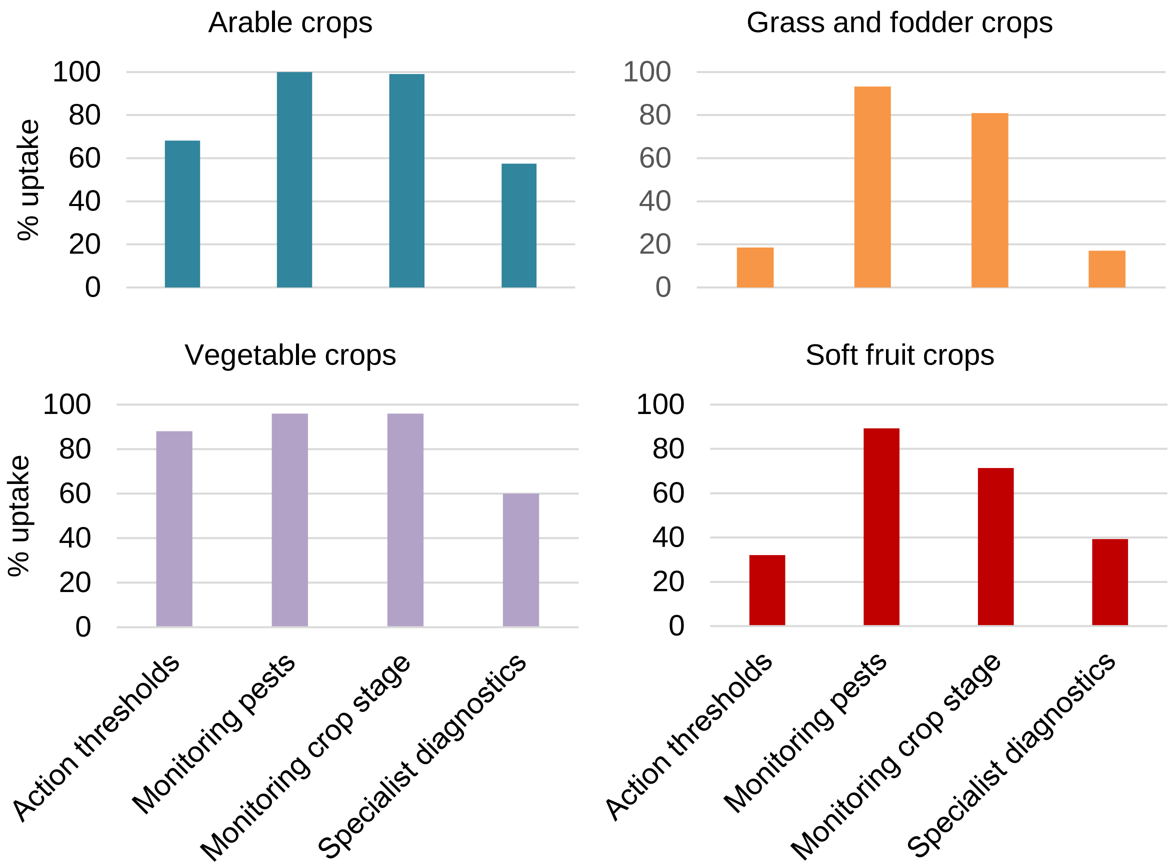

Figure 2. Adoption of pest monitoring activities

The last category of survey investigation was in relation to pest control strategies. If monitoring, identification and action thresholds indicate that control measures are required, IPM programmes aim to evaluate the best control method in relation to effectiveness and risk. This should incorporate non-chemical methods alongside, or instead of, chemical control. Where pesticides are used, they should be as targeted as possible, the risk of resistance development should be minimised, and their effectiveness reviewed. Almost all of the growers surveyed complied with at least one aspect of IPM compliant pest control (100, 100, 97 and 96% of arable, vegetable, grass & fodder and soft fruit growers respectively). Figure 3 displays a summary of the individual actions implemented in relation to this category.

The majority of growers, in all sectors, reported that they used non-chemical control in partnership or instead of chemical control (96, 87, 76 and 68% in soft fruit, grass & fodder, vegetable and arable sectors respectively). The type of non-chemical approaches used varied with sector. In grass and fodder settings, the primary non-chemical control employed was mechanical weed control (predominately mowing) and also hand rogueing and intensive grazing to control weeds. There was also, less frequent, reports of mechanical control of insect pests (rolling and harrowing for slugs and leatherjackets) and limited use of biocontrol & biopesticides.

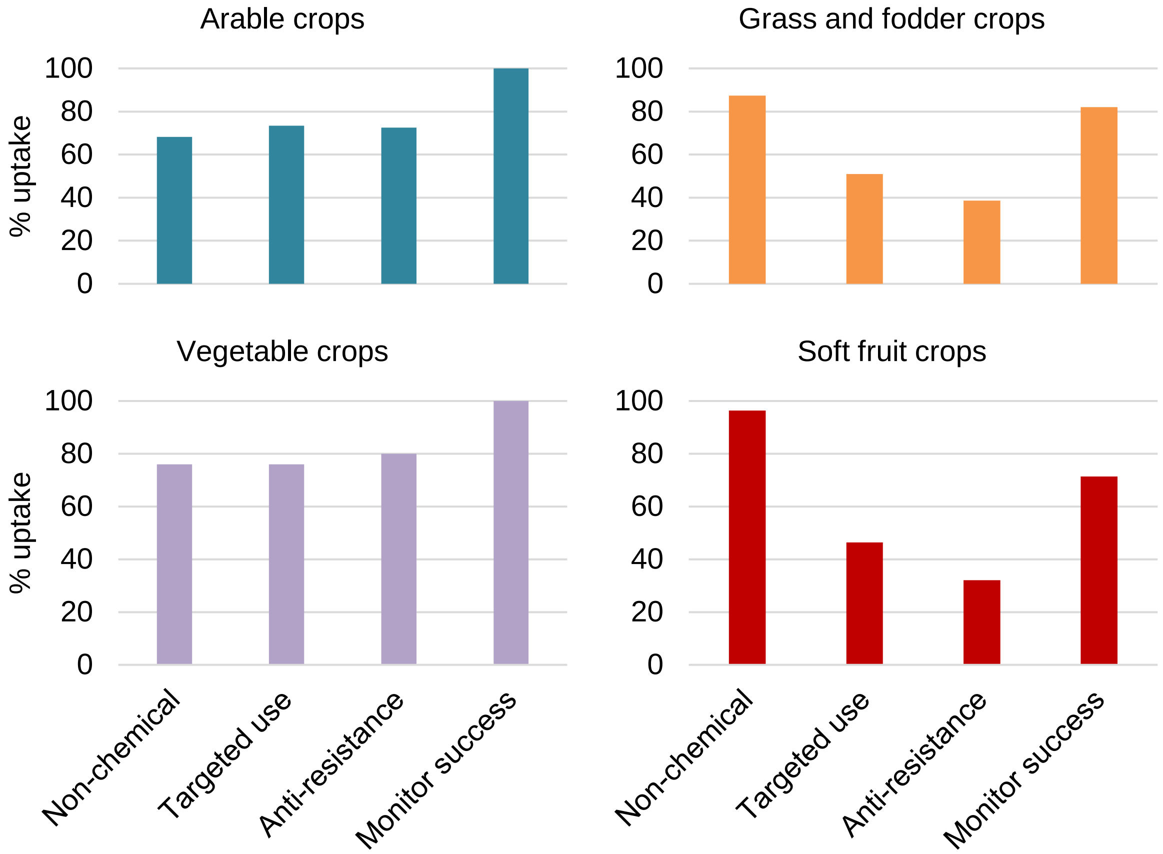

Figure 3. Adoption of pest control activities

In arable crops, relatively few non-chemical controls were reported. The most common technique encountered was hand rogueing (primarly for wild oats), with some use of mechanical weeding. In both soft fruit and vegetable crops, the most common non-chemical control measures were use of physical barriers such as netting, fleece and mulching. Both of these sectors also employed mechanical weed control and biological pest control. In addition, the soft fruit sector also reported a range of more sophisticated control measures, such as trapping, pheromone mating disruption and push-pull strategies using a combination of trap crops and repellent treatments on the main crop.

In relation to chemical pesticide use, 76, 73, 51 and 46% of vegetable, arable, grass & fodder and soft fruit growers respectively reported that they took measures to target and reduce pesticide applications. Common strategies included, in order of frequency of adoption, applying pesticides as spot treatments, applying herbicides using weed wipers and reducing dosage and frequency of applications where possible. Some growers also reported using drift reduction apparatus and, particularly in the vegetable production sector, precision application systems.

Adoption of anti-resistance strategies when using pesticides, to minimise the risk of pest resistance development, was reported by 80, 73, 39 and 32% of vegetable, arable, grass and fodder and soft fruit growers respectively. This was mainly achieved by minimising the number of pesticide applications used, but also by using pesticides with multi-site modes of action and/or a range of pesticides with multiple modes of action.

The final aspect of pest control recorded was whether growers monitored the success of risk management and crop protection practices to assess and improve regimes. All arable and vegetable growers reported that they did this, alongside 82 and 71% of grassland and soft fruit growers respectively. The most common assessment method in the arable, vegetable and grass and fodder sectors was conducting a regular review with an agronomist (91, 84 and 54% respectively), followed by regular self-inspection of outcomes and investigating causes of poor efficacy. In the soft fruit sector, as with pest monitoring, self-assessment of control measures (64%) was more prevalent than agronomist evaluation (25%). Around a third of vegetable growers reported that they conducted seasonal reviews of crop protection practice, a greater proportion than in the other crop sectors (16, 4 and 3% in arable, soft fruit and grass & fodder respectively). Other, less frequent, methods of evaulation included precision technology such as in-field yield mapping, or in the case of some vegetable growers, trapping to monitor the efficacy of insecticide treatments.

DISCUSSION

Plant protection products are an integral component of Scottish agriculture and horticulture, protecting crops from pests and pathogens and supporting the cost-effective production of a range of economically important produce and commodities.

The Scottish Government supports, as a commitment in its programme for government, the targeted and sustainable use of pesticides within an IPM framework. In addition to monitoring IPM uptake, it promotes the adoption of an IPM approach by a range of other activities, including hosting and promoting a Scottish IPM plan and by funding a variety of related research and knowledge exchange.

The pattern of pesticide use in Scottish agriculture and horticulture is well-defined and documented and, whilst trends in usage are complex and influenced by a number of inter-related drivers, recent pesticide statistics suggest that use of pesticides may be declining in some sectors (Monie et al, 2018; Reay et al, 2018; Wardlaw et al, 2019). This additional data collection about other aspects of crop protection, including risk reduction and alternative control measures, helps to complete this picture, providing the Scottish Government with a holistic overview of the practices involved in Scottish crop production.

The surveys described in this paper provide baseline data about uptake of IPM. These data sets show that all sectors undertake a wide range of crop protection and risk reduction strategies and growers have adopted many aspects associated with an IPM approach. It also highlights differences in uptake in different crop sectors, and where there are gaps in adoption. It is the intention that these IPM surveys are conducted, for each sector, every four years, to allow comparison over time. Data are currently being collected for the 2019 vegetable IPM survey, with arable and soft fruit surveys scheduled for 2020 and grass and fodder for 2021.

It is this sequential data collection, alongside the pesticide monitoring, that will provide valuable information about the evolution of Scottish crop protection strategies. It will inform government about the response to loss of approvals of pesticides, particularly where control gaps are left. For example, SASA IPM monitoring has documented the implementation of non-chemical control strategies for leatherjackets following the loss of chlorpyrifos and risk reduction strategies for cabbage stem flea beetle following the restriction of use of neonicotinoid insecticides. Future surveys will reflect the impact of more recent pesticide losses, both in relation to alternative pesticide use (for example to compensate for the loss of chlorothalonil) and in relation to non-chemical control (which is likely to result from the loss of diquat for potato haulm desiccation).

These data will also reflect the influence of other drivers such as developments in crop protection technologies, knowledge transfer activities and quality assurance requirements that occur over time. For example, in 2019 Scottish Quality Crops, the principal arable farm assurance certification body, introduced a requirement for those they certify to complete IPM, biodiversity, soil testing and water management plans. As only 24% of the arable growers surveyed completed a plan in 2016, and 90% of Scottish pesticide use is on arable crops, this should have a significant impact on grower awareness, and hopefully uptake of IPM measures, in the 2020 survey.

These initial baseline surveys are presented here as a descriptive, quantitative dataset, and do not assign weight to the types of measures adopted regardless of complexity, cost or impact on pesticide use. This data collection series is a work in progress and trend data would benefit from adopting a weighted metric, such as that described by Creissen et al (2019) to allow more nuanced comparison between sectors and over time. With this kind of analysis it may be possible to better describe, understand and influence changes in response to government initiatives to encourage greater sustainability in the use of pesticides.

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MEASURING THE UNMEASURABLE? A METHOD TO QUANITFY ADOPTION OF INTEGRATED PEST MANAGEMENT PRACTICES IN TEMPERATE ARABLE FARMING SYSTEMS

H E Creissen[1], P J Jones[2], R B Tranter[2], R D Girling[3], S Jess[4], F J Burnett[1], M l Gaffney[5], F S Thorne[6] and S Kildea[7]
[1]Department of Agriculture, Horticulture and Engineering Sciences, Scotland's Rural College, Edinburgh, UK.
[2]Centre for Agricultural Strategy, School of Agriculture, Policy and Development, University of Reading, Reading, UK.
[3]Centre for Agri-Environmental Research, School of Agriculture, Policy and Development, University of Reading, Reading, UK.
[4]Agri-Food Sciences Division, Agri-Food and Biosciences Institute, Belfast, UK.
[5] Horticultural Development Department, Teagasc, Ashtown, Dublin, Ireland.
[6] Agricultural Economics and Farm Surveys Department, Teagasc, Ashtown, Dublin, Ireland.
[7]Crop Science Department, Teagasc Oak Park Crops Research Centre, Carlow, Ireland.
E-mail: henry.creissen@sruc.ac.uk

Summary: Managing pests, whether insects, weeds or diseases is a key component of arable cropping in temperate climates and the integration of the key means of control is fundamental to achieving this. Using a survey of arable farmers across Ireland and the UK, in combination with a metric to determine levels of integrated pest management (IPM) practised, differences between the countries in levels and familiarity with IPM were identified. Levels of familiarity, relationships between the farmer and advisor, area of land farmed and country of origin were identified as key drivers in the levels of IPM practised.

INTRODUCTION

Arable production systems in temperate climates are often subjected to a variety of insect pests, weeds and diseases. To combat each of these threats control programmes utilising a combination of different measures including cultural, biological and chemical are widely employed. This concept, referred to as Integrative Pest Management (IPM) is increasingly promoted as a means of providing pest control in a manner that is environmentally and socially acceptable, but equally profitable (Barzman et al., 2015). Given the diverse range of pests that threaten arable crops in temperate climates, and the equally broad range of control measures that can be potentially used, gauging levels of IPM currently practiced can be difficult to determine. However if IPM is to be increased in arable systems the ability to determine what levels are practised, and equally what factors either promote or limit uptake must be determined. To achieve this Creissen et al. (2019) have devised, through stakeholder engagement, a metric based on farmer responses to key questions relating to their on farm practices. In the presented paper an overview of this metric and key findings from its application across arable farms in Ireland and U.K is described and discussed.

MATERIALS AND METHODS

Through consultation with stakeholders a list of six questions relating to varying aspects of arable production and their impacts on pest (insects, weeds and diseases) were identified. The potential responses to each question were subsequently ranked, and each question subsequently given a weighting depending on its perceived role in IPM. Combined, they provide a metric from 0-100 reflecting levels of IPM practised. Further details on the development of the survey questions and metric can be found in Creissen et al, (2019). The metric was applied randomly to farmers across Ireland and the U.K. using national farm statistic datasets in each of the respective countries. In addition to the six questions relating to IPM, an addition eight relating to IPM adoption and perception, and a further eight socio-economic questions relating to the farmer and farming enterprise was asked. Combined the levels of IPM practised on arable farms in Ireland and the U.K were determined, and the various factors that may influence these levels were explored.

RESULTS

Significant differences between the four countries in both levels of IPM adoption and familiarity were observed (P<0.0001). Levels of IPM familiarity, relationship with crop protection advisor, area of land farmed and country of origin were all identified as significant drivers of IPM levels detected. Similarly, country of origin was also identified as a factor in levels of IPM familiarity, alongside source of pest management information and levels of education of the respondent.

DISCUSSION

As the availability of effective pesticide chemistries continues to decline due to increased regulation and resistance development, alternative means of pest control is required. IPM is continually promoted as a means of achieving these goals; however for it to be successful its essential to first understand what levels are currently employed and second, what its potential is, but equally its limitations in arable cropping systems. The development of metric described provides a simple and effective means of achieving this. By using the metric the potential for further improvements in the uptake of IPM across arable cropping systems was identified, including the increasing the levels of IPM familiarity amongst farmers, promoting relationships between advisors and farmers in relation to pest management.

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THE IMPACT OF FIELD HEADLANDS ON THE PERFORMANCE OF SPRING BARLEY IN A TEMPERATE MARITIME CLIMATE

M Ward[1,2], K McDonnell[2] and P D Forristal[1]
[1]*Teagasc Crops Research Centre, Oak Park, Carlow, Ireland*
[2]*School of Agriculture and Food Science, UCD, Belfield, Dublin 4, Republic of Ireland*

Summary: Headlands are fundamental features of agricultural landscapes. Growers have adopted larger heavier machines which in a temperate maritime climate where field size can be small and irregular, could impact on crop performance. A survey of field headlands on cereal growers' fields was conducted to investigate the impact of headland zones (as defined by intensity of headland machinery traffic) on the performance and yields of spring barley. The experiment was conducted over two growing seasons (2016 and 2017) and included a number of soil textures. Significant differences between headland zones was documented for most crop growth parameters with the in-field zone having the highest yield and the zone along the field edge the lowest. The impact of zone on crop performance was influenced by soil texture.

INTRODUCTION

The term headland is commonly associated with the area of land found next to the field boundary and is of a size that encompasses all machine operations conducted parallel to this boundary (Sparkes et al., 1998a, Wilcox et al., 2000). They can be classified as 'turning' headlands where machine turns occur and 'non-turning where traffic patterns align with the rest of the field (Sparkes et al., 1998a). Turning headlands have been reported as areas of lower grain yields than the main in-field areas (Sparkes et al., 1998b, Kuemmel, 2003) for cereals (Sparkes et al., 1998a) and root crops (Cook and Ingle, 1997). The impact of competition for nutrients, light and moisture from weed ingress adjacent to the crop (Marshall and Arnold, 1995) or compaction due to trafficking by farm machinery (Speller et al., 1992) is also recognised. Surveys to date have primarily focussed on yields with respect to distance from the field boundary (Wilcox et al., 2000) and have not considered specific machinery patterns in the analysis.

The objective of this study was to quantify the impact of headlands on crop performance using a zone approach where headland areas are categorised based on the intensity of machine traffic.

MATERIALS AND METHODS

The study was conducted on 24 randomly selected cereal growers' fields in the main cereal growing region of Ireland (east of a line from 53°54'13.9"N 6°25'46.3"W to 52°06'43.8"N 7°48'05.2"W) (Figure 1) from a list populated of Teagasc cereal grower clients. An assessment of machine headland turning techniques was conducted that allowed zones based on machinery traffic intensities: field edge; turning; transition, and; in-field, to be defined between the headland and in-field areas (Figure 2). During the growing seasons of 2016 and 2017, crop measurements were taken from each of the four zones at four different transects, giving four replicated measurements per zone.

Post full plant emergence, plant population densities were determined, followed by light interception (Delta-T Devices Sunscan Canopy Analysis System) at two timings (GS 30 and GS32) as an indicator of biomass. Using intensive hand harvested sampling methods, final grain yield and its components were determined. Moisture and hectolitre weight were measured using a DICKEY-JOHN GAC®2100 GI and thousand grain weights (TGW) using a Haldrup GC-30 automatic seed counter. The numbers of ears/m^2 were counted and grains/ear and grains/m^2 were calculated. At each site, a soil texture analysis was also carried out on a composite sample using the sieve pipette method (Massey et al., 2014).

Data from this study met the assumptions necessary for a two-way Analysis of Variance. The effects of zone and soil texture on crop performance was analysed with blocking using SAS 9.4 statistical software.

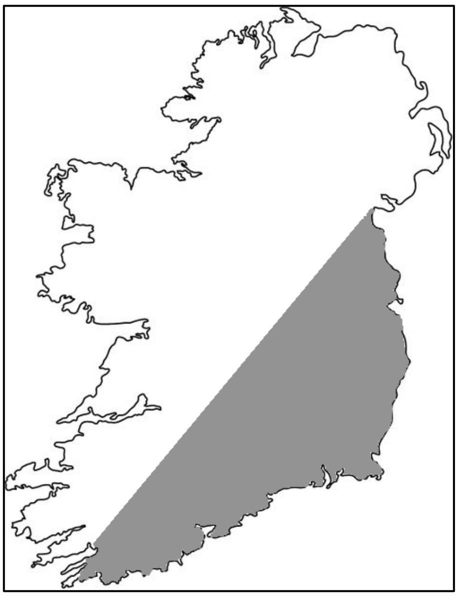

Figure 1. Location of the main cereal growing region of Ireland from which sample sites were randomly selected

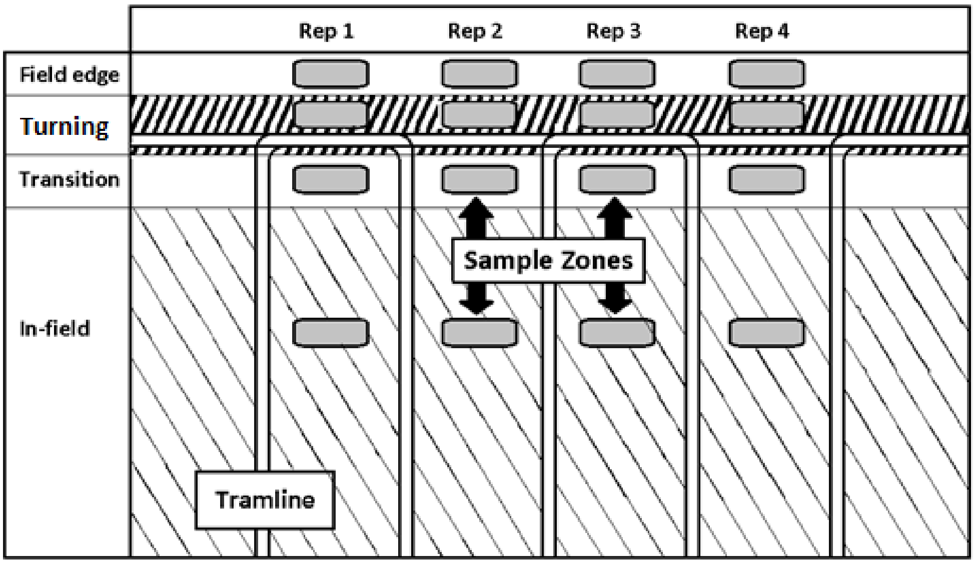

Figure 2. Illustration of zone approach and sample locations in grey

RESULTS

Zone had an effect (P<0.001) on the majority of measurements in this study (Table 1). Establishment plant density figures were higher at the in-field zone than all other headland zones. Light interception values varied between zones with greater light interception values indicating denser crops. At harvest, grain yields for spring barley ranged from 6.45 t/ha at the field edge to 8.53 t/ha at the in-field zone with statistical significance documented between each zone. This yield response was influenced by soil texture. On an individual site basis, the in-field zone was the highest yielding zone across all sites, while the field edge zone was the lowest yielding zone at 72% of the sites.

Table 1. The effect of zone and soil texture on plant densities, indicators of growth and final yields of spring barley (values within columns subscripted by different letters are significantly different at P=0.05)

		Yield	Plant Density	Light Intercepted		TGW	Ears/m²	Grains/ ear
		(t/ha)	(#/m²)	1 (%)	2 (%)	(g)	(n)	(n)
Zone	Field edge	6.45^{d}	243^{cb}	59^{c}	75^{d}	46.38^{a}	675^{c}	19.68^{a}
	Turning	7.19^{c}	246^{b}	60^{c}	78^{c}	46.46^{a}	752^{b}	19.59^{a}
	Transition	8.17^{b}	236^{c}	73^{a}	88^{a}	43.52^{c}	896^{a}	19.87^{a}
	In-field	8.53^{a}	256^{a}	64^{b}	82^{b}	44.70^{b}	896^{a}	21.11^{a}
Soil Texture	Clay loam (n=6)	8.71^{a}	287^{a}	57^{c}	74^{c}	46.67^{a}	968^{a}	17.99^{b}
	Loam (n=6)	8.31^{b}	224^{b}	70^{a}	88^{a}	46.15^{a}	792^{b}	21.05^{a}
	Sandy Loam (n=12)	6.66^{c}	235^{b}	64^{b}	81^{b}	44.12^{b}	729^{c}	20.61^{a}
		Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F	Pr > F
Zone		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.310
Site		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zone*Site		<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.885
Soil Texture		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zone*Soil Texture		0.031	0.421	<0.001	0.002	<0.001	0.463	0.983

DISCUSSION

The headland zone approach utilised for this study, based on various levels of headland machinery trafficking, better defined the variability in crop performance than previous studies that considered distance from the field boundary as a factor in yield. As this was a survey, the variability between sites is partially explained by soil texture but also may have been contributed to by management factors such as different turning techniques and equipment being used between sites, giving a zone and site interaction. All measurements of crop performance were impacted by the headland zone in which the measurement was taken, with significant yield differences recorded between all zones. Despite lower plant densities at the transition zone, the thickest crops for both timings as indicated by light interception were the transition and in-field zones also seen for many of the subsequently measured yield components. The yield differences were associated with differences in the number of ears produced per m^2 with a good relationship between ear numbers and final yield ($yield = 0.008 ears/m^2 + 1.1396$, $R^2 = 0.72$), and this was associated with a larger crop canopy through the season. While the differences between the headland and in-field areas were as hypothesised, the zone with the lowest recoded yield (field edge) was not the zone with the most traffic. This indicates that while machinery traffic may be contributing to yield loss, as both the field edge and the turning area have more traffic than the in-field area, it was not the only factor because the field edge is subject to less traffic than the turning area. Other factors at the field edge are contributing to yield loss and these need to be further quantified. For grain yields, light intercepted and Thousand Grain Weight (g) (TGW), the zone response was impacted by soil texture. The traffic effect suggests growers should be cautious about the management of headland traffic.

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IMPLICATIONS OF PESTICIDE WITHDRAWALS ON THE SCOTTISH AGRICULTURAL AND HORTICULTURAL INDUSTRY

K A Evans
Department of Agriculture, Horticulture and Engineering Sciences, SRUC, West Mains Road Edinburgh EH9 3JG
E-mail: andy.evans@sruc.ac.uk

Summary: The key questions addressed in this paper are the risks posed to Scottish plant health from the withdrawal of some of the key pesticide active substances used in Scotland for pest, weed and disease management across the agriculture and production horticulture sectors. The possible withdrawal of a range of active substances has the potential (in a worst case scenario) to have a significant impact on the ability to manage pests effectively and economically and the total value of output for all sectors will be affected. There will be a necessity to rapidly adopt alternative Integrated Pest Management (IPM) control methods in some crop sectors. For soft fruit and vegetable production, in a worst case scenario losses may be sufficiently severe as to make production uneconomic for some crops if no adaptations are made. Where non-chemical alternatives to pesticides are available this will add to complexity and cost for producers and land managers.

INTRODUCTION

The Scottish agricultural and production horticultural sectors face a significant threat to their plant health and productivity through the withdrawal of key pesticide active substances and products. Use of pesticides is just one of the options within the integrated pest management programmes that are promoted as part of the Scottish Government Integrated Pest Management (IPM) Plan (https://consult.gov.scot/cap-reform-and-crop-policy/9a1bb2d9/). However, current Scottish agricultural and horticultural pest management practices rely heavily on chemical pesticides. This paper is a summary of a report commissioned by the Plant Health Centre, and assesses the risk of the potential withdrawal of the main active substances used in Scotland across the agriculture and production horticulture sectors, and where possible provides an assessment of the impact of key active substance withdrawals in the relevant Scottish sectors.

MATERIALS AND METHODS

The approach adopted in this assessment of at-risk active substances used Scottish pesticide use data gathered from the most recent Pesticide Usage Survey Reports (http://www.sasa.gov.uk/pesticides/pesticide-usage/pesticide-usage-survey-reports) to identify the most frequently used active substances in Scottish sectors (where available). This data was cross-referenced with an analysis of active substances at greatest risk of loss of authorisation provided by SASA and a database maintained by the Agriculture and Horticulture Development Board (AHDB) that tracks and assesses the risk of withdrawal of active substances currently approved in the UK and in the EU. In addition, information and data from sources listed in the references were taken into account when identifying active substances that are at risk of withdrawal. The data were sub-divided into: Agriculture: arable crops (cereals, potatoes, oilseeds and legumes); and Production horticulture: soft fruit crops (strawberries, raspberries and other soft fruit), and field vegetable crops (carrots, turnips/swedes, Brussels sprouts and other crops).

The criteria that was used to get to a rating of either: High risk of withdrawal, Medium risk of withdrawal, No/low risk of withdrawal, was based on European Union Regulation EC 1107/2009, which covers both the approval of new active substances and the renewal of existing substances within the EU. Specifically, active substances that receive classifications of 1A & 1B – for Reproduction, Carcinogenic and Mutagenic risks all warrant a non-renewal from the European Food Standards Agency (EFSA), and as such all of these active substances receive a High risk of Withdrawal rating. If a substance is given a classification of 2 in more than one of these categories: Reproduction, Carcinogenic or Mutagenic, this gives the risk as Medium risk of Withdrawal, as this is often stated as a reason for non-renewal. Active substances with a classification of 2 in all three categories (Reproduction, Carcinogenic or Mutagenic) receive a High risk of Withdrawal rating. Active substances determined to be endocrine disruptors are given a non-renewal, and subsequently a High risk of Withdrawal rating. Pesticides where there are specific reviews underway or pending for renewal of approvals by EFSA were considered. 'No Safe Use' suggested by EFSA early in the review process are given either a Medium risk of Withdrawal or High risk of Withdrawal rating depending on areas of concern. Critical areas of concern are given a High risk of Withdrawal rating. Determining whether a non-safe use is given a Medium Risk of Withdrawal rating depends on the nature of the concern and the number of areas of concern. If the area of concern is simply overcome by filling a data gap the risk of withdrawal is downgraded (e.g. if based on one data gap, or a simple data gap).

The potential economic impacts of yield loss for the withdrawal of key active substances on Scottish crops were estimated based on the Total Value of Output data for each commodity from the Scottish Government Economic Report on Scottish Agriculture 2018. Additional data was obtained for the breakdown of cereals into winter barley, spring barley and winter wheat derived from a combination of data from DEFRA, Scottish Government, AHDB Cereals, MAGB and SRUC (compiled by Julian Bell, SAC Consulting).

Derivation of the potential financial impact (£) of the withdrawal of active substances on the Scottish Total Value of Output, utilised the estimated percentage impact of withdrawal specified by Wynn (2014) in the AHDB review of the impacts of the withdrawal of active substances on crop sectors in the UK. This approach using the Total Value of Output (where known) was adopted for both sectors to provide an estimate of the economic impact of the withdrawal of an active substance(s). A caveat here is that for the Scottish (as opposed to the UK) situation, pest pressure could be different (higher or lower), which was not considered as part of the analysis. For example, the Total Value of Output of Scottish winter wheat is £120.01M (Julian Bell, SAC Consulting). Wynn (2014) estimated that the withdrawal of the active substance prothioconazole would lead to a withdrawal of 2% of the UK farmgate value of winter wheat. So assuming this 2% withdrawal to Total Value of Output of Scottish winter wheat, withdrawal of prothioconazole would reduce the Total Value of Output of Scottish wheat (£120.10M) by £2.40M (2%).

This approach using the Total Value of Output (where known) was adopted for both sectors to provide an estimate of the economic impact of the withdrawal of an active substance.

RESULTS

Cereals

The loss of chlorothalonil in May 2020 coupled with the potential loss of at-risk azole fungicides would make the management of diseases such as Septoria leaf blotch on Scottish winter wheat crops harder, and Ramularia on barley crops impossible. It has been estimated that the loss of prothioconazole would reduce the annual Scottish Total Value of Output of winter wheat (£120.01M) by 2% - £2.40M. The additional loss of chlorothalonil (2% reduction) and epoxiconazole (0.4% reduction) would together reduce the Scottish Total Value of Output of Scottish wheat by £2.88M. Winter barley (Scottish Total Value of Output of £46.54M) and spring barley (Scottish Total Value of Output of £274.83M) will also be impacted by the loss of prothioconazole (both 1% of Total Value of Output), resulting in £0.46M and £2.75M losses respectively.

The loss of the insecticide chlorpyrifos in 2016 has raised the risk of leatherjacket damage in spring barley, potentially taking 0.5% (£1.37M) off the Scottish Total Value of Output (£2.74M). There are currently no pesticide options available to manage this pest, growers having to rely on techniques such as rolling the crop. Due to the revocation of the neonicotinoid cereal seed treatments, there will be a predicted increase in the use of pyrethroid insecticides to manage aphids and Barley Yellow Dwarf Virus (BYDV). The potential loss of lambda-cyhalothrin (medium risk of loss) and other pyrethroid insecticides, coupled with limited alternatives could see a resurgence of BYDV and significant yield losses – estimated to be in the region of 1% (Total Value of Output of £0.47M for winter barley, £1.2M for winter wheat and £2.75M for spring barley), particularly in light of grain aphid resistance to pyrethroids. The probable loss of metaldehyde in the near future is mitigated by the availability of ferric phosphate as a straight alternative. Whilst some herbicides used in cereals are at high or medium risk of loss, there are several alternatives available and economic impact on cereals is likely to be minimal.

Fig. 1 summarises the potential reduction in the Total Value of Scottish Outputs of Cereals, Oilseed rape, Legumes and Potatoes (£M) if high and medium risk active substances are lost.

Oilseed rape

The scheduled loss of the seed treatment thiram in January 2020 will leave oilseed rape at risk of diseases at crop emergence, such as damping off. New fungicide seed treatment options are in the pipeline and may be available for autumn 2020 sowing. In winter oilseed rape (Scottish Total Value of Output of £44.75M), the potential loss of azole fungicides in particular; prothioconazole (6% - £2.69M), metconazole and tebuconazole (both 0.5% - £0.22M each) would increase the risk of light leaf spot and sclerotinia.

There is widespread pyrethroid insecticide resistance in the cabbage stem flea beetle, which, whilst not currently a serious issue in Scottish oilseed rape crops, has caused serious issues in English rape crops where resistance is rife. There are no other approved alternatives to the pyrethroids to manage this pest. Peach-potato aphid is also resistant to the pyrethroid insecticides. With the loss of pymetrozine and impending loss of thiacloprid, only flonicamid will be available for managing peach-potato aphid/turnip yellows virus on oilseed rape. In the spring pollen beetles are demonstrating resistance to the pyrethroid insecticides. With the loss of pymetrozine and thiacloprid due for withdrawal, acetamiprid and indoxacarb (medium and high risk of loss respectively) will be the only alternatives for the control of pollen beetle. As with cereals, growers have an alternative molluscicide available to mitigate the probable loss of metaldehyde in the form of ferric phosphate. Whilst some herbicides used in oilseed rape are at high or medium risk of loss such as glyphosate, clomazone and propyzamide, there are several alternatives available and economic impact is likely to be minimal.

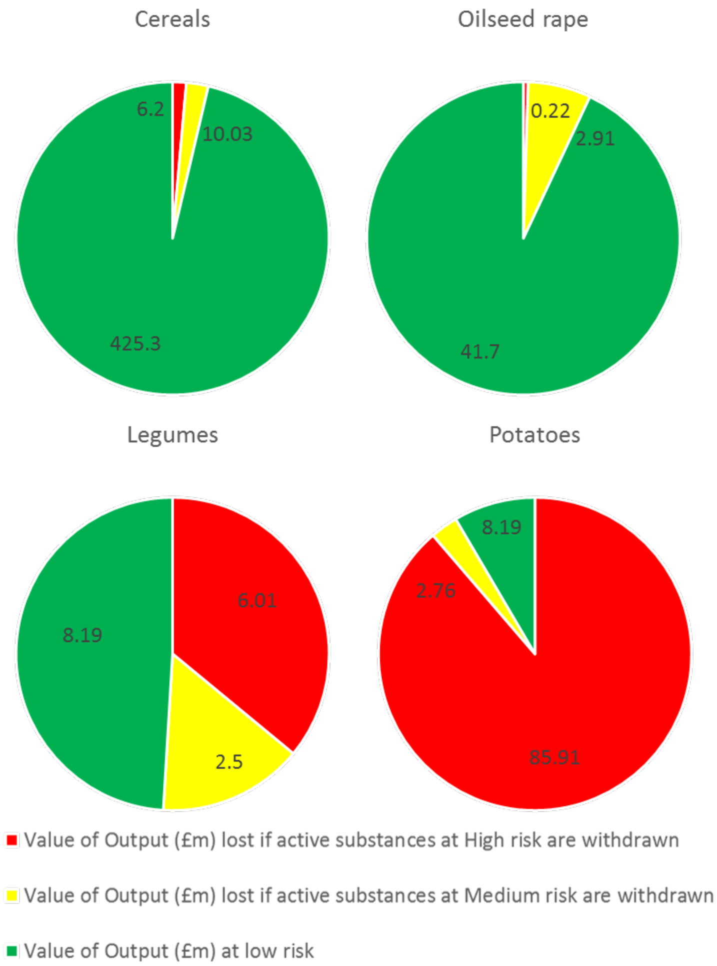

Figure 1. The potential reduction in the Total Value of Scottish Outputs of Cereals, Oilseed rape, Legumes and Potatoes (£M) if high and medium risk active substances are lost.

Legumes

Scottish legume crops lost the fungicide iprodione in 2018 and thiram will be unavailable after 2020. Alternative fungicide seed treatments are particularly limited, with a biopesticide (*Gliocladium catenulatum*) the only alternative. Key foliar applied fungicides are at medium or high risk of loss for the management of diseases such as botrytis, sclerotinia, damping off, chocolate spot and downy mildew. The scheduled loss of chlorothalonil alone has been estimated to reduce farm gate values of legumes by 18% (£3.01M for Scottish peas), with the other fungicides an estimated reduction of 16% (£2.67M for Scottish peas). The loss of the herbicide active substance linuron in 2018 is estimated to reduce farmgate value of combining peas and beans by 4% (equivalent to £0.67M of Scottish Total Value of Output). Alternatives such as glyphosate, pendimethalin, diquat and clomazone are all at high risk of loss. There will be a need for growers to look at Extensions of Authorisation for Minor Use (EAMUs) rather than on-label approvals for weed management in the short term.

Potato

The potential loss of mancozeb will put pressure on other active substances for potato blight management and with it the risk of fungicide resistance. The cost of production will increase through the use of more expensive products to maintain yields, estimated to have an impact of £0.66M on the Total Value of Output of Scottish potato crops. The additional issue of fluazinam resistance in blight has been estimated to lead to an extra £2.1M in UK costs through the use of more expensive active substances (£0.41M for Scottish potato crops).

The loss of diquat in Feb 2020 to 'burn off' and desiccate potatoes will have a significant impact especially in the Scottish seed crop. Alternatives such as flailing and other desiccants are only around 60% as effective as diquat in burning down crops. Increased risk of blackleg is a concern in these scenarios and would lead to yield and quality losses and might also impact on the marketability of the Scottish crop.

Virus management in Scottish seed potatoes is reliant on non-pyrethroid active substances due to the main virus vector (peach-potato aphid) being resistant to pyrethroid insecticides. With the loss of pymetrozine and the impending withdrawal of thiacloprid there will potentially be just three active substances effective against this aphid. This will restrict the number of aphicide applications to a maximum of 8 in total. This poses a risk of increased virus levels in the Scottish seed potato crop which would have a significant impact on seed health and seed exports. The loss of key herbicides in potatoes such as linuron (already withdrawn) and potential loss of metribuzin (15% of the Scottish Total Value of Output of £214.7M - £32.21M) in Jan 2022 would have left a significant gap in weed management options in Scottish potatoes, especially with other herbicides also at risk or being withdrawn such as diquat, glyphosate and pendimethalin. However, in March 2019 a new active substance, aclonifen, was granted approval for use on potatoes, and will help to mitigate the loss when metribuzin and other herbicides are unavailable.

Note that the additional loss of the potato sprout suppressant chlorpropham (CIPC) from October 2020 has been estimated to lead to a 25% loss in the Total Value of Output for potatoes due to the inability to store potatoes for as long, and the increased cost of alternatives. This 25% loss in the total Value of Output equates to £53.7M for Scottish potatoes.

Soft fruit

Scottish raspberries have a Scottish Total Value of Output of £20.2M. The loss of key fungicides such as myclobutanil (3% loss), tebuconazole (10%), bupirimate (2%) and fluazinam (10%), totalling an estimated £5.05M in Total Value of Output losses, will put pressure on the few alternatives available, and likely lead to more expensive treatments for powdery mildew and increased use of fenhexamid and biopesticides such as *Bacillus subtilis*.
The loss of the insecticide chlorpyrifos in 2016, which was estimated to contribute to a 20% Total Value of Output loss (£4.04M), will be exacerbated by the loss of the insecticide thiacloprid (15%) and potential loss of abamectin (7%), deltamethrin (15%) and spinosad (3%), and will leave growers with no active substances for the management of capsids, raspberry beetle, clay-coloured weevil, raspberry cane midge, small raspberry sawfly and substantially reduced options for aphid control. The loss of these insecticides would reduce the farmgate value of raspberries by up to 50% (£10.1M).

The loss of the herbicide diquat in 2020, and potentially propyzamide in 2021 and glufosinate-ammonium in 2020 will leave a significant gap in the management of weeds, estimated to be 8% of the Scottish Total Value of Output (£1.62M), putting pressure on active substances currently at low risk of loss such as isoxaben.
The worst case scenario is that raspberry production could become uneconomic if all the possible pesticide losses outlined above occur and alternative chemical or non-chemical control strategies are not available.

Fig. 2 summarises the potential reduction in the Total Value of Scottish Output (£M) of Raspberries and Strawberries if the High and Medium risk active substances are lost.

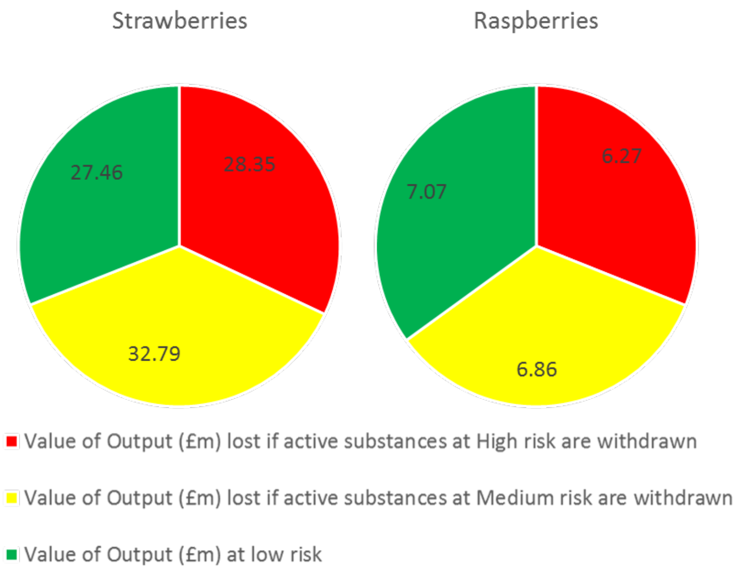

Figure 2. The potential reduction in the Total Value of Scottish Output in Strawberries (£88.6M) and Raspberries (£20.2M) if high and medium risk active substances are lost.

Scottish strawberries (Scottish Total Value of Output of £88.6M) will be significantly affected (Fig. 2) by the loss of the fungicides iprodione (8% of the Total Value of Output), myclobutanil (5%) and bupirimate (13%), leading to more expensive or difficult management of botrytis and powdery mildew. The loss of myclobutanil (already withdrawn in 2017), bupirimate and iprodione alone could reduce the farmgate value of strawberries by 26% (£23.04M for Scottish crops). As with raspberries, the loss of thiacloprid (19%) and potential loss of the insecticides abamectin (13%), and deltamethrin (11%) will severely compromise the management of aphids, weevils, mites, and spotted wing drosophila, potentially reducing the Total Value of Output by 43% (£38.1M). The worst case scenario is that strawberry production could become uneconomic if all the pesticide losses outlined above occur and alternative chemical or non-chemical control strategies are not available.

Field vegetables

Four fungicide seed treatment active substances are at high or medium risk of loss, leaving brassicas and carrots with few or no options against establishment diseases such as damping off and pythium. The loss of several foliar applied fungicides will severely hamper the management of diseases such as downy mildew, powdery mildew, sclerotinia, botrytis and stem canker in Scottish field vegetables. Growers would need to increasingly turn to using biopesticides alongside the few active substances left available, which are primarily EAMUs rather than fully approved substances.

The list of herbicides potentially at high or medium risk of loss is a particular cause for concern as alternative options tend to be EAMUs. Reliance on active substances with limited on-label approvals and/or EAMUs may have to suffice in the short-term. Fig. 3 summarises the potential reduction in the Total Value of Scottish Output (£M) in Brassica field crops (turnips/swedes and Brussels sprouts) and Carrots if high and medium risk active substances are lost.

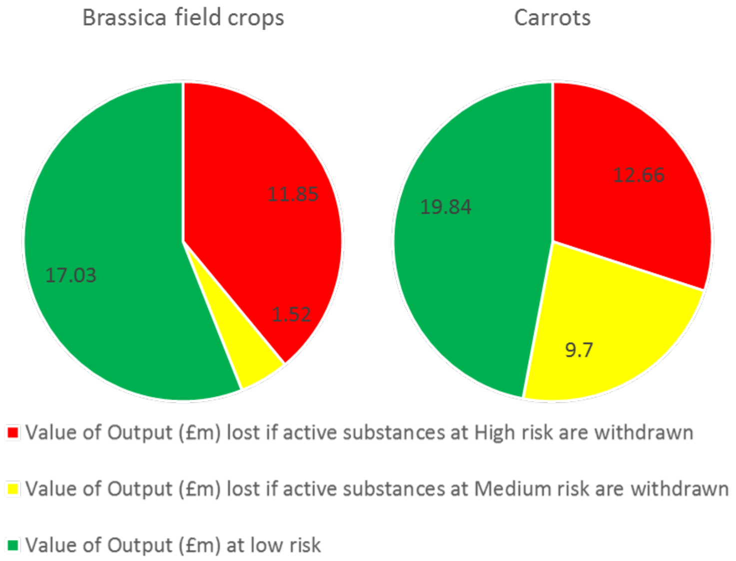

Figure 3. The potential reduction in the Total Value of Scottish Output (£M) in Brassica field crops (turnips/swedes and Brussels sprouts) and Carrots if high and medium risk active substances are lost.

The Scottish Total Value of Output for brassica field crops (turnips/swedes and Brussels sprouts) was estimated at £30.4M. The loss of the fungicides tebuconazole (leading to a loss of 5% of the Total Value of Output) and difenoconazole (5%) would reduce the Total Value of Output by £3.04M. The potential loss of the insecticides thiacloprid (15%) and spinosad (16%) would significantly compromise aphid management and cabbage root fly management respectively, leading to a reduction in Scottish Total Value of Output of 31% (£9.42M). The loss of pymetrozine in 2020 will limit options for aphid management, particularly with thiacloprid being withdrawn and if spinosad is also lost. Whilst there are short-term alternatives when it comes to herbicides, there are question marks over the continuing approval of pendimethalin and clomazone. Consequently, use of S-metolachlor may become more important in the future. However, S-metolachlor is at high risk of loss, and its loss could reduce the farm gate value of brassicas by 3% (£0.91M).

Carrots have a Scottish Total Value of Output of £42.2M. The potential loss of the azole fungicides tebuconazole, difenoconazole and prothioconazole would reduce the Total Value of Output of carrots by 5% (£2.11M), 4% (£1.69M) and 6% (£2.53M) respectively. Loss of the pyrethroid insecticides deltamethrin (6% - £2.53M) and lambda-cyhalothrin (7% - £2.95M) would have a marked effect on the ability to manage carrot fly, and with the additional loss of thiacloprid (5% - £2.11M) lead to management of willow-carrot aphid and virus reliant on just one or two active substances, increasing the pressure for insecticide resistance. The loss of the herbicides linuron (which has already been withdrawn) and potentially metribuzin are estimated to reduce the Total Value of Output of Scottish carrots by 20% (£8.44M) due to a lack of options for post-emergence chemical control of broad leaved weeds.

DISCUSSION

For both Agricultural and Horticultural sectors, the loss of key substances would have a significant impact on the ability to manage pests, weeds and diseases effectively and economically. The Total Value of Output for production horticulture in particular will be negatively affected. Alternatives for many of the active substances at risk of loss are either limited, are short-term EAMUs, more expensive or require a further shift into the use of biological pesticides. Further adoption of integrated approaches to pest management will be necessary to achieve effective management. The number of management options available may well be less than growers are used to, and this may have an impact on the quality and yield of specific crops. It remains to be seen whether the markets will adjust to take into account the increased costs associated with crop protection in particular sectors. The cost of production may increase as alternative approaches for managing crops such as use of pest and disease monitoring, forecasting pest and disease outbreaks, biopesticides, release of biological controls, potentially more expensive chemical pesticides and so on are utilised.

This shift to a more integrated approach to pest, weed and disease management (IPM) whilst welcomed, will not happen overnight, particularly in sectors where there may still be sufficient active substances available to reduce the pace of IPM adoption. Consequently, there is a role for Scottish Government, the MRP's and agronomy consultants to engage with stakeholders and encourage the adoption of IPM approaches across all sectors in preparation for the loss of pesticides that growers have relied on over many years. Successful implementation of IPM will require more accurate and detailed crop surveillance and consideration of factors such as weather and climate which drive risk. It will also require training and support on the use of alternative control measures such as biologicals and greater appreciation and understanding of whole-systems based approaches to reduce pest risk. Enhanced host resistance will provide some mitigation but will require dialogue with breeders (and end-users). Consequently, knowledge exchange between stakeholders within the Scottish plant health sector is to be encouraged as a matter of priority to ensure that Scotland maintains and improves its plant health in the future.

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SOIL REGENERATIVE AGRICULTURE GROUP

S Dolan[1], R S Audsley[1] and P Lindsay[2]
[1]*SAC Consulting, Environment Team, JF Niven Building, Auchincruive Estate, Ayr, KA6 5HW*
E-mail: rebecca.audsley@sac.co.uk
[2]*SAC Consulting, Farm and Rural Business Services, 77 North Street, Forfar, DD8 3BL*

Summary: The Scottish Government established the Soil Regenerative Agriculture Group under the Farming for a Better Climate (FFBC) programme in order to trial and develop ideas on farms which could provide practical and innovative solutions to help mitigate climate change in agriculture. Five farmers are taking part in the initiative and soil health tests have been conducted in three of each of their fields to investigate nutrients levels, soil structure, earthworm numbers and potentially mineralisable nitrogen. The soil health of the five farms was on target for phosphorus, magnesium and soil pH with some issues with low potassium levels which could be addressed. Soil structural quality requires further consideration as fields ranged from poor to good structure although none were very heavily compacted. Finally, earthworm populations were typically poor or patchy across most of the fields, with some showing healthy populations of earthworms throughout, indicating that the impact of management strategies should be considered moving forward. Soil health testing has helped the farmers to benchmark their fields so that they can focus on targeted approaches to regenerating their soils to mitigate the effects of climate change on their farms.

INTRODUCTION

Sustainability in the agricultural industry is important in order to provide food security for a growing world population which is estimated to reach almost 10 billion by 2050 (United Nations, 2017). Climate change and land management practices pose a risk to soil health (Doran, 2002) and structure (Ball, 2013). Nutrient losses are a significant issue, and inefficient use of fertilisers and manure result in losses and eutrophication of nearby water bodies (Sharpley et al, 2001) as well as financial losses. Tillage practices can have harmful effects on soil biology, resulting in the decline of earthworm populations for example (Stroud, 2019). Innovative and practical solutions are required to help farmers adapt and mitigate to the effects of climate change in agriculture. In order to address this issue, new soil health tests are being developed with the aim of considering not only the nutrient (chemical) status of the soil, but also to investigate biological indicators and analyse soil structural quality to inform management practices (FAS TN721, 2019). The Scottish Government recognises the importance of soil quality in sustainable agriculture and has committed to supporting a new farmer-led initiative, the Soil Regenerative Agriculture group under Farming for a Better Climate, to collaborate and test potential solutions for tackling climate change.

The aim of this investigation was to benchmark the soil health of five farms taking part in the Scottish Government's Farming for a Better Climate initiative on 'Soil Regenerative Agriculture'. The objective was to use the new 'Soil Health Test' developed by Scotland's Rural College (SRUC) to determine the chemical, physical and biological status of the soils. Through reference to guideline values in SRUC and Scotland's Farm Advisory Service (FAS) Technical Notes (TN's) where applicable, this work highlighted areas in the farms that are performing well and identified areas where consideration should be given to future soil management practices to improve overall soil health and promote soil regeneration.

MATERIALS AND METHODS

Farms

The FFBC Soil Regenerative Agriculture Group comprises five farms in North-East Scotland growing a range of crops including cereals, potatoes, oil seeds, legumes, soft fruits and daffodils; one farm has beef cattle. Most of the farm soils are direct drilled and crop residues are removed although in some fields they are chopped and left on the field.

Soil sampling and analysis

One bulked soil sample (1 kg) per field was collected in March 2019 using a 20 cm Dutch hand auger in a 'W' pattern across each field post-harvest and before cultivation, sowing, chemical or organic material applications. Three fields per farm were sampled, giving 15 soil samples in total. A sub-sample (200 g) of each was sent to SRUC Analytical Services Department for a Soil Health Test analysis. This included sample processing using field moist soil for potentially mineralisable nitrogen (PMN) then air-drying and sieving soil for the determination of Modified Morgan's extractable phosphorus (P), potassium (K) and magnesium (Mg), soil pH (distilled water) and loss on ignition (%) for organic matter determination. At three points across each field, an earthworm count and a Visual Evaluation of Soil Structure (VESS) (Ball *et al*., 2007) was conducted in a 20 cm x 20 cm soil pit.

RESULTS

The results collected across the fifteen fields are summarised in Table 1 for soil pH, main nutrients (P, K and Mg), loss on ignition, potentially mineralisable nitrogen, earthworm populations and VESS. Loss on ignition was used as an indicator of organic matter and ranged from 3.59 to 7.02%. Potentially mineralisable nitrogen was on average 26.8 mg/kg (± 3.26 standard deviation) with a range of 19.9 to 30.8 mg/kg. The highest populations of earthworms were found in fields 1 and 3 showing earthworms of 8+ in all three soil pits. Fields 7, 8 and 11 had the lowest worm populations (< 2 in total in the field across all three pits).

Table 1. Summary of the main results from the SRUC Soil Health Test performed on fifteen fields from the five farms.

Field	pH	P (mg/L) (P Status)	K (mg/L) (K Status)	Mg (mg/L) (Mg Status)	LOI (%)	PMN (mg/kg)	Earthworms	VESS
1	6.1	4.81 (M-)	71.2 (L)	119 (M)	4.64	23.9	8+, 8+, 8+	3.55
2	6	7.68 (M-)	148 (M+)	66.2 (M)	4.5	29.8	7, 7	3.6
3	5.9	5.09 (M-)	138 (M-)	57.2 (L)	5.4	30.8	8+, 8+, 8+	1.67
4	6.3	5.39 (M-)	61.1 (L)	72.9 (M)	4.36	27.7	8, 6, 3	2.3
5	6.5	4.84 (M-)	79 (M-)	158 (M)	4.51	30.1	5, 4, 4	2.6
6	5.9	5.64 (M-)	138 (M-)	107 (M)	3.6	25.2	3, 6, 5	2.4
7	6.2	9.24 (M-)	375 (H)	195 (M)	7.02	25.6	0, 0, 2	2.33
8	6.2	6.83 (M-)	98.6 (M-)	90.8 (M)	4.6	19.9	1, 0, 1	2.53
9	6.1	11.9 (M+)	168 (M+)	147 (M)	5.39	23.3	8+, 8+, 4	2.3
10	6.2	8.4 (M-)	111 (M-)	116 (M)	5.47	28.9	3, 2, 8	2.64
11	5.9	7.89 (M-)	86.1 (M-)	91.9 (M)	5.86	30.5	1, 0, 0	1
12	5.9	7.64 (M-)	80.6 (M-)	84.3 (M)	5.01	27	2, 1, 5	1
13	6	8.15 (M-)	106 (M-)	164 (M)	4.93	27.3	8+, 3, 5	2.22
14	5.9	8.79 (M-)	142 (M+)	141 (M)	4.95	29.4	7, 8+, 7	2.02
15	6.1	12.8 (M+)	86.5 (M-)	125 (M)	3.59	23	2, 0, 4	1.67

The soil pH results are shown in Figure 1 in relation to the optimum pH range for arable crops grown on mineral soils in Scotland (pH 6.0 to 6.2) (FAS TN714, 2019). The range of soil pH values was low with the minimum soil pH at 5.9 and the maximum at 6.5.

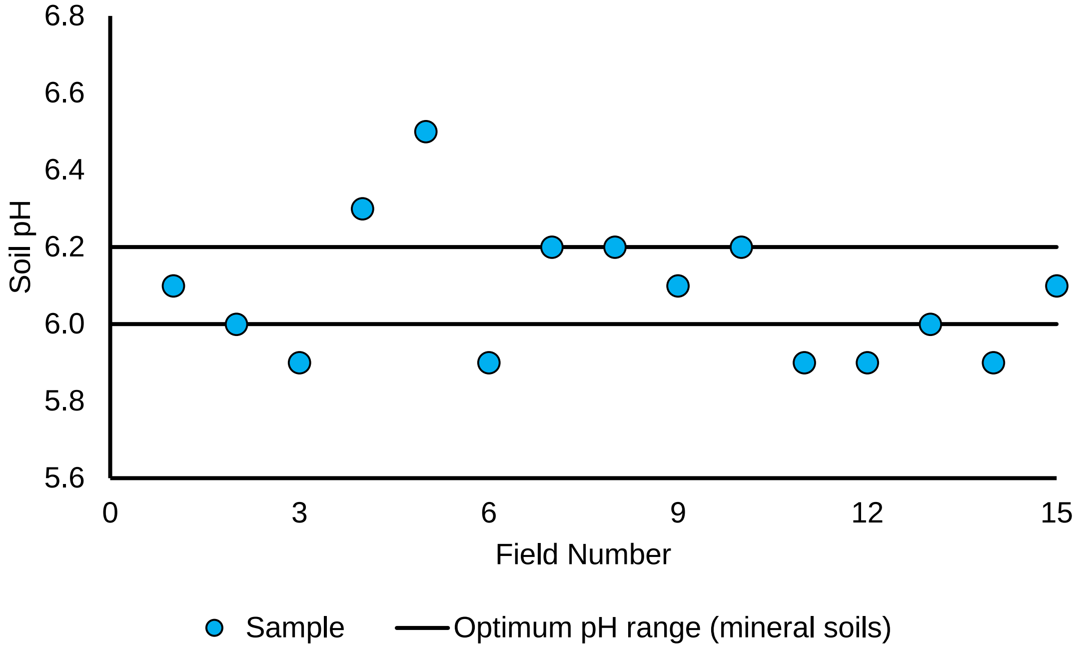

Figure 1. Soil pH results for the fifteen fields showing the optimum pH range of 6.0 to 6.2 for mineral soils in Scotland (FAS TN714, 2019)

The average field VESS Soil Quality (Sq) scores for the fifteen fields are shown in Figure 2. Nine out of the fifteen fields were between Sq 2 (intact) and 3 (firm). Two fields were between Sq 3 and 4 (compact), two were between Sq 1 (friable) and 2 and the final two fields had an average score of Sq 1. None of the fields were Sq 5 (very compact).

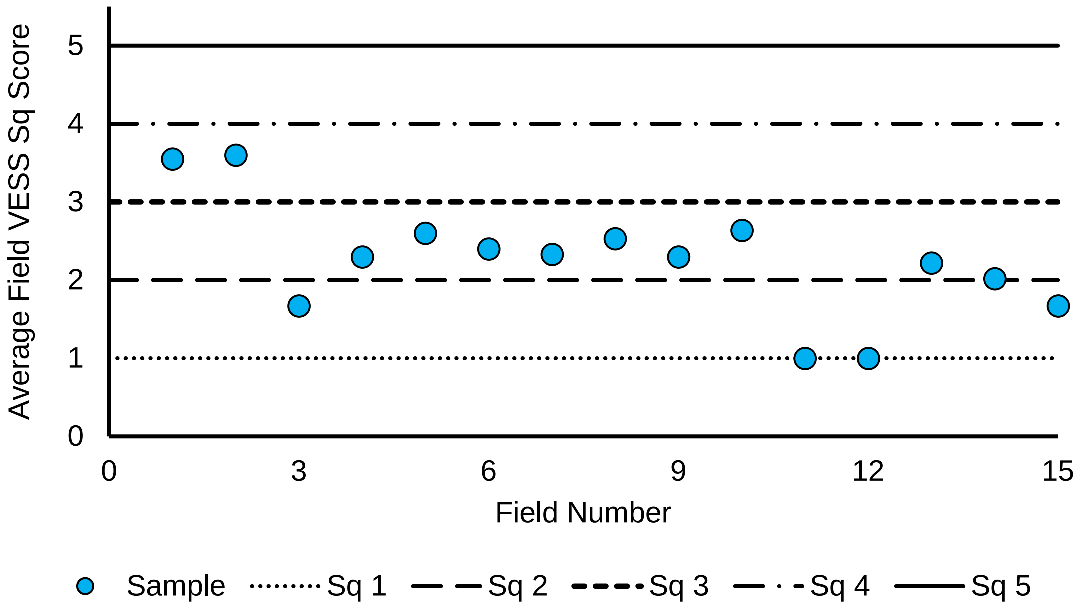

Figure 2. Average Field VESS Sq Scores for fifteen fields tested across the farms showing where the samples lie within the Sq Score bands.

All soil samples had a Modified Morgan's P concentration within the Moderate range, with 92% given a Status of M- (Figure 3). This shows that the farmers are managing their P concentrations well and should apply maintenance application of P fertilisers unless there are potatoes in the rotation then the farmers should look to increase their Status to M+ taking into account their soil phosphorus sorption capacity (PSC) (FAS TN717, 2019).

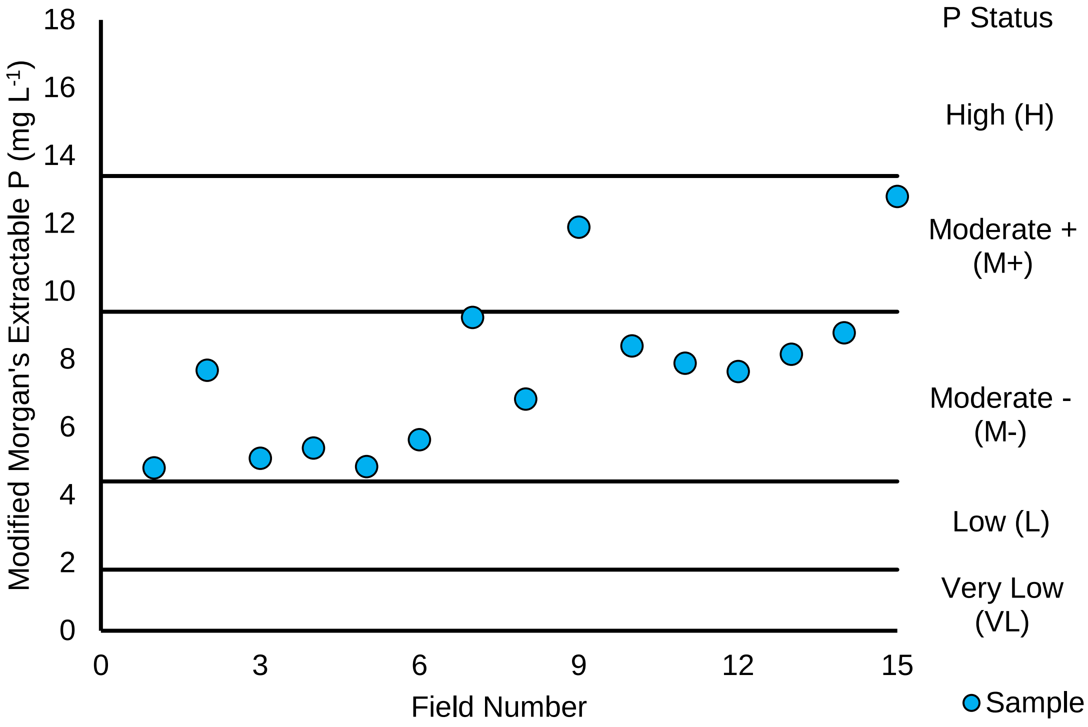

Figure 3. Modified Morgan's extractable P concentration in the fifteen fields compared to the reference soil P Status ranges (FAS TN717, 2019).

The soil Modified Morgan's K results showed a wide spread across the guidelines for K in Scotland (FAS TN717, 2019) with an average K of 126 mg L^{-1} and a standard deviation of 76 mg L^{-1} (Figure 4). One sample with a concentration of 375 mg L^{-1} was found to be of High K Status (between 201 to 400 mg L^{-1}) with two samples falling into the Low Status range (40 to 75 mg L^{-1}) with values of 61.1 and 71.2 mg L^{-1}. Most samples were within the target moderate ranges as either M- (76 to 140 mg L^{-1}) or the M+ range (141 to 200 mg L^{-1}).

The Modified Morgan's extractable Mg concentration is shown in Figure 5 and the results for the soil samples are compared to guidelines in Scotland (SRUC TN633, 2013). Almost all samples were within the target Moderate Mg Status range (61 to 200 mg L^{-1}) with one sample only just in the Low range (20 to 60 mg L^{-1}) at 57 mg L^{-1}.

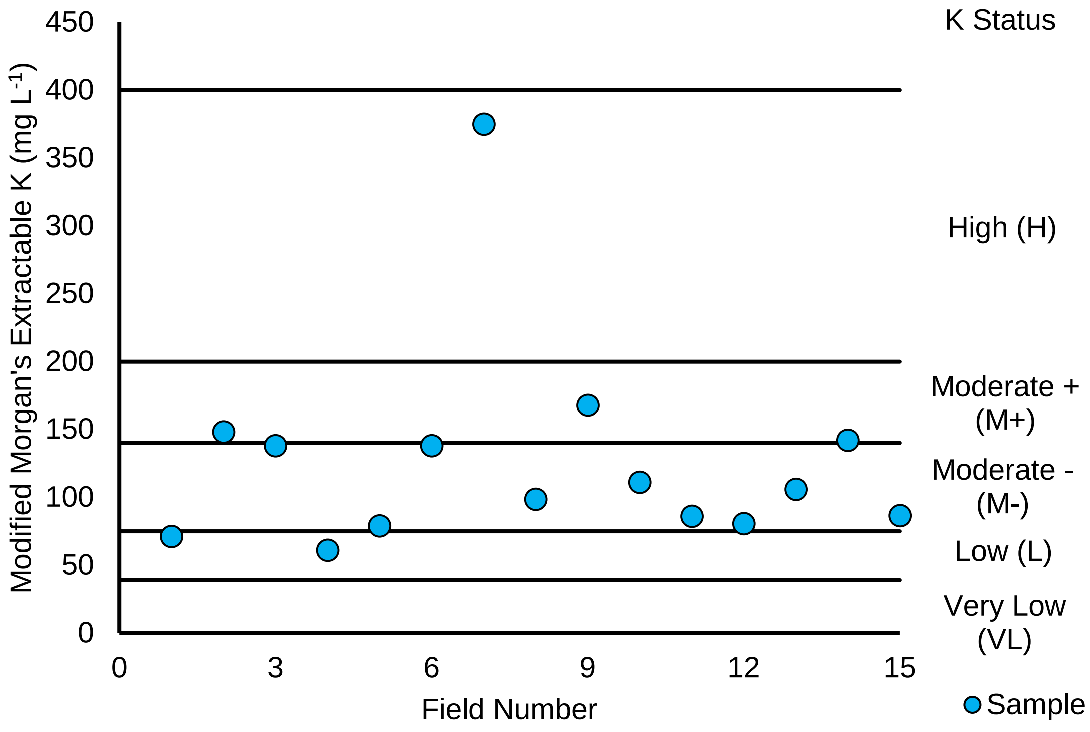

Figure 4. Modified Morgan's extractable K concentration in the fifteen fields compared to the reference K Status ranges (FAS TN717, 2019).

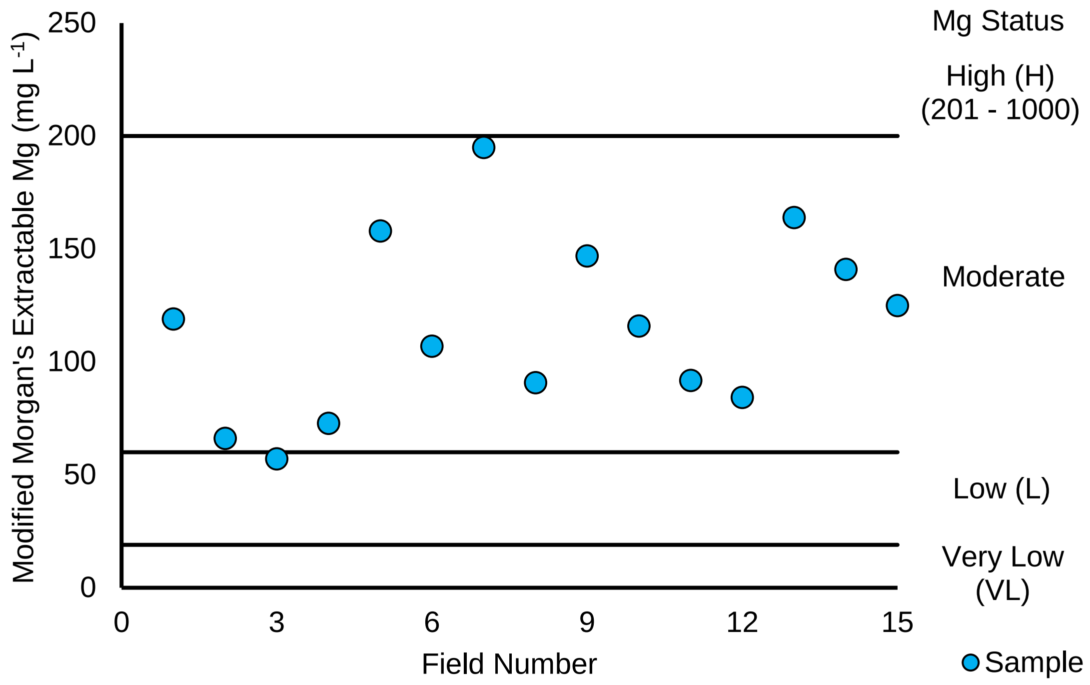

Figure 5. Modified Morgan's extractable Mg concentration in the fifteen fields compared to the reference Mg Status ranges (SRUC TN633, 2013).

DISCUSSION

Chemistry

The conventional chemical testing of the soils showed that most fields were on target Status for P (M-), Mg (M) and soil pH (5.9 to 6.5) with the most variation seen in the K results (L to H). Soils with a K Status of L require more than a maintenance application of K fertiliser to raise the K status to the recommended levels necessary for growing crops in North East Scotland and Tayside (FAS TN717, 2019). Financial savings on lime could be made in those fields with a soil pH above 6.2 by reducing the application amounts in line with agronomic requirements but overall the results indicate that the soil pH and nutrient status are well managed across the five farms. The LOI indicate total organic matter content and the results showed that most samples were between 3.50 and 7.02% with an average of 4.92% across the 15 samples.

Currently there are no guideline values as to what a soil LOI should be as this depends on a range of factors including rainfall amounts, soil texture and underlying soil series as well as soil use e.g. arable or grassland (Griffiths et al, 2018). Potentially mineralisable nitrogen is another chemistry technique used to indicate soil health however, as with loss on ignition, no guideline values exist for Scotland. The method was recently considered by the Agriculture and Horticulture Development Board (AHDB) as a potential indicator but did not make the shortlist for testing to produce a ‘scorecard’ of recommended levels (Griffiths et al, 2018). Based on the scorecard which SRUC uses for PMN results in their soil health test, below 20 mg kg^{-1} PMN is not ideal (red), 20 to 50 mg kg^{-1} would be acceptable (amber) and a PMN over 50 mg kg^{-1} would be considered good (green). The results in this study revealed that most of the soils were within the amber category, at an average of 26.8 mg kg^{-1} with a standard deviation of 3.26 mg kg^{-1} across the 15 samples. The lowest value was 19.9 mg kg^{-1}, just before the amber threshold, and the highest was 30.8 mg kg^{-1}. Until further work has been done on the PMN test to clarify guideline values it is difficult to determine management strategies that would be effective in terms of N management on farm.

Physics

The VESS Sq scores showed that 9 out of 15 soils were intact (Sq 2) or firm (Sq 3) and for soil structure this is considered to be good and moderate respectively. Two of the soils had a Sq score of 1 (good, friable) and another two scored between Sq 1 and 2 which means the soils require no management (Ball *et al*., 2007); all four soils require only to be monitored. Those two soils which were between 3 (moderate, firm) and 5 (very compacted) require immediate management changes to be considered such as residue or traffic management (Ball *et al*., 2007) although some farms already manage their residue inputs. None of the Soil Regenerative Agriculture farms had field Sq score of 5 (poor, very compacted) which is positive but those fields which are between Sq 3 and 4 will require changes to be made to ensure they do not become heavily compacted which could affect productivity. Compaction in agricultural fields can lead to increased soil erosion and runoff leading to nutrient loss in Scotland (Hallett et al, 2016). The farms are currently investigating the use of cover crops as a soil regenerative method. Cover crops can be useful in mitigating the effects of climate change, provide ecosystem services and can be used to mitigate soil structural issues such as compaction and reduce erosion and loss of soil (Blanco-Canqui *et al*, 2015).

Biology

The earthworm population recording and guidance document produced by AHDB (2018) recommends that earthworm populations per soil pit should at least be between 7 and 10, which indicates a good presence of earthworms across the field. A range of 0 to 3 earthworms in a soil pit is considered to be ‘poor’ and 4 to 6 is ‘patchy’. These numbers indicate that improvements could be made in the fields were there are low numbers and may suggest underlying issues with soil structure or chemistry such as pH, although in this study pH would not appear to be an issue. Those fields with populations over 7 are likely to be benefit from improved plant productivity. The earthworm results showed populations were ‘poor’ or ‘patchy’ in 46.6% of fields across the three replicates. In 33% of fields, populations ranged from ‘poor’ to ‘good’. Conventional tillage practices can have a detrimental effect on earthworm populations (Stroud, 2019) although most of the farms have recently become no-tillage or reduced tillage systems. Earthworm populations might be expected to improve if the system undergoes reduced tillage combined with the leaving of crop residues in the field for more than 10 years (Briones & Schmidt, 2017).

CONCLUSION

Conducting a soil health test on the fields has helped the farmers to produce a benchmark from which to start soil regenerative agricultural practices to combat the effects of climate change. Further research is required to understand and recommend management practices based on the results of certain tests used in soil health testing such as loss on ignition and potentially mineralisable nitrogen. However, management advice exists in the form of technical notes and guideline values for certain parameters such as P, K, Mg, soil pH and soil structure which can be used by farmers to act on the results of testing. There are a number of management practices which can be used to improve soil health and help mitigate climate change effects on soil structure including the use of cover crops, diversifying crop rotations, using organic fertilisers and altering tillage practices for example (FAS TN721, 2019). Through investigating the biological and physical parameters of the soil in addition to nutrient and chemical status, farmers can make informed management decisions particular to their fields and business needs and focus on potential problem areas which will save time and money. The use of the VESS to investigate soil compaction issues alongside routine testing should improve soil and nutrient management practices on the farms which will ultimately improve soil structure and help earthworm populations.

ACKNOWLEDGEMENTS

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THE IMPACT OF AGRICULTURAL MANAGEMENT STRATEGIES ON CROP MICROBIOMES

N Holden, J Marshall, Laura Pugh and A Newton
The James Hutton Institute, Invergowrie, Dundee DD2 5DA
E-mail: Nicola.holden@hutton.ac.uk

Summary: The microbial communities associated with crop plants play a pivotal role in their development and health. Microbes inhabit the developing rhizosphere in a process of recruitment that is driven by the plant exudates, and the resulting composition is also heavily influenced by soil type. The phyllosphere community then reflects the rhizosphere community composition, in addition to influences from above-ground environmental factors. Different agricultural management strategies can profoundly alter the local crop environment, ranging from soil structure alterations to foliar pesticide application, which inevitably impact the microbial taxonomic composition and therefore potentially their function. Here, we investigated the impact of different tillage strategies on the microbiome of barley. In parallel, the impact of tillage was related to the extent of fungal disease. Tillage had a strong impact on the rhizosphere microbiome taxonomy of spring barley as well as the outcome of fungal disease on winter barley.

INTRODUCTION

Soil microbial communities are diverse across different soil types and in different environmental contexts, where they carry out a range of fundamental biological and chemical functions. Plants select or enrich communities from the surrounding soil microbiome into the rhizosphere and endosphere environments through the action of root exudates (Hartmann *et al.*, 2009), with well-known examples such as plant growth promoting bacteria (PGPRs) and arbuscular mycorrhizal fungi (AMF). Research into the microbiome communities have demonstrated how their taxonomy changes dependent on the soil type and plant species (Bulgarelli *et al.*, 2015). Technological advances in DNA sequencing and bioinformatics mean that we are now at the point of better understanding the functional capacities of rhizosphere and endosphere microbiomes.

Soil communities are prone to anthropogenic disturbance that arise from different agricultural practices. Intensive agricultural practices that employ high tillage frequency, low crop diversity and high nutrient input correlate with reduced AMF mycelium formation and faster spore formation (Verbruggen & Toby Kiers, 2010). Tillage, in particular, is known to reduce mycorrhizal diversity, which in turn impacts functional complementarity between AMF species, but it is unclear how spatial alterations affect inter-species competition and functional evolution. While spatial disturbances have obvious impacts for fungi that form macro-scale networks, less is known about the impact on soil and rhizosphere bacteria. There is evidence that community diversity and functional traits such as nitrogen distribution are affected (Sun *et al.*, 2014).

Here, we wanted to determine the impact that different agricultural practices have on rhizosphere communities and associated functional roles in plant health. To address this, we tested the hypothesis that arable plants grown under regimes that inflict the least amount of soil disturbance have more diverse phytobiomes, which is reflected in the diversity of the rhizosphere microbiome and positive impacts on plant health. A field trial that was designed to investigate the impact of conservation farming was employed (Spring-Summer 2018), enabling barley rhizosphere bacterial and fungal taxonomic compositions to be characterised. Two tillage approaches were compared: minimum disturbance of direct drill and the conventional approach of inversion tillage, in a single field site split into parallel plots. In parallel, fungal disease assessments were made, enabling a correlation analysis with taxonomic composition. Barley was selected as a common arable crop in Scotland.

MATERIALS AND METHODS

Field Trials

A field trial (Leven, Fife, Scotland) was established for multiple cultivars of spring barley, winter barley and winter wheat in east Scotland, to compare the impacts of long-term non-inversion direct drilling (Fig. 1A) ('direct') with conventional inversion tillage ('plough') on crop disease and yield.

Disease Assessment

Mildew disease was scored on a series of winter barley cultivars in the trial site, using established scoring criteria (Newton & Hackett, 1994). 30 cultivars (anonymized) were assessed.

Rhizosphere Sampling

Barley plants (cvs. Concerto & Laureate) were sampled from four replicated plots treated by direct drill or ploughing, at the tillering stage (GS20: May 2018) and stem elongation (GS39-49: June 2018), generating 32 samples. Between 5 – 8 plants per plot were pooled per sample. Bulk soil was removed and the rhizosphere collected from the top 6 cm of root in 15 ml PBS (Robertson-Albertyn *et al.*, 2017). Total genomic DNA was extracted using a commercial kit (FastDNA SPIN Kit for Soil, Invitrogen).

Microbiome Sequencing and Analysis

Amplicon libraries were generated for bacterial and fungal communities by PCR of rRNA regions, 16S (Klindworth *et al.*, 2013) and ITS2 (Ihrmark *et al.*, 2012), respectively, using the Nextera 600 amplicon and indexing kits (Illumina Ltd). Samples were pooled into a single run on an Illumina MiSeq sequencer and data cleaned, clustered and filtered using Mothur (Schloss *et al.*, 2009) on a Galaxy bioinformatics platform (Goecks *et al.*, 2010). Reads were aligned using standard 16S databases into operational taxonomic units (OTU), which represent a taxonomic distinction below the level of genera, but normally above the species level. OTU quantification and distributions were analysed using the phyloseq package in the R environment (McMurdie & Holmes, 2013).

RESULTS

Soil management practices had an obvious impact on crop development at the early (tillering) stage, with a growth lag apparent in the direct drill plots compared to the ploughed plots (Fig. 1A). A rhizosheath was more evident in plants recovered from the conventionally-treated ploughed plots, especially at later growth stages (Fig. 1B, C).

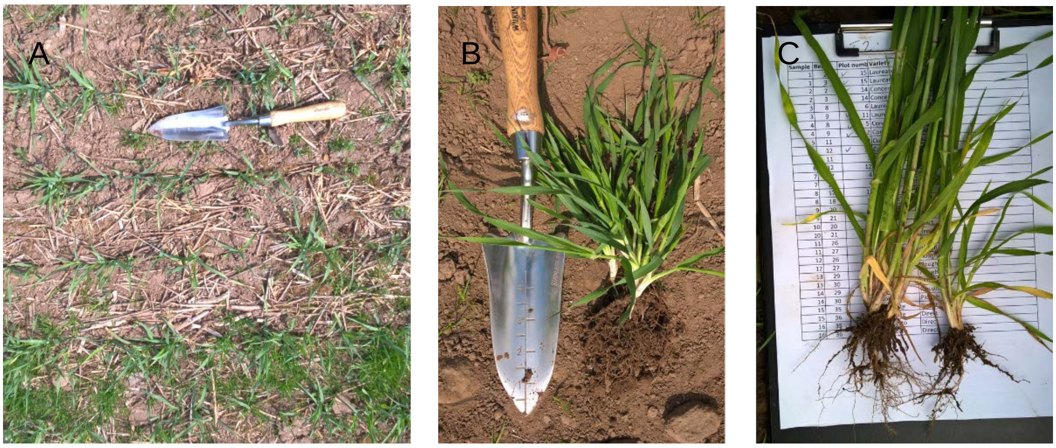

Figure 1. Field Sampling for barley rhizosphere. (A) Direct drill plot: Spring barley (May 2018). Plants were sampled at (B) GS20 and (C) 39-49 developmental stages.

The presence of bacteria and fungi genomic DNA was validated for all rhizosphere samples prior to library generation by conventional PCR (Fig. 2). Generation of amplicon libraries for both kingdoms required optimization of PCR on a per sample basis, due to the degree of heterogeneity between the samples. Amplicon libraries were generated for both bacteria (16S) and fungal (ITS2) communities, but since PCR amplification of the ITS2 region was not universal across all samples (in contrast to the bacterial 16S region), analysis of the fungal communities is not discussed further here. Short-read sequencing of the amplicon libraries generated ~ 20 M reads that passed the quality filtering step, with 70 % reads with a score of > Q30 (i.e. 99.9 % base accuracy). The aligned reads were clustered into operational taxonomic units (OTU) using the Silva database and filtered for contaminating plant (i.e. plastid, mitochondrial) DNA.

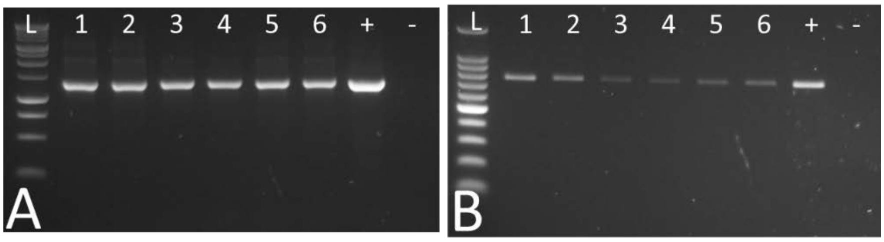

Figure 2. Detection of bacterial (A) and fungal (B) communities from PCR amplification of 16S and ITS2 target sequences, respectively, for templates 1-6, with controls (+, -) and ladders (L: 1 kb – A, and 100 bp - B).

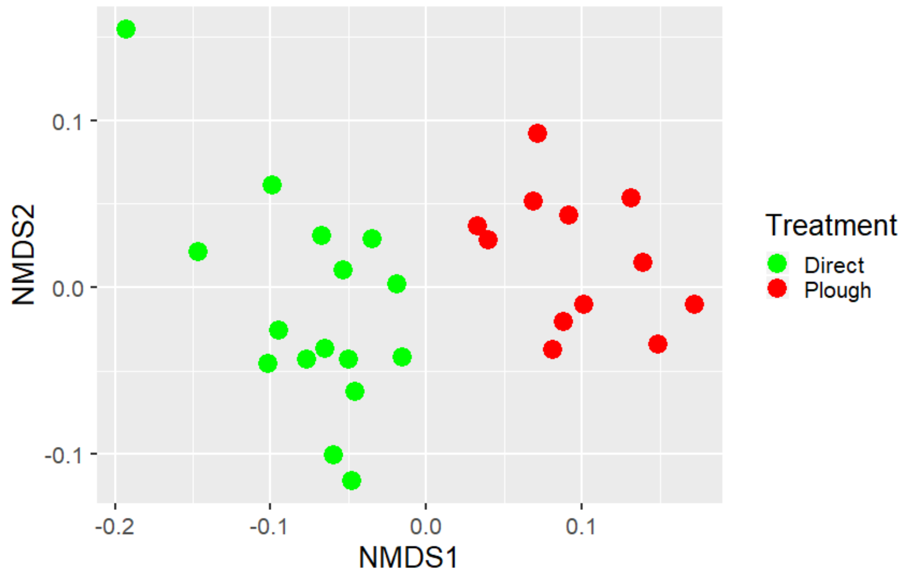

Figure 3. OTU samples grouped by treatment. Ordination plot of all OTU sample coloured by treatment type, for direct drill (green) or ploughed (red).

Diversity in taxonomy (OTUs) occurred between samples (i.e. beta diversity) at the level of soil management practice (plough 'vs' direct drill) (Fig. 3). Diversity was also evident at the level of growth stage (GS20 'vs' GS39-49), but not between the genotypes (not shown).

Disease assessment for powdery mildew on winter barley showed a significant and extensive increase in disease associated with increased tillage (Fig. 4) (the spring cultivars were not included here as they were not susceptible to the disease). A similar impact was recorded for winter wheat (not shown).

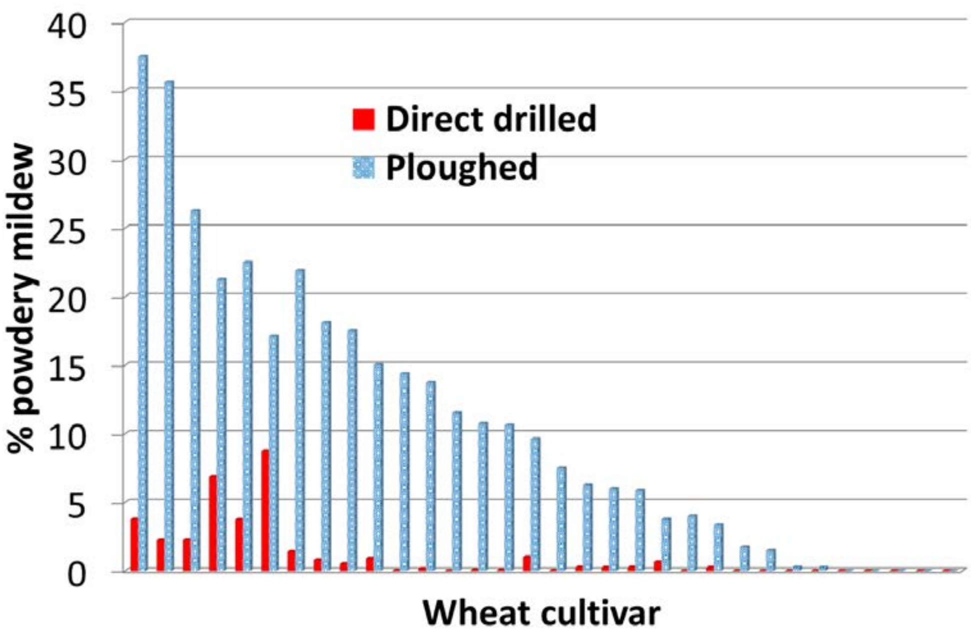

Figure 4. Powdery mildew on winter barley. Symptomatic disease was scored (% disease) for a series of winter barley cultivars in direct drilled (blue) and ploughed (red) plots.

DISCUSSION

Conservation agriculture is practiced in commercial production as a means to increase biodiversity, improve soil health and reduce soil erosion, with reductions in tillage as one of the most important components (Hobbs *et al.*, 2008). Although the practice has been in use for some time, on-going technological developments in DNA sequencing are permitting a much deeper understanding of the effect on and functional role of the microbiome communities. Thus, it is possible to determine the impact that different tillage or conservation practices have on soil microbiome communities. Since the plants play an active role in recruitment of specific members of the soil microbiome through the action of root exudates (Wagner *et al.*, 2016), any impact of tillage will most likely be reflected in the rhizosphere microbiome. In turn, this community is related to the above-ground communities, and plays a role in plant health.

Here, a microbiome analysis pipeline was established to investigate the impacts of conservation agricultural practices on microbiomes, specifically the effect of tillage on the rhizosphere of spring barley. The large degree of heterogeneity, inherent in complex soil and rhizosphere microbial communities, necessitated PCR optimisation to generate sufficiently robust amplicon libraries for both bacterial and fungal communities. Amplicons for fungal communities were only detected in a sub-set of samples, indicative of either poor recovery of fungal gDNA from the samples, or potentially, technical issues with amplification. The latter is less likely since PCR validation prior to amplicon library generation also showed lower levels of rRNA for fungi than for bacteria (Fig. 2). Thus, it appeared that the rhizosphere DNA preparations were dominated by bacterial rather than fungal-derived material.

Tillage practices impacted on barley rhizosphere community composition, resulting in a clear distinction in taxonomic diversity between the treatments. There was significant enrichment in different genera and the OTU levels (*to be shown elsewhere*). This is consistent with other studies that have identified particular OTUs that are differentially impacted in 'organic' and intensive agricultural practices, termed as 'cropping sensitive' OTUs (Hartman *et al.*, 2018). Thus, an extension of our work is to carry out comparative analysis on the OTUs that are similarly 'tillage sensitive'. Plant developmental growth stage also impacted taxonomic diversity, which is consistent with reports for other plant species (Chaparro *et al.*, 2014). There was no impact from the spring barley cultivar, but this is perhaps not surprising since the two cultivars investigated here are closely related, sharing the same parental line.

The impact of tillage on plant disease was investigated in parallel in the field study site. Here, a strong treatment effect was seen for powdery mildew on winter barley, with a reduction in disease for the direct drilled plots. The basis for the effect is currently under investigation but is likely to be a combination of the impact of the endemic rhizosphere community on induced systemic resistance and disruption of AFM networks with consequential changes in the plant-fungi interaction and fungi-soil microbiome interaction. Powdery mildew (*Blumeria graminis* f.sp. *hordei*) is a foliar disease, but the plant rhizosphere community is well known to influence plant health, and indeed is a target environment for application of biocontrol inoculants. As such, our longer-term plans aim to correlate any relationship between the rhizosphere microbiome and incidence of plant disease.

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VALIDATION OF A REAL TIME DECISION SUPPORT SYSTEM (PREDICTION SYSTEM) TO CONTROL STRAWBERRY POWDERY MILDEW WITH THE USE OF FEWER FUNGICIDES

A M Hall, H Wileman and B Liu
Biological and Environmental Sciences, University of Hertfordshire, Hatfield AL10 9AB
Email: a.m.hall@herts.ac.uk

Summary: The parameters used to predict disease conducive conditions for strawberry powdery mildew development are described and then used in a real-time web-based system to predict when a grower should spray with fungicides. This keeps the initial inoculum to a minimum and prevents epidemic build up with the use of fewer fungicide sprays than the advised weekly or fortnightly fungicide sprays. The results of the successful 2018 and 2019 trials in Scotland are given in this paper. The cost / benefit analysis from the final validation of the system in 2019 on farms in Scotland will be presented in February 2020.

INTRODUCTION

The strawberry crop in Britain is a successful soft fruit crop, the hectarage has remained static for over 20 years, but the yield has doubled. This has been achieved using polythene tunnels, precision watering and nutrition coupled with the judicious use of cultivars, both June bearers and ever bearers. This has resulted in a lengthening of the harvest season from 6 or 8 weeks to six months. However, the environment created (temperature and relative humidity) in the polythene tunnels has resulted in strawberry powdery mildew (caused by *Podosphaera aphanis*) to become the most feared disease of strawberries (Figure 1). *P. aphanis* can cause up to 70% yield loss. One grower reported a loss in one year of £750,000, due to this disease. To control strawberry powdery mildew, some growers are spraying weekly resulting in up to 24 fungicide sprays in a season. This number of sprays a season has environmental and financial consequences. Hall *et al.*, 2017 gives an overall description of integrated control of this disease, including information on clean up spraying at the start of the season and venting tunnels, however, multiple fungicide sprays are still required. The life cycle of the fungus is shown in Figure 2.

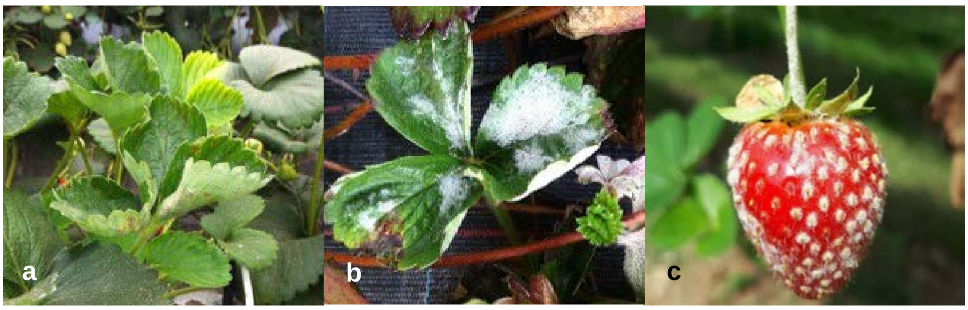

Figure 1. Symptoms of strawberry powdery mildew, caused by *Podosphaera aphanis* including leaf cupping, (a) mycelium on both leaves (b) and mycelium on ripe fruit (c).

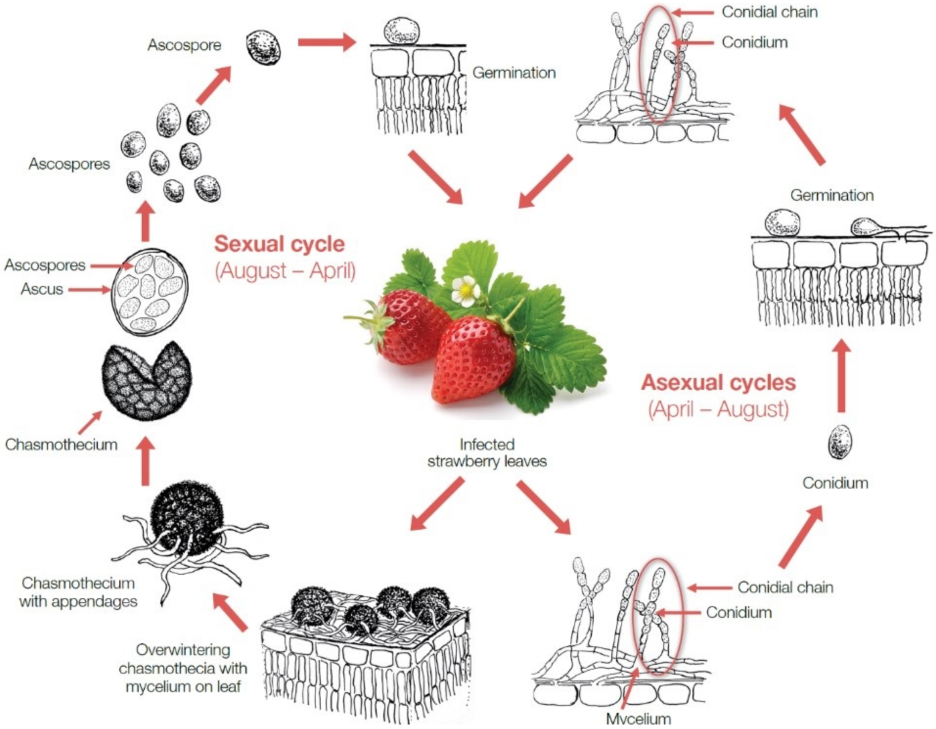

Figure 2. Life cycle of *Podosphaera aphanis,* including both an asexual and sexual cycle (Jin, 2016).

Work at the University of Hertfordshire since 2004 has resulted in the development of a decision support system based on the temperature and humidity for asexual fungal growth and sporulation that predicts when growers should spray with fungicides against strawberry powdery mildew. The aim of the system is to prevent sporulation of the fungus. The prediction system is based on the parameters shown in Figure 3, using temperature and humidity sensors within the crop. At the start of the season the grower assumes that there may be some disease and does a clean-up spray. The prediction system accumulates the hours which have the correct temperature and humidity conditions for the fungus to grow from conidiospore germination, through 'elongating secondary hyphae' to sporulation, i.e. it is accumulating 'disease conducive' hours. This appears as an ascending green line until it reaches 115 hours, when the line turns to amber, which is an indication to the grower that they should start thinking about making a fungicide application. At 125 hours the line turns to red; at 144 hours, the fungus can start to produce new spores and so initiate an epidemic if the grower has not sprayed.

After spraying, the grower enters fungicide details and resets the system which then starts to accumulate disease conducive hours again. Risk is defined by the number of disease conducive hours that have occurred. If only 50 disease conducive hours have occurred, then there is a low risk, as the fungus will not have grown very much. If 115 hours of disease conducive conditions have occurred, the fungus will be growing and there will be a high risk of disease development. When the ascending line is between 125 and 144 hours it is advised that the grower sprays a fungicide.

The work reported here is of the validation of the real time, web-based system on farms in Scotland in 2018 and 2019.

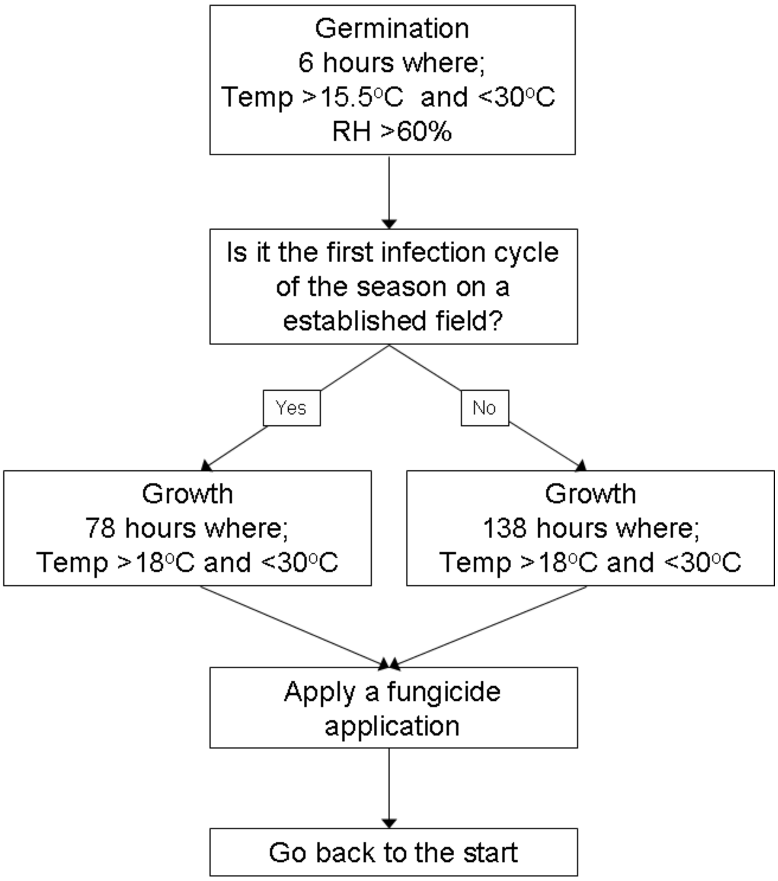

Figure 3. Flow chart showing parameters used to predict when fungicides should be sprayed (Dodgson, 2007).

MATERIALS AND METHODS

The validation criteria of the prediction system were to have a range of geographical locations (England and Scotland), a range of cultivars (June bearers and ever bearers), and a variety of growing methods i.e. the use of soil or coir, on raised beds or tabletops.

The decision support system was used on one farm in Scotland in 2018, and two farms in Scotland in 2019 (cost benefit analysis not available at time of writing for 2019). In 2018, the farm located at DD2 5 used the prediction system from March to October on an area of 15 hectares. Both ever bearer (cv. Islay and Murano) and June bearer (cv. Sonata) strawberry crops were grown in coir on tabletops in Seaton tunnels. The June bearers were grown as two successive crops, the second was planted in June and overwintered into the 2019 season. A Davis temperature and relative humidity sensor was placed within the crop. The normal routine spray programme for this farm was to apply fungicides every 14 days. Disease assessments were carried out throughout the season, to achieve commercially satisfactory disease control.

In 2019, two farms in Scotland used the prediction system. The first strawberry crop, located near PH12 8 was sprayed with fungicides guided by the prediction system from July to October 2019 on a hectare of covered everbearer crops (cv. Murano), grown in coir bags on tabletops. A second strawberry crop, located near DD11 3 was sprayed with fungicides guided by the prediction from June to July 2019 on a covered June bearer crop (cv. Malling Centenary™), grown in soil. The routine spray programmes for both farms was to apply fungicides every 10 days.

RESULTS

The prediction system gave commercially satisfactory disease control in 2018, confirmed by routine disease assessments. On the ever bearers, the routine spray programme used 13 fungicide sprays whereas the prediction system only used 10 fungicide sprays, thus giving a saving of three sprays. The first June bearer crops received 5 fungicide sprays, and the second crop received 3 when using the prediction system (the advised routine spray was 7 fungicide sprays on the first crop and 4 on the second). The use of the prediction system used three fewer sprays than the routine programme advised. Table 1 shows the cost benefit analysis.

Table 1. Cost benefit analysis for DD2 5 (2018)

Cultivar type	Cost for routine commercial spray programme (£ ha^{-1})	Cost for prediction system (£ ha^{-1})	Total saving (£ ha^{-1})
Ever bearer	1,194.60	918.92	275.68
June bearers	1,029.44	748.68	280.76

Table 2. Fungicide spray programmes from PH12 8: using the prediction system from 2nd July to 2nd October 2019; and routine spray programme (2019)

Prediction System			**Routine Spray Programme**		
Application Date	Fungicide Used	Active Ingredient	Application Date	Fungicide Used	Active Ingredient
21st Jun	Amistar	azoxystrobin	21st Jun	Amistar	azoxystrobin
2nd Jul	Topas	penconazole	2nd Jul	Talius	proquinazid
10th Jul	Topas	penconazole	12th Jul	Takumi	cyflufenamid
23rd Jul	Luna Sensation	fluopyram + trifloxystobin	22nd Jul	Topas	penconazole
			1st Aug	Takumi	cyflufenamid
			11th Aug	Topas	penconazole
			31st Aug	Charm	fluxapyroxad + difenoconazole
			10th Sep	Luna Sensation	fluopyram + trifloxystobin

Table 3. Fungicide spray programmes from DD11 3: using the prediction system from 5th June until mid-July. The routine spray programme is given as approximate spray dates based on a ten-day spray programme (2019)

Prediction System			Routine Spray Programme
Application Date	Fungicide Used	Active Ingredient	Approximate Application Date
29th Apr	Topas	penconazole	29th Apr
13th May	Potassium Bicarbonate	potassium hydrogen carbonate	9th May
	Kumulus DF	sulphur	19th May
25th May	Luna Sensation	fluopyram + trifloxystobin	29th May
5th Jun	Potassium Bicarbonate	potassium hydrogen carbonate	8th Jun
	Kumulus DF	sulphur	18th Jun
15th Jun	Luna Sensation	fluopyram + trifloxystobin	28th Jun
29th Jun	Potassium Bicarbonate	potassium hydrogen carbonate	
	Kumulus DF	sulphur	

In 2019, the prediction system also gave commercially satisfactory disease control on both farms. When guided by the prediction system at the farm located at PH12 8 (Table 2), four fungicide sprays were applied, whereas following the routine spray programme eight fungicide sprays were applied. The use of the prediction system has saved four fungicide sprays, on this everbearer crop. At the farm located near DD11 3 (Table 3), when guided by the prediction system six fungicide applications were made, whereas if a ten-day routine spray programme had been used seven fungicide applications would have been made (based on application dates). The use of the prediction system has saved a fungicide spray on this June bearer crop.

DISCUSSION

The results from the 2018 and 2019 trial in Scotland showed that the growers who used the prediction system had commercially satisfactory disease control (i.e. minimal amount of disease observed, and no epidemic build-up) but this was achieved with fewer fungicide sprays than the advised fortnightly spray, routine spray programme or ten-day spray programme. The growers had the confidence to not spray with fungicides when they could observe on the prediction system that the disease pressure was low (low risk). In 2018, the grower also benefited from the use of the system by making financial savings on both crops (>£200 per hectare), due to the reduced number of fungicide applications and saved labour costs. Additionally, the reduced number of fungicide sprays when using the prediction system will be beneficial to the environment. In 2018, the grower found the system to be reliable and user friendly, therefore, a final validation of the system was conducted in 2019. In 2019 validation was carried out on two farms in Scotland, which also achieved reduced fungicide applications by using the prediction system. These results of the 2019 cost-benefit analysis will be available in February 2020. Both the 2019 growers reported that the system was easy to follow and use as well as being a reliable decision support system.

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We are very grateful to all the farms participating in the validation of the prediction system in both 2018 and 2019. Many thanks for present funding from Ceres. Many thanks to Kisanhub for their work in proof of concept in 2016 to 2018 and Agri-tech Services for seeing the great potential of a web-based prediction system and for their partnership in the Ceres grant.

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XYLELLA FASTIDIOSA: AN OVERVIEW OF RESEARCH AT SASA

K Lester[1], K Murphy[2], A McCluskey[1], R Cairns[1], K Fraser[1] and D Kenyon[1]
[1]SASA, Roddinglaw Road, Edinburgh, EH12 9DD
[2]LumiraDX Technology Manor Farm Business Park, Stirling, FK9 5QD
E-mail: katherine.lester@sasa.gov.scot

Summary: *Xylella fastidiosa* is a bacterial pathogen responsible for various plant diseases such as Pierce's disease in America and Olive Quick Decline Syndrome in Europe. The introduction and subsequent spread of the disease in Europe presents a significant risk to the UK both in terms of commercially grown plants and plant health in the natural environment. The highest risk of introduction of *Xylella* to the UK is through the movement of infected plants by humans, however the natural route of infection is through xylem feeding insects such as *Philaenus spumarius* belonging to the Order Hemiptera, sub-order Auchenorrhynch. Research of *Xylella* continues with projects such as EUPHRESCO and BRIGIT (Vector borne diseases of plants) studying hosts, vectors and the diagnosis of *Xylella fastidiosa* improving our knowledge of the disease and its vectors in order to mitigate the effects of this potentially devastating disease.

INTRODUCTION

Xylella fastidiosa (X.f) is a bacterial pathogen responsible for several serious plant diseases across the world such as Pierce disease within grapevine in California and Citrus Variegated Chlorosis in Brazil. In 2013 *Xylella* was detected in Europe associated with Olive Quick Decline Syndrome in olive trees in Apulia, Southern Italy and since then there have been positive identifications in Tuscia, Italy, France, Germany, Spain, and Portugal (Denance *et al*., 2017 Saponari *et al*., 2013; Olmo *et al*., 2017). The bacterium has been tentatively subdivided into six subspecies (*fastidiosa, pauca, multiplex, sandyi, tashke* and *morus*). There are three formally accepted subspecies of *X. fastidiosa*, i.e.*fastidiosa, pauca* and *multiplex* (Schaad *et al*., 2004), based on DNA–DNA hybridization data, however currently only two subspecies, *fastidiosa* and *multiplex*, are officially recognised (Bull *et al*., 2012; EFSA, 2018). Genetic analysis of bacteria isolated from Europe suggests there has been more than one introduction of disease into Europe from both Central and North America. The *X. fastidiosa* subsp. *pauca* detected in Italy in 2013 is related to bacteria in Costa Rica, whereas isolations from France in 2015 are from two differing strains of the subspecies *multiplex* related to bacteria originally found in almond trees in California (Martelli *et al.*, 2016).

The initial introduction of *Xylella* to Europe was through movement of infected plant material, however the natural spread of the bacteria from plant to plant is via xylem feeding insects belonging to the Order Hemiptera (Redak *et al*., 2004). In the Americas the primary vectors are the glassy-winged and blue-green sharpshooters where as in Europe the common meadow spittlebug or froghopper *Philaenus spumarius* has been identified as the main vector (Cornara *et al.*, 2017). The vector carries the bacteria within its foregut or mouthparts, and it is for this reason that the insect can be used as a 'spy' insect to screen for presence of *Xylella* (Yaseen *et al*., 2015; Craud *et al*., 2018).

Development of disease after infection with the bacteria is a complex interaction between the bacterium, host plant, insect vector and environment therefore the impact and response to infection and disease varies (EFSA, 2018). The most recent list of *Xylella* host plants produced by EFSA details over 560 plant species reported to be infected by *Xylella* (EFSA, 2019a). Asymptomatic periods are highly variable dependent on host species and subspecies of bacterium meaning successful control of infection could be limited if reliant only on visual inspection for disease (EFSA, 2018).

At the time of writing, detection of *Xylella* within a plant legally requires destruction of all host plants within 100m and a 5km movement ban for 'specified' plants for five years as well as application of appropriate phytosanitary treatments against all stages of the vector population of the specified organism identified at the site of removal. The consequences of which would have severe economic impact on trade not to mention the potential impact of the disease itself on the wider natural environment.

Xylella has not been detected in the UK and the greatest risk of introduction and spread of the pathogen is through movement of inflected plant material into the UK. Due to this several research projects have been initiated to improve our understanding of the pathogen and the biology of the vectors specifically with respect to the UK's cooler climate.

MATERIALS AND METHODS

Enhancing diagnostic capabilities

From 2016 to 2019 SASA was involved in a EUPHRESCO project 2015-F-146 harmonizing methods for the detection of *Xylella* in both plants and insects across Europe and as part of this participated in Proficiency Tests and Test Performance Studies alongside 24 other laboratories across Europe and further afield including USA and Russia.

In collaboration with the Royal Botanic Gardens *Philaenus spumarius* were collected from two gardens, Edinburgh and Dawyck, and tested for the presence of *X. fastidiosa* using the Harper QPCR methodology detailed in PM 7/24 (EFSA, 2019b, Harper *et al*., 2010). These sites are considered to be at higher risk of exposure to an introduction of *Xylella* than those in the natural environment.

Most recently SASA has collaborated on the BBSRC UK Strategic Priorities funded BRIGIT project for further harmonisation of current testing procedures, evaluation of potential technological approaches to targeted sampling, source tracing of *X. fastidiosa* in the event of an outbreak through optimising multilocus sequence typing (MLST) techniques and whole genome sequencing (WGS) of bacterial isolates. MLST is a genetic typing methodology based on the comparison of a set of seven housekeeping genes which can be used to compare subtypes of *X. fastidiosa* (Yuan *et al*., 2010).

Vector biology

In 2018 a citizen science project was run to collect information on host plant preferences of *Philaenus spumarius*. The 'spittlebughunt' hashtag was originally used in 2017 by the International Plant Sentinel Network (https://plantsentinel.org/news/1493/) in corroboration with Fera Science Ltd, Royal Botanic Gardens (Kew), Botanic Gardens Conservation International (BGCI) and Defra to collect information from their gardens in the UK and in 2018 SASA utilized a similar method as part of EUPHRESCO project (F-221) to gather further information (http://www.sasa.gov.uk/sites/default/files/spittlebughunt%20poster.pdf). Individuals were encouraged to tweet images of 'cuckoo spit' using the hashtag 'spittlebughunt' including information on the host plant and location within the tweet. Volunteers also collected nymphs along with a sample of the host plant.

Identification of the nymphs and plants was carried out using a combination of classical identification techniques and molecular barcoding.

Abundance of Aphrophoridae insects (*Phileanus*, *Neophilaenus* and *Aphrophora* species) known as spittlebugs or froghoppers was recorded every two weeks over a period of four calendar months at two different sites; a grassy meadow and the understorey of broadleaved woodland. Data was collected by observing the number of 'spittle' in thirty quadrants along a 100m transect. Adults were sampled from herbaceous plants using a sweep net; 4 sweeps 30 times over the 100m transect. A selection of trees and hedges were also sampled for adult spittlebugs at one timepoint in August.

RESULTS AND DISCUSSION

Enhancing diagnostic capabilities

The inter-laboratory comparison demonstrated that best sensitivity was found using a CTAB extraction method in conjunction with a Harper real-time PCR (Harper *et al.*, 2010; erratum 2013). The accuracy, repeatability and reproducibility of these methods was also high. It should be noted that the QuickPick extraction also produced high quality results but only when using an automated extraction method, repeating the same methodology with a manual extraction step resulted in lower sensitivity (Saponari *et al*., 2019).

No *Xylella fastidiosa* was detected from any of the *Philaenus spumarius* collected from the two sites in Scotland.

Methods have been standardised between official diagnostic laboratories in the UK and further development of detection methods such as improving sensitivity for low bacterial titres and bulking of plant material have been investigated. This also includes in-house validation of tetraplex Q-PCR for rapid characterization of a different *Xylella* subspecies (Dupas *et al*., 2019 preprint).

The advantages of MLST techniques for typing bacteria are well-recognised however there are still gaps in the methodology with respect to *X. fastidiosa*. Although MLST techniques can be used directly from plant material in order to quickly source the origin of any outbreak, work is still required to improve the robustness of the technique. For instance, there is over-representation of some sequence types and a lack of others in NCBI; therefore, a *X. fastidiosa* ssp. *multiplex* strain which has produced conflicting results and is not currently well-represented in existing databases was selected for WGS. Alongside work carried out at other institutes such as Fera and Forest Research this will eventually allow the creation of a new scheme for identification of *X. fastidiosa* subtypes based on genes involved in selection and adaptation to host and environment.

Vector Biology

Records of 'spittle' and host plants were received from both the public and volunteers with 90% of samples being contributed by plant health professionals based at SASA or within the Agriculture and Rural Economy directorate of the Scottish Government (Table 1).

Table 1. Response to Citizen Science Survey

Response	No of respondents	No of locations	No of records
Twitter	32	32	94
Email	13	11	27
Nymph & host plant sample	38	32	219
Total	83	75	340

The spittlebug nymphs and associated plant material were identified to a species and genus taxonomical level respectively. The survey carried out in 2018 identified 93 plant genera ranged across 39 botanical families and it was possible to differentiate which of those records were from gardens *i.e.* managed habitats and those which were observed from un-managed habitats *i.e.* natural parks, waste ground (Table 2).

Table 2. Number of host plants for *Philaenus spumarius*

Managed Habitat *i.e.* garden, urban		Natural Habitat including parks & waste ground		Total	
No of families	31	No of families	26	No of families	39
No of genera	57	No of genera	57	No of genera	93

Overall the botanical families presenting the highest number of plants hosts with *P. spumarius* nymphs were Lamiaceae (10%), Rosaceae (10%), Onagraceae (9%) and Poaceae (9%). The most common garden host recorded was lavender (*Lavandula* sp) and within unmanaged areas such as waste ground, parks, natural areas grasses (Poaecea) were most common. Adult *Philaenus spumarius* were also collected from trees and hedges such as birch (*Betula* sp), hawthorn (*Crataegus* sp) and willow (*Salix* sp) in August. *Philaenus spumarius* are highly polyphagous and therefore should be considered our most important vector.

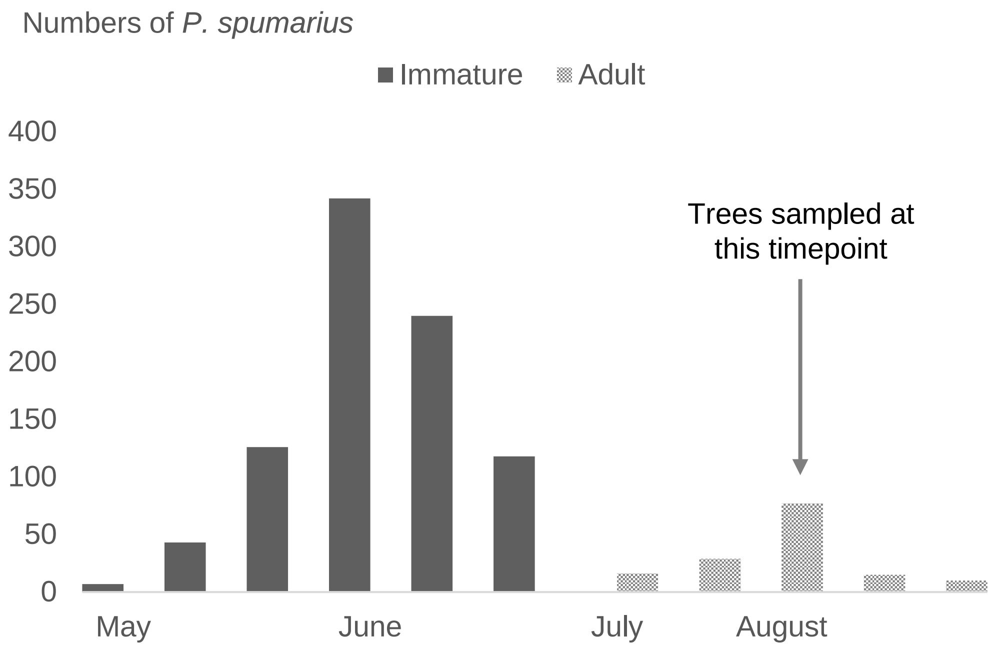

Figure 1 Number of *P.spumarius* nymphs and adults from May 2018 to September 2018.

The number of *P. spumarius* nymphs present steadily increased from early May peaking in mid-June with the first adults emerging in July. This data provides invaluable information for when collection of adults of *P. spumarius* is optimal if *Xylella* were to be detected in Scotland.

SUMMARY

Through these projects Scotland is now better prepared to deal with the threat of *Xylella,* however further research is needed to understand the potential host plants, insect vectors and bacterium interactions especially in our cooler climate. As the highest risk of introduction of *Xylella* into the UK is through the import of an infected plant into a managed habitat, understanding the potential movement of the disease from a garden environment to the wider natural environment or agricultural systems is essential.

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PROMOTING PLANT HEALTH IN SCOTLAND AS PART OF THE UN INTERNATIONAL YEAR OF PLANT HEALTH

K Pearson, G Saddler and D A'Hara
SASA, Roddinglaw Road, Edinburgh, EH12 9JF
E-mail: Karen.pearson@sasa.gov.scot

Summary: The United Nation's Food and Agriculture Organisation have designated 2020 to be the International Year of Plant Health (IYPH). With the tag line "Protecting plants, protecting life", IYPH is a once in a lifetime opportunity to raise the profile of plant health on the global stage. This paper outlines the key messages that the FAO have identified for the year, gives information on some of the Scottish Government initiatives that support these messages, and finally encourages others to think about how they can help promote the plant health message in 2020 and beyond. To find out about Scottish activities planned to celebrate IYPH please see www.sasa.gov.uk/iyph.

INTRODUCTION

The United Nation's Food and Agriculture Organisation (FAO) have designated 2020 to be the International Year of Plant Health (IYPH). Countries all around the world are taking part in this once in a lifetime opportunity to raise global awareness of plant health issues. Scottish Ministers have long supported plant health resilience and the rural industries it underpins. For example, in 2016, the Scottish Plant Health Strategy was published which sets out the Government's approach to protecting Scotland's plants; in 2017, the Chief Plant Health Officer for Scotland was appointed to provide strategic leadership across all plant health sectors and, in 2018, the Plant Health Centre was commissioned to provide call down expertise to inform policy. Now, in 2019-2020, Ministers have marked the International Year of Plant Health as a Scottish Programme for Government commitment to raise the profile of plant health issues, particularly amongst young people and gardeners. IYPH is a key opportunity for government, scientists and industry stakeholders to work together to raise the profile of plant health and all of the industries, landscapes, biodiversity, and recreational activities which are supported by it.

KEY MESSAGES

The FAO have identified 6 key messages, each of which is discussed below.

1. Be careful when bringing plants and plant products across borders

Scottish Government (SG) regularly promotes the European and Mediterranean Plant Protection Organisation's (EPPO) "Don't risk it" campaign to advise on the risks of bringing plants or plant products home from overseas. In December 2019, new legislation removed the passenger baggage allowance for plants. Now travellers are not entitled to bring any plants into the UK from outwith the EU (except for banana, coconuts, dates pineapples or durians for consumption only), which will significantly strengthen our biosecurity. We will continue to reinforce these messages throughout IYPH at events such as SASA's participation in the Edinburgh "Doors Open Day".

2. Make trading in plants and plant products safe without setting up unnecessary barriers.

The International Plant Protection Convention (IPPC), international standards and phytosanitary legislation help to protect our plants whilst facilitating trade. During this year we will continue working with industry to discuss key trade risks and how we can best improve our biosecurity practices at Ministerial round table events across each plant health sector. We will also offer advice similar to "Don't risk it!" to the public for internet sales, where we ask people to be aware of the provenance of any plants they buy, and to ensure that they buy from reputable local sources.

3. Keep plants healthy to protect the environment and biodiversity.

Healthy plants constitute the foundation for all life on earth by providing 98% of the oxygen we breathe and 80% of our food. Plants enhance our landscapes, improve biodiversity, and support our recreational activities. Healthy plants are also essential to a healthy economy - plants are estimated to add £19.2 billion to the Scottish economy every year across forestry, crops and the natural environment (Anonymous, 2016; Anonymous, 2015; Anonymous, 2008) In order to protect these assets and the industries they underpin, Scotland has strategies in place for Plant Health, Forestry and Biodiversity. Working with industry and public stakeholders, we aim to promote the value of our plants, for example, through targeted publications and social media.

4. Strengthen monitoring and early warning systems to protect plants & plant health

Monitoring for pests and diseases is key to preventing their introduction into Scotland. Our plant health service monitors imports and plant based businesses in the agriculture, horticulture and forestry sectors. Industry are also key players in protecting plant health with initiatives such as the industry-led Safe Haven Certification Scheme for seed potatoes helping to protect our plant-based industries. Citizen science also has a part to play in monitoring for plant pests and diseases. For example, Observatree is an initiative which encourages public surveillance and reporting of pests and diseases. In the first 10 months of 2019, Observatree volunteers spent more that 4,800 hours on the project and submitted over 3,600 tree health survey reports from across the UK. IYPH is an opportunity to raise public and industry knowledge of plant pests and diseases, and thus improve our resilience.

5. Invest in plant health organizations and phytosanitary research and development.

Scottish government officials regularly participate at UK and European meetings to keep abreast of plant health developments. Closer to home, the Scottish Government invests in the region of £7 million/annum on plant health research. This investment also includes the virtual Plant Health Centre, comprised of expertise from across 10 UK organisations, to provide the call-down evidence to inform policy decisions across all plant health sectors.

6. Keep plants healthy to achieve zero hunger and the sustainable development goals.

Attacks from pests destroy up to 40% of food crops globally every year. Plant pests can leave people without enough food to eat and the FAO want to increase the understanding of the link between policies and actions to support plant health and the global development goals. Scottish Government fully supports the UN's Sustainable Development Goals (SDGs) with its own National Performance Framework (NPF) designed to localise and implement the SDGs. The NPF has a focus on tackling inequalities so that no one in Scotland is left behind as we work together to achieve the Goals. The SG also co-funds the BBSRC's Global Food Security Programme, which provides vital research to help us be better informed to meet the challenges of providing the world's growing population with access to safe, affordable and nutritious food in a sustainable manner.

IYPH ACTIVITIES

IYPH has encouraged organisations and nations to work together to promote plant health. Internationally there will be two highlights to the year, the high-profile launch of International Year of Plant Health at the FAO headquarters in Rome, and the International Plant Health Conference “Protecting plant health in a changing world” which will bring together global experts in plant health to discuss the challenges and opportunities ahead. For further information see the FAO IYPH website at http://www.fao.org/plant-health-2020/en/. The IYPH logo will be used by partners all over the world to promote International Year of Plant Health.

Figure 1. The IYPH logo will be used by partners all over the world to promote International Year of Plant Health

Scottish Government IYPH activities

Scottish Government, in conjunction with the Plant Health Centre, is planning a range of activities to promote IYPH in Scotland. We are also working with the UK Plant Health Service and collaborating with stakeholders from agriculture, forestry, horticulture and natural environment sectors to ensure a coherent programme of activities across the UK. Defra is organising the first UK National Plant Health Week in 2020 (19th-26th April 2020) and intend to hold this week in future years as part of the legacy of IYPH.

Scottish Government and the Plant Health Centre have organised a range of activities, events and publications for IYPH:

One of our focuses for the year is raising knowledge and understanding of plant health in children and young people based around the idea “plants get sick too”. We have a bug hunt trail touring a number of sites around Scotland, and also plant health activities to take to a number of Scottish science festivals.

At the Royal Botanic Garden Edinburgh, we have a year-long biosecurity exhibition in the John Hope Gateway which should reach almost one million visitors and we are planning other awareness raising events at RBGE's three other Scottish gardens.

To raise the profile of plant health within the Scottish Parliament we will hold a stakeholder reception on the 22nd April.

Further details on these and our other planned activities can be found at: www.sasa.gov.uk/iyph.

The best impacts will result from collaborations, so if you've any ideas to promote IYPH to strengthen Scotland's biosecurity against plant health threats, please get in touch.

CONCLUSION

We hope that stakeholders across science, industry and the public will help us use this year to promote the importance of healthy plants in ensuring sustainability of our food and the environment for future generations.

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EARTHWORMS, THE FARMERS FRIEND, UNDER THREAT

B Boag and R Neilson
The James Hutton Institute, Invergowrie, Dundee, DD2 5DA
E-mail: brian.boag@hutton.ac.uk

Summary: Earthworms are found in nearly all soils and are known to have a beneficial effect on crop yields and soils: improving soil structure and stability, nutrient turnover, porosity and drainage. However modern intensive agriculture, which relies on cultivating the soil every year and typically monoculture production e.g. cereals, has led to soil quality and biodiversity being significantly eroded and this has been exemplified by the reduction in earthworm populations. Mitigating procedures will need to be implemented to reverse the reduction in soil quality seen in recent years and if agriculture, as we know it, is going to survive. Earthworms make up the bulk of the soil faunal biomass and therefore are ideal candidates to be used to monitor the success of any mitigating procedures.

INTRODUCTION

Earthworms are an integral part of the soil fauna of most farms, the exception being those farms which have strongly acidic soils such as hill farms or those on peaty soils. There are 31 species of earthworm in natural environments in the British Isles, 29 in Great Britain (Sherlock 2018) and many have a cosmopolitan distribution (Carpenter et al. 2012). In Scotland the results of a stratified random sample of 100 farms found only 13 species of earthworms of which most were widely distributed (Boag et al. 1997). Results also showed that fields which had been cultivated for arable cropping had significantly lower earthworm populations compared with those grass pastures on the same farm and supports data from experiments using different cropping regimes (Fraser et al., 1994). Earthworms are considered “ecosystem engineers” and have been shown to have a beneficial effect on agricultural productivity (Sinha et al., 2010a; van Groenigen et al., 2014), soil structure (Edwards & Shipitalo 1998), soil biodiversity (Plaas et al., 2019) as well as wildlife as they make up a significant part of the diet of many birds and mammals (Alford et al., 1995).

Agricultural intensification has increased (Rudel et al., 2009) and been associated with a loss of soil biodiversity (Tsiafouli et al., 2015) as well as a decrease in farmland birds (Donald et al., 2001), insects (Shortall et al., 2009) and flora (Storkey et al., 2012). However, the impact on below ground organisms has not been monitored in a similar chronological manner and the impact on soil fauna including earthworms has usually only been assessed by comparison with land which has been extensively managed.

This paper examines some of the factors which influence earthworm populations, the impact they have and suggests measures which might be used to mitigate the loss of earthworms in the future.

BENEFICIAL EFFECTS OF EARTHWORMS

The extent to which earthworms increase crop yields has not been extensively researched in the field, but significant increases have been reported especially where earthworms have been added to soil where they had been absent. For example, Stockdill (1959, 1982) found crop yields were increased by 29-72% in New Zealand when European lumbricid earthworm were introduced to fields which had been devoid of earthworms. Baker et al (1997) found earthworms could increase grain yields in Australia by up to 35% and clover biomass by 21%. These increases in productivity are due to a number of factors (Zaller & Arnone 1999) including improving soil aggregation (Shipitalo & Le Bayon 2014) and porosity (Shipitalo & Protz 1987; Bottinelli et al., 2010). Whalen & Costa (2003) also found earthworms stabilised organic matter within their casts and accelerated nutrient cycling.

Earthworms can decrease the negative impact of some pests and pathogens e.g. nematodes and fungi (Bertrand et al., 2015). They have been used to aid the reclamation and restoration of industrial sites in Britain where they play an important part in soil reconstruction (Butt 2008) for example after open cast mining (Boyer and Wratten (2010). Earthworms are also important in helping drain soils, especially since extreme rainfall events are becoming more frequent (Andriuzzi et al., 2015). Earthworms have the potential ability to convert sewage sludge into pathogen-free, safe fertiliser for farms (Sinha et al., 2010b)

EARTHWORMS IN SCOTTISH FARMLAND

The Scottish earthworm survey (Boag et al., 1997) found representatives of the three generally recognised ecological groups (Bouche 1971) though earthworms can demonstrate trophic plasticity (Neilson and Boag 2000). Epigeic species generally live near the surface in mineral soils beneath a litter layer and do not burrow very far into the soil. Endogeic species live deeper in the soil and derive their nourishment from humidified organic matter. They play an important role in topsoil mixing. Anecic species form permanent or semi-permanent vertical burrows which are open to the soil surface. They emerge from these burrows to feed on dead leaves and play an important role in buying surface litter as well aiding soil drainage. These three ecological groups can be affected differently depending upon the activity/predators which threaten e.g. the New Zealand flatworm has a disproportionally detrimental impact on anecic species (Jones et al, 2001, Murchie and Gordon 2013). The Scottish earthworm survey found an average of 218 earthworms per m^2 in arable fields compared with 313.2 per m^2 in grass fields (Boag et al., 1997). These results are comparable with those found across Europe (Rutgers et al., 2016).

AGRICULTURAL PRACTICES IMPACTING EARTHWORMS

Compaction

Hamza and Anderson (2005) reviewed the nature and causes of compaction and found that it was mainly due to overuse of machinery, intensive cropping, short crop rotations and intensive grazing. Soil compaction has a detrimental impact on the quality of the environment (Soane & van Ouwerkerk 1995), including increasing soil bulk density which in turn has a detrimental impact on invertebrates e.g. earthworms and nematodes (Boag 1985, Langmaack et al., 1999) as well as soil processes, root growth and crop yields (Oussible et al., 1991).

Cultivation

The mechanical disruption of soil by ploughing, rotary cultivation etc. has a potential detrimental impact on soil invertebrates (Fraser et al., 1994) especially earthworms (Chan 2001). Berry and Karlen (1993) found that "as the amount of tillage increased the number of earthworms generally decreased". However, Lofs-Holmin (1983) reported that while ploughing adversely affected the larger anecic species e.g. *Lumbricus terrestris* which feed on surface litter, ploughing may benefit some smaller endogenic species e.g. *Allolobophora chlorotica* which benefit from ploughed in organic residues. The consensus is that generally any mechanical disruption of the soil will lead to a reduction in earthworm populations. Curry et al. (2002) reported that "earthworm populations could be virtually eliminated within a single season by drastic forms of cultivation" and found this was particularly associated with potato and spring barley crops which were grown in this experiment. Of the common cultivation processes Bostrom (1995) found that rotary cultivation accounted for a reduction of 61-68% of the earthworms killed while ploughing caused a further 9-12% loss in earthworm biomass.

Pesticides

The detrimental impact of pesticides on earthworms affects in particular those which feed on the soil surface (Bertrand et al., 2015) however herbicides are usually sprayed on foliage and earthworms are unlikely to come in direct contact with them. Pelosi et al. (2014).found that certain pesticides disrupted gene expression, physiology, life history traits, population densities, behaviour community biomass and densities. Of the pesticides reviewed including herbicides they found under insecticides and fungicides had the most toxic effect impacting survival and reproduction. Results of experiments in this field are complicated by the possible interactions of agrichemicals applied, how the soil has been cultivated and previous crop history. However, laboratory experiments found that of 24 insecticides tested neonicotinoids were the most acutely toxic (they have now been restricted and no longer used on field crops) while pyrethroids were least toxic (Wang et al., 2012).

Grazing

Watkin & Wheeler (1966) reported that the presence of dung produced by grazing animals increased numbers and weights of earthworms and that there was also a significant correlation between pasture production and earthworm numbers and their weights. However, if heavy tramping by livestock occurs, then this can have a detrimental impact on earthworm populations especially the surface-dwelling species (Piearce 1984).

Predators

Earthworms make up a major part of the biomass in soils (Badgett & Cook 1998) and are the major source of food for many birds and animals. Some mammal species rely on earthworms for their survival e.g. moles (*Talpa europaea*) while it can make up a major part of the diet of many other mammals and birds e.g. badgers, foxes, hedgehogs, snipe and woodcock (Alford et al., 1995).

Alien Species

Threat to our native earthworms may also come from other introduced alien invertebrates e.g. it has been suggested that the introduced earthworm *Dendrobaena attemsi* may possibly have a negative impact of our native *D. octaedra* (Smith 2009). Another threat comes from alien planarians which have become established in the British Isles. So far, of the 16 introduced planarian species to the UK, the majority are known to feed on earthworms but only the New Zealand flatworm *Arthurdendyus triangulatus* has proved to have a deleterious impact on earthworms and more specifically the anecic species (Jones at al., 2001; Murchie and Gordon 2013). The New Zealand flatworm, which was probably introduced into Scotland in the 1950s (Jones and Boag 2001), can now be found throughout the British Isles but is more prevalent in the west and north of Scotland including the Scottish islands (Boag and Neilson 2019) than in arable land found in southern and eastern Scotland. The total potential future costs to the Scottish agriculture extrapolated from the value put on earthworms (Bailey et al., 1999) would be £24m in 2019 (Boag and Neilson 2006)

MITIGATION

A number of management interventions have significantly increased earthworm populations (Bertrand et al. 2015). Edwards & Lofty (1982) reported that, after 8 years of replacing conventional ploughing by direct drilling, populations of the deep burrowing earthworms *Lumbricus terrestris* and *Allolobophora longa* increased by 17.5x and 37.3x respectively. Haines and Uren (1990) also found that the introduction of direct drilling doubled the biomass of earthworms compared with that found under conventional cultivation. The addition of organic matter was reported by Estevez et al. (1996) to significantly increase earthworm populations, as well as soil diversity, biological activity, structural stability compared with synthetic fertiliser treatments and the control. Fraser et al. (1996) found that continuous arable production reduced earthworm numbers to below 100 m^{-2} in contrast to over 800 m^{-2} under the same 3 years period under grass pasture. The inclusion of grass/clover leys in crop rotations greatly enhanced earthworm numbers and increased cereal crops yields which followed the grass/clover leys (Watson et al., 1999). Riley et al (2008) recommended that "50% leys in the rotation was desirable for the maintenance of satisfactory soil structure and earthworm activity" and without a ley in the rotation there was high pressure on soil fauna. Intensive agricultural practices have also led to the decrease in soil pH and the addition of lime has been suggested to increase earthworm abundance on agricultural farms and to aid the survival and recovery of birds which forage on upland grasslands e.g. northern lapwing (McCallum et al., 2016).

DISCUSSION AND CONCLUSION

It is difficult to assess the value of earthworms to agriculture but Bailey et al. (1999) valued the impact of earthworms to agriculture at £0.08- £0.48 per kilo depending upon the crop. Although earthworms have been used as bioindicators of soil quality (Fusaro et al., 2018), there are arguably other possibly better indicators of soil health e.g. nematodes (Neher 2001). However, earthworms make up the bulk of the soil faunal biomass (Bardgett and Cook 1998), are the most conspicuous component of the soil fauna and able to reflect the impact of change on other microscopic members of the soil fauna. Undoubtably, intensive agriculture is responsible for a major part of the drastic reduction in earthworm population (Curry et al., 2002) which has occurred. Earthworms could be used in the future as one of a suite of biological indicators to monitor the success or otherwise of mitigating measures to maintain and protect and improve soils. Reversing the decrease in soil health and improving it is essential to maintain crop yields and ultimately for human health (Wall et al., 2015).

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THE IMPACT OF FIELD HEADLANDS ON VISUAL SOIL STRUCTURE IN IRISH ARABLE FIELDS

M Ward[1,2], P D Forristal[1], J Grant[3], K Metzger[4] and K McDonnell[2]
[1]*Teagasc Crops Research Centre, Oak Park, Carlow, Ireland*
[2]*School of Agriculture and Food Science, UCD, Belfield, Dublin 4, Republic of Ireland*
[3]*Teagasc Food Research Centre, Ashtown, Dublin, Ireland*
[4]*Environment, Soils and Land Use Department, Teagasc, Johnstown Castle Research Centre, Wexford, Ireland*

SUMMARY: Field headlands are integral components of agricultural landscapes and are used for machinery turns. This poses a threat to soil structure with previous research indicating differences between headlands and in-field areas. In the post-harvest period of 2016 and 2017, soil structure was examined on 40 arable fields across the main cereal growing region of Ireland (Figure 1). A headland zone approach (Figure 2) as defined by intensity of headland machinery traffic was used for soil measurements. Visual (VESS and Double Spade) soil assessments were carried out. The impact of zone on soil structure was influenced by soil texture. Significant differences between the two outermost headland zones and the transition and in-field zones were documented for VESS and DS. The turning headland zone tended to have the poorest soil structural scores highlighting the negative impact of machinery traffic.

INTRODUCTION

In arable fields, the area of land found next to the field boundary is commonly referred to as the headland (Wilcox et al., 2000; Welch et al., 2016) and extends to contain work conducted parallel to field boundary (Sparkes et al., 1998; Wilcox et al., 2000). Headlands can be categorised into 'turning' headlands where all non-working machinery manoeuvres occur and 'non-turning' representing in-field traffic patterns (Sparkes et al., 1998). The threat of soil compaction is generally greatest on headlands not only due to high rear axle loads (Burke et al., 2018) but also repeated wheelings (Botta et al., 2009) that can induce subsoil compaction. Visual soil structural assessments are useful indicators for growers to monitor their soil structural condition. Such assessments allow timely soil management decisions (Askari et al., 2013) be made to limit variability between headlands and main in-field areas (Sparkes et al., 1998). Visual Evaluation of Soil Structure (VESS) (Ball et al., 2007) and Double Spade (DS) (Emmet-Booth et al., 2019) are accepted visual soil evaluation methods that have been proven to identify soil structural issues to below plough depth (Emmet-Booth et al., 2019). In Ireland, because of smaller field size, more frequent rain and the adoption of heavier machines, the impact of headland traffic may be greater. The objective of this research was to quantify the impact of headland turning traffic on visual soil structure using a zone approach based on the intensity of machine traffic.

MATERIALS AND METHODS

In the post-harvest period of 2016 and 2017, soil structure was examined on 40 randomly selected sites from a list populated of Teagasc cereal grower clients. All sites were located in the main cereal growing region of Ireland (Fig. 1) and subject to conventional plough-based crop establishment systems. An assessment of machine headland turning techniques was conducted that allowed zones based on machinery traffic intensities: field edge; turning; transition, and; in-field, to be defined between the headland and in-field areas (Fig 2). Visual soil assessments were conducted at two depths: 0-250 mm for VESS, and; 250-400 mm for DS at each of the four zones at four different transects, giving four replicated measurements per zone.

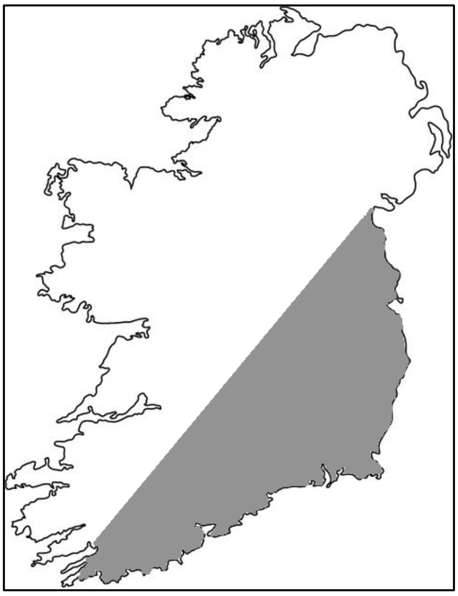

Figure 1: Location of the main cereal growing region of Ireland from which sample sites were randomly selected

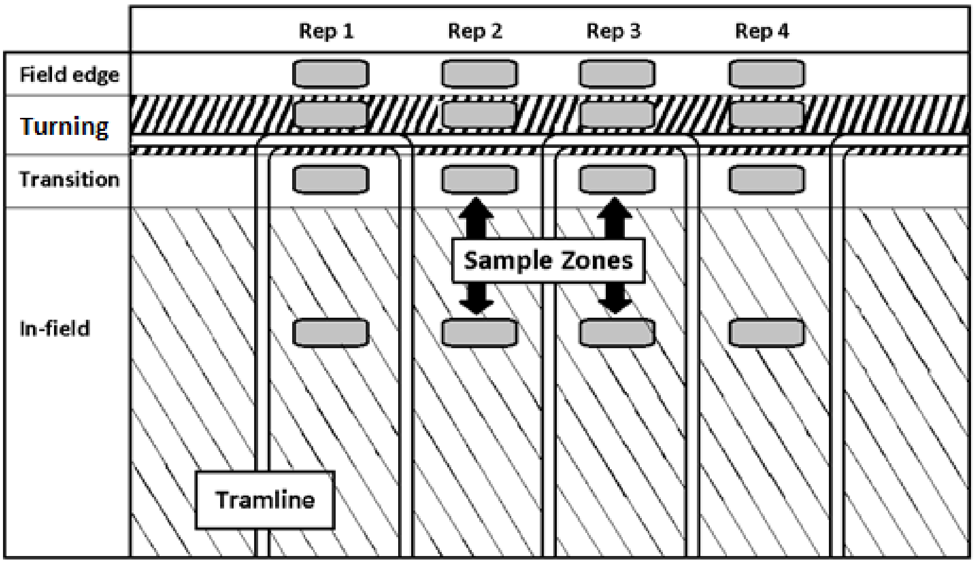

Figure 2: Illustration of zone approach and sample locations in grey.

Data from this study met the assumptions necessary for a two-way Analysis of Variance. The effects of zone and soil texture on visual soil structure was analysed with blocking using SAS 9.4 statistical software.

RESULTS

There was a zone effect (P<0.001) on all visual soil evaluation scores (VESS and DS) in this study (Table 1). The best visual scores for both methods were found at the in-field zones, while the poorest were documented at the turning zones. There was a visual trend of increasing scores in the zone order of in-field, transition, field edge and turning which follows increases in perceived intensities of machine traffic. The in-field and transition zones produced statistically better scores than the main headland zones (field edge and turning) for VESS and DS.

Table 1. The effect of zone and soil texture on VESS and DS (values within columns subscripted by different letters are significantly different at P=0.05)

		VESS (*Sq*)	DS
Zone	**Field Edge**	3.18[a]	2.82[b]
	Turning	3.31[a]	2.91[a]
	Transition	2.94[b]	2.54[c]
	In-field	2.81[b]	2.48[c]
Texture	**Clay loam (n=6)**	3.13[a]	2.83[a]
	Loam (n=17)	3.05[a]	2.67[b]
	Sandy loam (n=17)	3.04[a]	2.66[b]
		Pr > F	**Pr > F**
Zone		<0.001	<0.001
Site		<0.001	<0.001
Zone*Site		<0.001	<0.001
Soil Texture		0.415	<0.001
Zone*Soil Texture		0.123	0.008

DISCUSSION

In this study, soil structure at two soil depths was assessed according to zones based on various levels of headland machinery trafficking. Due to the survey aspect, the variability between sites is partially explained by soil texture but also may have been contributed to by management factors such as different turning techniques and equipment was used between sites giving a zone and site interaction. VESS and Double Spade methods were both impacted by the headland zone in which the measurement was taken. Scores for both methods followed the same visual zone order of increasing scores agreeing with the hypothesis where zones of greatest machinery traffic gave the poorest visual soil scores. Soil texture was seen to influence visual soil scores in the lower soil layer for DS and not in the cultivated layer for VESS that is directly influenced by crop management operations. The traffic effect suggests growers should be cautious about the management of headland traffic.

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SOIL REGENERATIVE AGRICULTURE GROUP – SUPPORTING FARM SOILS

R S Audsley[1] and P Lindsay[2]
[1]*SAC Consulting, Environment Team, JF Niven Building, Auchincruive Estate, Ayr, KA6 5HW*
[2]*SAC Consulting, Farm and Rural Business Services, 77 North Street, Forfar, DD8 3BL*
E-mail: rebecca.audsley@sac.co.uk

Summary: Five arable farmers in the North East of Scotland are working together as the Soil Regenerative Agriculture Group under the Scottish Government's Farming for a Better Climate initiative. Together the group aim to develop ideas and practices which could provide practical and innovative solutions to share with other farmers, support farm soils and help mitigate climate change in agriculture.

INTRODUCTION

Soil health is at risk from both a changing climate and routine land management practices (Ball, 2013). Not only can healthy soils improve agricultural production and business resilience, but if managed correctly are also widely recognised as a carbon store.

The Scottish Government has committed to supporting a new farmer-led soils group as part of the Farming for a Better Climate initiative. Five mainly arable farmers in North-East Scotland are working together as the Soil Regenerative Agriculture Group to share ideas and information which could help both them and the wider agricultural sector protect and enhance farm soils.

Ideas and findings from the group will be widely disseminated across the agricultural sector to help other farmers consider alternative options and ideas to improve and protect their farm soils, benefit their farm business and increase future resilience to climate change.

MATERIALS AND METHODS

In April 2019, five farmers in North-East Scotland were approached to participate in a group to look at regenerative agricultural practices and how they could integrate and develop these on their farms. An introductory meeting was organised to explain the initiative and seek farmers' comments and participation. All five farmers accepted the challenge.

Once participation was agreed and funding secured, each farmer hosted the group on their farm to showcase their current soil management and cropping practices, highlighting areas they would like to improve, change or investigate in more detail. Between them, the farms grow a range of crops including cereals, potatoes, oil seeds, legumes, soft fruits and daffodils; one farm has beef cattle. The farmers in the group were already moving away from traditional tillage systems to support farm soils, favouring a direct drill or reduced tillage approach.

Following the introductory visits, a group 'wish list' was compiled reflecting areas of interest where more information, support, research or trials would be beneficial.

Baseline data has been collected on the host farms including a detailed SAC Soil Health Test on three fields at each farm, looking at nutrients, soil structure and soil biology (Dolan *et al*, 2020). Soil biology has been further explored using the FERA Soil Health Test.

The group is supported by an SAC Agricultural Consultant and meets around six times per year either on each other's farms or through trips to other industry events. The group is in regular contact via WhatsApp, allowing questions to be posed, comments made and photos shared.

RESULTS AND DISCUSSION

In the first eight months of the project, meetings have been held on the host farms to share the range of activities undertaken within the group. Soil sampling for nutrient and pH status and assessments of soil structure have been taken across a number of target fields with results shared and discussed within the group (Dolan *et al*, 2020). Some of the farm visits have also invited a guest speaker, for example a bio-agronomist or a specialist plant and soil health consultant to share more insights about what is happening within the farm soils, subsequent effect on crops and how practices could be enhanced or improved.

The farmers came to the group with a keen focus on the need to improve soil biological health and the benefits this could transfer to the growing crop and farm biodiversity. The group have engaged with researchers, hearing some of the current soils-focused work underway at Scottish Institutions and how it could apply to them.

Small scale working trials are happening across the farms including cropping oilseed rape with an understorey of beans and clover, cover crop establishment methods and species mix and altering spray solution pH to increase accessibility of target compounds to the growing crop.

Information is being shared at www.farmingforabetterclimate.org and via FFBC social media. Videos and podcasts will highlight findings and encourage discussion within the sector. This approach could have a wide reach, highlighting the value of soil regenerative agriculture techniques across the sector.

CONCLUSION

Healthy farm soils are key to a sustainable agricultural sector and contribute to climate change mitigation measures. The small group allows participants to be more open about sharing data and information and what works and what doesn't. Key messages can be distilled and disseminated via social media and the project website, sharing information with a wide audience and allowing others to learn from the group and share their knowledge and experience. To date, working with both researchers and specialists has highlighted the need for more knowledge and focus to be placed on soil biological health.

ACKNOWLEDGEMENTS

With thanks to The Scottish Government for funding the Farming for a Better Climate Soil Regenerative Agriculture group; the host farmers and SAC Consulting's P Lindsay and Z Reilly for facilitating and supporting the farmer group.

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HOW TWO SCOTS BUILT EUROPE'S LARGEST ARABLE FARM

K P Dawson[1] and M Laird[2]
[1]SustainAgro Ltd, Peebles, Scottish Borders. EH45 9HF
[2]CPG Ltd, Memus, Angus, DD8 3TY
Email: drkpdawson@aol.com

Summary: In 2005 the authors set up a farming business in Western Ukraine to produce 90 ha of potatoes. Climate and soils were favorable to employ a farming model developed successfully in Poland based on Scottish IFM techniques such as LEAF, TIBRE, and SQC. Availability of good fertile land at an acceptable rent allowed expansion of the farming business to 45,000 ha and a successful IPO on the London Stock Exchange in 2012. Following the sale of the business to a sovereign wealth fund in 2015 and with further acquisition of land and businesses the farm grew to 200,000 ha in 2019 making it the largest arable farming business in Europe. This demonstrates the success of IFM techniques in a new global farming model as we move toward Peak Farmland.

INTRODUCTION

Global food security is a pressing issue in the 21st century, as population growth from 2 bn to 7 bn in the last sixty years has increased demands on both quality and quantity of foodstuffs. It is important to maximise production from all available farmland, whilst protecting the environment. By maximising production per ha of existing farmland this reduces pressure on natural ecosystems being converted to farmland. It is important that in this farming process all techniques used to improve crop production also aim to minimise adverse internal and external effects on the environment. By regenerating and utilising run down farmland in Western Ukraine this case study shows how good environmental practice can be used in a new large scale model of arable agriculture combining the benefits of the two previous global arable models; extensive and intensive arable agriculture. This is particularly important as we move closer to global peak farmland.

Background

During the late 1990's a Scottish farmer Mark Laird developed a successful Polish farming business in Northern Poland aided by techniques and consultancy developed by CSC CropCare (K Dawson) in Scotland during that period. These included new technologies covered in Scottish Natural Heritage's TIBRE (Targeted Inputs for a Better Rural Environment) and protocols developed by the Scottish Quality Cereals Assurance Scheme and LEAF. Whilst this IFM (Integrated Farm Management) model worked well in both Scotland and Poland, further expansion of the farming business from 5000 ha in Poland was limited due to availability and cost of land. A detailed appraisal of global wheat production by SAC Consulting (Kerr, 2004) showed that the lowest costs of production per tonne for cereals were in Ukraine and Australia. The climate risk and the tyranny of distance ruled out Australia and so Ukraine was examined closely as an area where expansion of the successful model could be employed in a larger farming business. Following an evaluation of climate, soils and land availability in 2005 a company KRMG was set up by Mark Laird and Keith Dawson with two Perthshire farmers Russel and George Taylor to set up a pilot project of 90ha of potatoes near Lviv in Western Ukraine. The initial capital investment was $375,000.

In global agriculture in 2005 there were two main global models for arable agriculture. The first was the Extensive model e.g. Australia/US, characterised by low intensity of crop inputs over very large farm sizes 50-100,000+ ha. The second model is the Intensive model e.g. Scotland/UK/Germany, characterised by high intensity of crop inputs but over relatively small farm sizes 50-300 ha. Our shared vision was to develop a third successful global model with a high intensity of IFM inputs but on a much larger farm size 50-100,000+ ha area. This large scale production model based on IFM had never been attempted before.

Misson statement

The key to achieve our aim was to have a clear mission statement that all staff embraced and took ownership for. It covered four complementary and overlapping aims

1. To produce the highest yield
2. Of a product the market wants
3. At the lowest cost of production per tonne
4. With good social and environmental stewardship

It is important to note that a core belief of the business is that there is no conflict between the first three aims and the fourth of social and environmental stewardship.

CLIMATE AND SOILS

The soils in Ukraine are globally renowned for high quality and production potential. These black chernozem soils, formed over thousands of years under steppe grassland vegetation are deep, stone free, well drained and have relatively high soil organic matters. Whilst the best chernozem soils are situated in the East of Ukraine, production here is limited by low rainfall of around 300 mm per annum. Whilst not quite as high quality as soils in the East, the soils in West Ukraine would be classified as Grade 1 or 2 on the UK Soil classification system. Due to the proximity of the Carpathian Mountains, rainfall is higher in this Lviv oblast (region) at 650-750 mm/annum. Analysis of actual versus potential production showed a huge shortfall in production in Ukraine despite excellent soils and in the west at least, adequate rainfall for a range of arable crops. Whilst actual cereal yields were below 2 t/ha, the biological/environmental potential was around 7 t/ha. Whilst Western Ukraine was not recognized by conventional wisdom as a good area for arable production, we ascertained with the correct production programmes, then yield and quality could be markedly improved. A key area of risk was the ability of autumn sown crops to survive long hard winters where air temperatures can drop to minus 30^{o}C for prolonged periods. The adequate winter precipitation in Western Ukraine falling as snow provides a reliable insulating winter cover to reduce the risk of frost kill for autumn sowings. The late spring and early ingress of winter give a shortened growing season than Scotland. A key aspect of the business has been to improve soil health in line with IFM principles, In particular soil structure has been improved markedly due to the removal of soil compaction caused by decades of poor Soviet cultivations. Drainage and soil biological health has been improved by the use of an IFM based crop rotation and remediation of Soviet drainage systems.

LAND AVAILABILITY

Ukraine is the largest country entirely in Europe. Prior to the formation of the independent nation of Ukraine the land was part of the Soviet Union and the land was collectivized in 1931 by Stalin who forced individual small farmers to give up their land to the collective farm. This led to one of the world's largest genocides with around 7 m Ukrainians dying of starvation in the winters of

1932/3. This tragedy known as the Holodomor, translates as “Death by Hunger.” These large 30-50,000 ha farms existed until Russia was thrown out of Ukraine and an independent nation formed in 1991.
At this point there was a major structural fragmentation of these collectives. Each villager was granted ownership of 1-2 ha to farm themselves. Very quickly due to lack of capital, breakdown of machinery and emigration these previously farmed fields were left uncropped and fallowed and quickly became mainly moribund and weed infested (Fig.1).


Figure 1. (l) Unregenerated abandoned farmland Western Ukraine 2005;
(r) Remediated land prior to sowing 2006

These fields had remained in this state in Western Ukraine until our arrival in 2005. In tandem with this major fragmentation of the collective farms, a moratorium on sales of agricultural land was enacted to avoid “recollectivisation” of ownership by oligarchs. This allowed the villager owners to own an asset, which they could work themselves or rent to others but not sell, even to fellow Ukrainians. For many villagers this left them with a moribund asset they could not work themselves and no one wished to rent. There was a clearly designated right of ownership and detailed maps of plot positions within the larger old collective fields. The rental value was set by the national government and represented excellent value at $30-50 ha/annum compared to over $1,500 ha/annum for comparable Scottish land. A 100 ha field might require up to 100 individual rental leases, but these were made for 10-15 years, via public village council meetings, with a right to extend the lease plus an option to buy should the moratorium end. It was of critical importance to gain the confidence and support of the local villagers and provision of employment, timely payment of rents, social programmes for the villages and a stated intent to steward their land well were all essential for trust and confidence.

COMPETITIVE ADVANTAGE

There were clear competitive advantages to crop growing in Ukraine compared to Scotland/EU.

1. Availability of uncropped land at a very competitive rent
2. Excellent deep, fertile, well drained soils of global renown
3. A good climate with good seasonal distribution of rain/snow
4. Actual yields well below potential yields-low cost of production
5. Availability of a low cost, hardworking skilled workforce
6. Supportive regional and local government

TECHNOLOGY SHIFT AND DEVELOPMENT

The existing agricultural landscape in Western Ukraine in 2005 was of moribund and deserted farmland over large areas of Lviv oblast. Around the villages was a pattern of inefficient, small plot peasant subsistence farming using horse drawn or human power, with only a few old dilapidated Russian tractors to cultivate and harvest. Village unemployment was at around 90%. Local oblast average wheat yields were only 1.5 t/ha despite the quality of land. Our vision was move rapidly towards modern Western technology and working practices in a marked technological leap forward, whilst protecting the soil and environment as per the terms of our rental agreements.

It was clear at an early stage that the first major step was to improve the quality and provenance of genetic material planted. Locally sown potato seed was of older varieties home saved for up to twenty years, so viral loads were high and seed quality was low. Due to the widespread use of unlicenced home saved seed, there was little advantage for plant breeders to introduce new plant material into Ukraine. Introduction of improved crop genetics, by importing good quality Scottish and Dutch potato seed multiplied up in our Baltic Polish seed potato business, was a major step forward in yield, quality and crop health. This was introduced in tandem with a "Scottish" model of production, with optimum fertiliser rates based on Scottish soil analysis results, and superior crop protection and cultivation and rotational strategies. Consultancy and technology transfer links were developed between Continental Farmers Group Ltd (CFG) and Scottish Agricultural College (SAC) and James Hutton Institute (JHI) to help professional development of our Young Agronomist Programme developed in conjunction with Lviv Agrarian University. Field and Village Open Days were used to help the development of new technology and techniques on local farms.

After over a decade of fallow both weed and pest control were problematic on this land, despite the use of glyphosate and deep ploughing to remediate (Fig. 2). Small scale peasant potato growers with crops unprotected from blight, together with a warm humid summer climate, created challenges for blight control, even with a modern blight control programme targeted with field weather station based blight models. For the first two seasons 2006 and 2007 90 ha and 700 ha potatoes was the main crop to clean the land and provide both profitable return for re-investment and local employment. Scottish second-hand modern tractors, harvesting and cultivation equipment was imported and every penny was a prisoner. The 2006 harvest showed an increase from local potato yields of 13 t/ha to 38 t/ha which allowed area expansion and confident investment in potato storage facilities with the help of Irish investors in 2007.

TIBRE technologies utilized in the production programme included targeted low dose crop protection applications, tank mixtures, adjuvants, reduced water volumes and low drift nozzles, in field weather stations and disease prediction models, use of varietal resistance, minimal tillage systems, GPS precision farming techniques, and pest, disease and weed thresholds. The storage and production models closely followed the SQC Cereal Assurance scheme and the use of LEAF techniques such as crop rotation, risk assessment, soil structure and health protection and water course protection were followed through a detailed programme of SOPs (Standard Operating Procedures) with relevant detailed staff training, education, buy in and countersigned ownership.

Figure 2. (l) Soil profile 2005 prior to CFG soil remediation clearly showing soil compaction and limited rooting (r) The same soil profile 2007 post CFG soil remediation clearly showing removal of soil compaction and increased rooting zone

An IFM based rotation incorporating oilseed rape as a cleaning crop, breadwheat and maize was established in 2008. This rotation was subsequently broadened by the introduction of sugar beet for the local factory, feed barley and soyabeans as the cropped area expanded. In 2009 the Irish agricultural conglomerate Origin PLC, owners of Agrii, purchased 25% of the business to allow further expansion and development of the business to 13000 ha in 2010 (Fig. 3). During this period wheat yields increased from local yields of 1.5 t/ha to 6 t/ha, maize from 3.5 t/ha to 7.5 t/ha, sugar beet from 22 t/ha to 50 t/ha and oilseed rape from 1.9 t/ha to 3.2 t/ha average. Further important investment was made in drying and storage facilities at three sites on farm around Lviv. Logistics were challenging with sub optimal rail and road infrastructure and with up to 300,000 t of sugar beet, 80,000 t of potatoes and 150,000 t of combinable crops to store and market effectively.

In 2012 with a cropped area of 23000 ha in Ukraine and 3000 ha in Poland, the two businesses were amalgamated under CFG and an IPO (Initial Public Offering) was made of the company on the London Stock Exchange to raise funds for further expansion. This was successful leading to a further 30% of the company sold on the Alternative Investment Market. In 2013 following this successful expansion an offer from SALIC, a Middle Eastern Sovereign wealth fund became keen to purchase the business for a 50% premium over the stock market, and a valuation of $200m was accepted and completed prior to the Euromaidan Revolution in early 2014. Despite the war with Russia in the East from 2014, which continues to this day, expansion of the CFG business continued reaching 45,000 ha in 2018. The lack of attractiveness of Ukrainian assets, due to the conflict, caused liquidity problems for many companies in Ukraine. This allowed CFG to acquire the troubled Myria farming business in autumn 2018 increasing the cropped arable area to 200,000 ha. This is an area three times the next largest European arable farm in Romania.

Figure 3. Potato Crop on remediated land 2010

SUMMARY AND CONCLUSIONS

Whilst the CFG production model is capital intensive, the resultant increased yields with good climate and exceptional soils in Ukraine have made the model a financially viable third successful global arable production model from 90 ha to 200,000 ha in thirteen harvests. Key to this success has been the implementation of Scottish based IFM principles, TIBRE Technology and SQC assurance standards by an experienced team of Scottish farm business managers and technologists. This has led to the four aims in the CFG mission statement to be achieved with good stewardship of the land and communities. A notable success has been the social "ripple effect" improving the prosperity of both individuals within and the entire Lviv oblast

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INTEGRATED CONTROL OF DISEASE IN BARLEY CROPS

N D Havis, H Creissen and F J Burnett
[1]*Carbon, Crop and Soil Group, SRUC Edinburgh, West Mains Road, Edinburgh EH9 3JG*
E-mail: neil.havis@sruc.ac.uk

Summary: Barley is an important crop, both globally and locally. It is important to both animal production and the brewing and distilling industry. Diseases can devastate crops and reduce yield and quality. A robust, sustainable progamme to control disease utilises a number of techniques. Here we report on the potential to alter sowing date to protect crops from disease, and also the use of biological control agents and resistance elicitors to replace conventional fungicides.

INTRODUCTION

Barley is the second most important cereal crop grown in the UK and production reached a new peak in 2019 of over 8.1 million tonnes (DEFRA, 2019). In Scotland alone over 430,000 ha of barley were grown and production rose to a record 3.2 million tonnes (Scottish Government, 2019). Barley is subject to many diseases which can cause devastating economic losses e.g. *Rhynchosporium commune* (Henly, 2015), *Ramularia collo-cygni* (Havis *et al.,*2015). Disease control has traditionally been based on varietal resistance and the use of fungicides. However, legislation passed by the European Union in 2012 (Directive 2009/12/EC) encouraged all governments to promote Integrated Pest Management (IPM) and minimize fungicide usage.

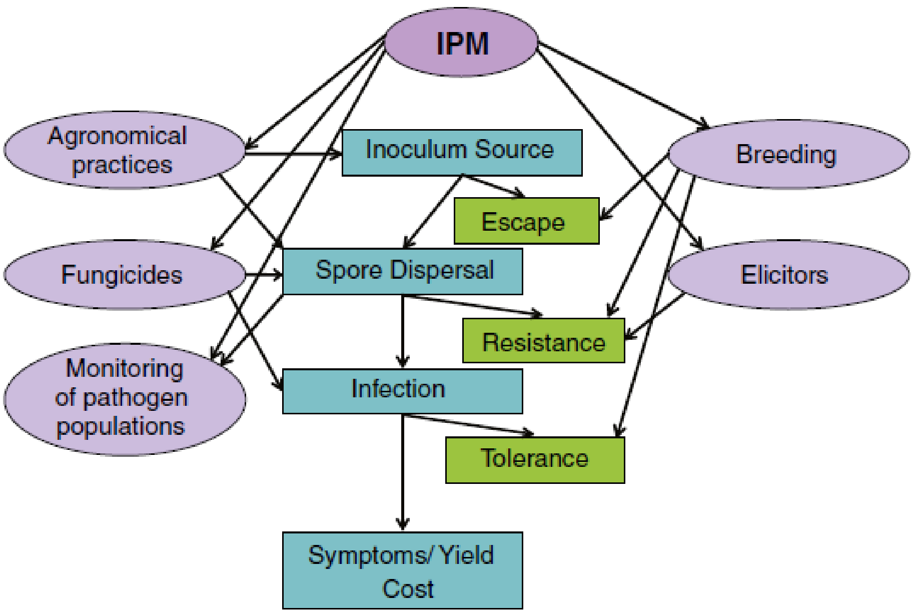

Figure 1. The components of IPM and their influences on pathogen epidemiology and host response (from Walters *et al.,* 2012).

There have been a number of issues with fungicide resistance in barley crops in recent years and this has increased the pressure to find durable control measures (Havis *et al*., 2018; Rehfus *et al.,* 2019). Resistance elicitors are compounds such as polysaccharides and plant extracts. Their efficacy against barley foliar diseases has been reported previously (Walters *et al*., 2014). There are a number of biofungicides with reported activity against mildew (Young-Sook *et al.,* 2013). Their use in barley disease programmes was evaluated in a series of field trials.

MATERIALS AND METHODS

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Seed treatment trials

Table 1. Elicitors and biological products applied as seed treatments to winter and spring barley prior to sowing.

Product	Active ingredient	Abbreviation
Raxil Star®	Prothioconazole, tebuconazole, fluopyram	Commercial std
Regalia®	*Reynoutria sachalinensis*	Elicitor 1
Laminarin ™	Brown algae storage glucan	Elicitor 2
Companion®	*Bacillus subtilis*	Biological 1
Rizoderma ™	*Trichoderma spp.*	Biological 2

Winter barley and spring barley trials (cvs. WB- KWS Cassia; cvs SB- Propino and Concerto) were sown in 2017 and 2018. Seed was sown in 10m x 2m plots at Boghall farm, Midlothian, Drumalbin farm, Lanarkshire, and Cauldshiel farm, East Lothian. Untreated seed and the treated seed were sprayed with the foliar sprays listed in Table 2.

Table 2. Foliar disease control programmes in winter and spring barley trials.

Treatment	T0 spray (GS 24)	T1 spray (GS 31)	T2 spray (GS 53)	Abbreviation
1	Untreated	Untreated	Untreated	Unt
2	Untreated	Prothioconazole (Pro), bixafen (bix) (Siltra XPro®)0.5l/ha + pyraclostrobin (pyr) (Comet®) 0.5 l/ha	Prothioconazole (Pro)(Proline®) 0.4 l/ha + chlorothalonil (chlor) (Bravo ®) 1.0 l/ha	Full fung
3	Regalia 2.5 l/ha	Pro + bix 0.25l/ha + pyr 0.25 l/ha	Pro 0.2 l/ha + chlor 0.5 l/ha	Elic + fung

Disease and green leaf area in the crop were assessed throughout the growing season. The trials were taken to harvest and yields expressed at t/ha at 85% dry matter.

Integrated trials

Winter and spring barley seed was either untreated or treated with Laminarin (3.7m dose rate per kg seed). Winter barley and spring barley trials (cvs. WB- California; cvs. SB- Propino and Laureate) were sown in 2019. Seed was sown in 10m x 2m plots at Boghall farm, Midlothian, Drumalbin farm, Lanarkshire and Cauldshiel farm, East Lothian. Both crops from untreated and treated seed were sprayed with the foliar sprays listed in Table 3.

Table 3. Foliar disease control programmes in winter and spring barley trials.

Treatment	T0 spray (GS 24)	T1 spray (GS 31)	T2 spray (GS 53)
1	Untreated	Untreated	Untreated
2	Kayak (cyprodinil) 0.3l/ha	Pro+bix 0.25l/ha + pyr 0.25l/ha	Pro 0.25l/ha + chlor 1.0l/ha
3	Elicitor 1 (2.5 l/ha)	Pro+bix 0.25l/ha + pyr 0.25l/ha	Pro 0.25l/ha + chlor 1.0l/ha
4	Biological 2 (0.06 l /ha)	Pro+bix 0.25l/ha + pyr 0.25l/ha	Pro 0.25l/ha + chlor 1.0l/ha
5	Elicitor 2 (0.75l/ha)	Pro+bix 0.25l/ha + pyr 0.25l/ha	Pro 0.25l/ha + chlor 1.0l/ha
6	Biological 2 (6l/ha)	Pro+bix 0.25l/ha + pyr 0.25l/ha	Pro 0.25l/ha + chlor 1.0l/ha
7	Bion (acibenzolar-S methyl)(0.175g/l)	Pro+bix 0.25l/ha + pyr 0.25l/ha	Pro 0.25l/ha + chlor 1.0l/ha
8	Sitko-SA (Salicylic acid, Phosphites, Silicates) 2.5 l/ha	Pro+bix 0.25l/ha + pyr 0.25l/ha	Pro 0.25l/ha + chlor 1.0l/ha
9	Chitosan (mix of oligosaccharides)1.67 g/ha	Pro+bix 0.25l/ha + pyr 0.25l/ha	Pro 0.25l/ha + chlor 1.0l/ha

Disease and green leaf area in the crop were assessed throughout the growing season. The trials were taken to harvest and yields expressed at t/ha at 85% dry matter.

Sowing date trials

Winter barley trials were sown in both 2017 and 2018 at Boghall farm, Midlothian. Two varieties with differing disease resistance profiles were selected for the trial (Surge – generally good resistance and KWS Tower – generally weaker against disease). Sowing date was delayed in half of the plots to investigate the influence of this factor on disease development and yield production. Sowing dates (2017 – early 19/Sep/17, late 03/Oct/17; 2018 – early 26/Sep/18, late 10/Oct/18). Plot size was 2m x 10m. 4 fungicide treatment regimes were used in the trial. Disease was assessed in the trial throughout the growing season and trials taken to harvest. Yields were expressed as t/ha at 85% dry matter.

Table 4 Treatment regimes used in sowing date trials.

Treatment	T0 (>GS30)	T1 (GS31)	T2 (GS39-45)
1 (untreated)	Untreated	Untreated	Untreated
2 (low)	Untreated	Pro+bix 0.6L/ha	Untreated
3 (medium)	Untreated	Pro + bix 0.6L/ha	Pro + bix 0.4L/ha
4(high)	Metrafenone (Flexity®) 0.25 L/ha Cyflufenamid (Cyflamid®) 0.3L/ha + pyr 0.4L/ha	Pro + bix 0.6L/ha	Pro + bix 0.4L/ha

RESULTS

Seed treatment trials

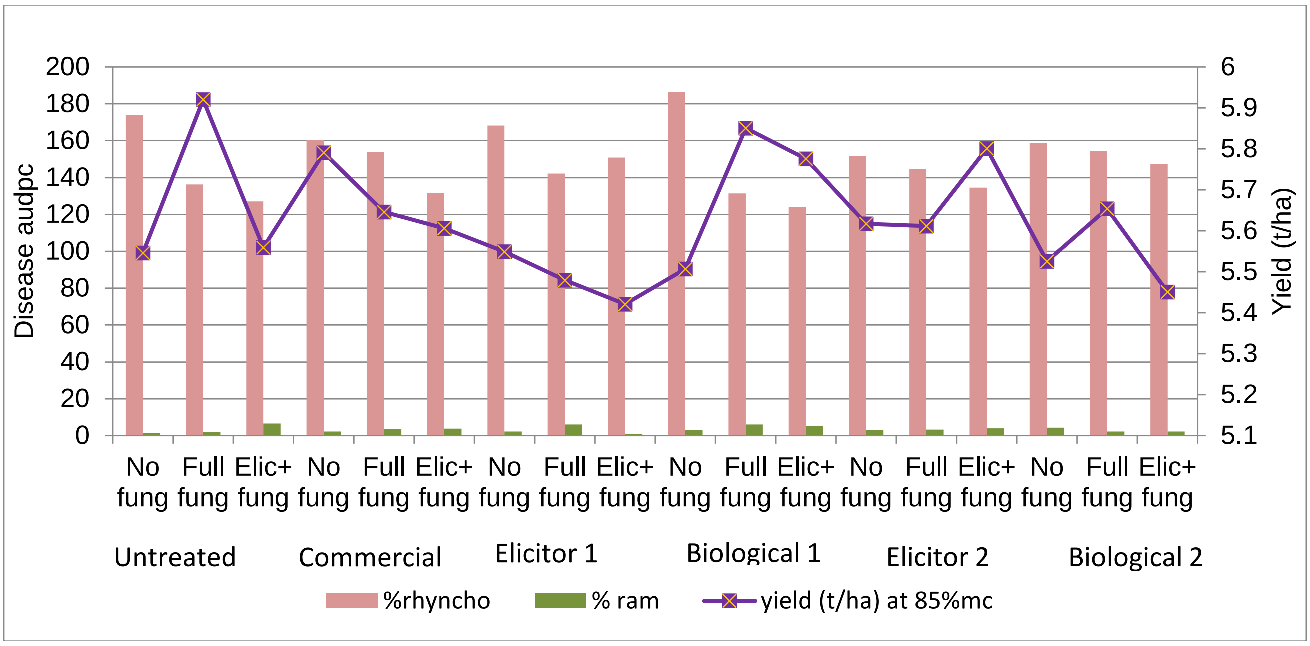

Figure 2. Levels of rhynchosporium, ramularia and grain yield in winter barley trials, 2017-18. Rhyncho LSD (P=0.05) 36.21; Ramularia LSD (P=0.05) 2.16, Yield LSD (P=0.05) 0.51

Integrated trials

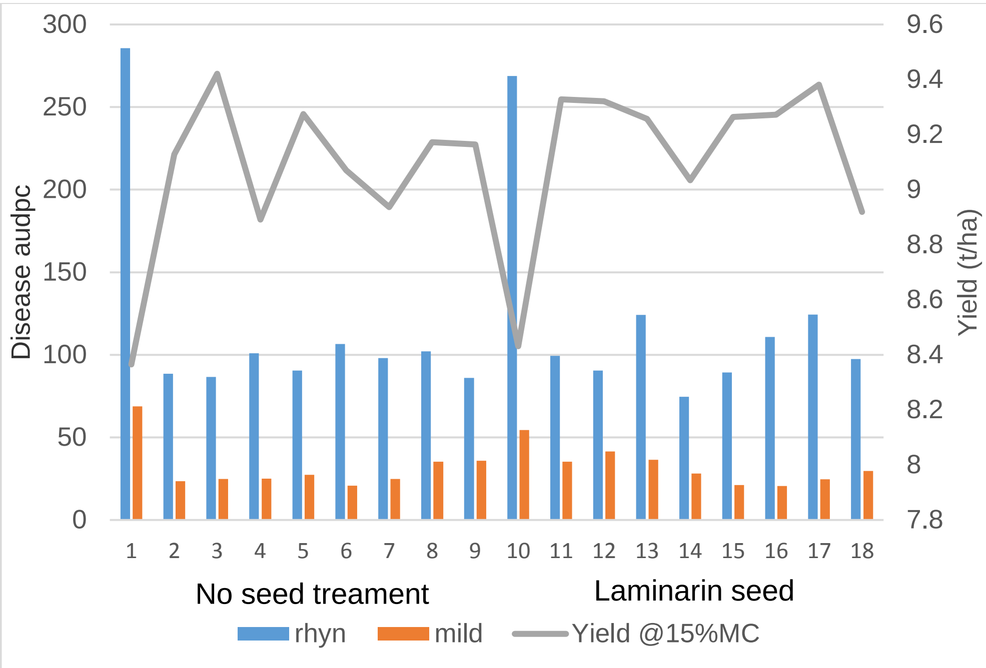

Figure 3. Levels of rhynchosporium, mildew and grain yield in winter barley trials, 2019. Rhyncho LSD (P=0.05) 73.96; Mildew LSD (P=0.05) 30.3 Yield LSD (P=0.05) 0.57

The winter barley trials had a number of diseases present. Control of both was significant. Lowest rhyncho levels were recorded by treatment 14 (Laminarin seed treatment + Elicitor 2 at T0 then reduced fungicides. The highest yielding treatments had no seed treatment then elicitor 1 at T0 the reduced fungicide or laminarin seed treatment followed by Sitko SA at T0 then reduced fungicides. All of the treatments gave superior control compared to the plots with no foliar sprays.

Sowing date trial

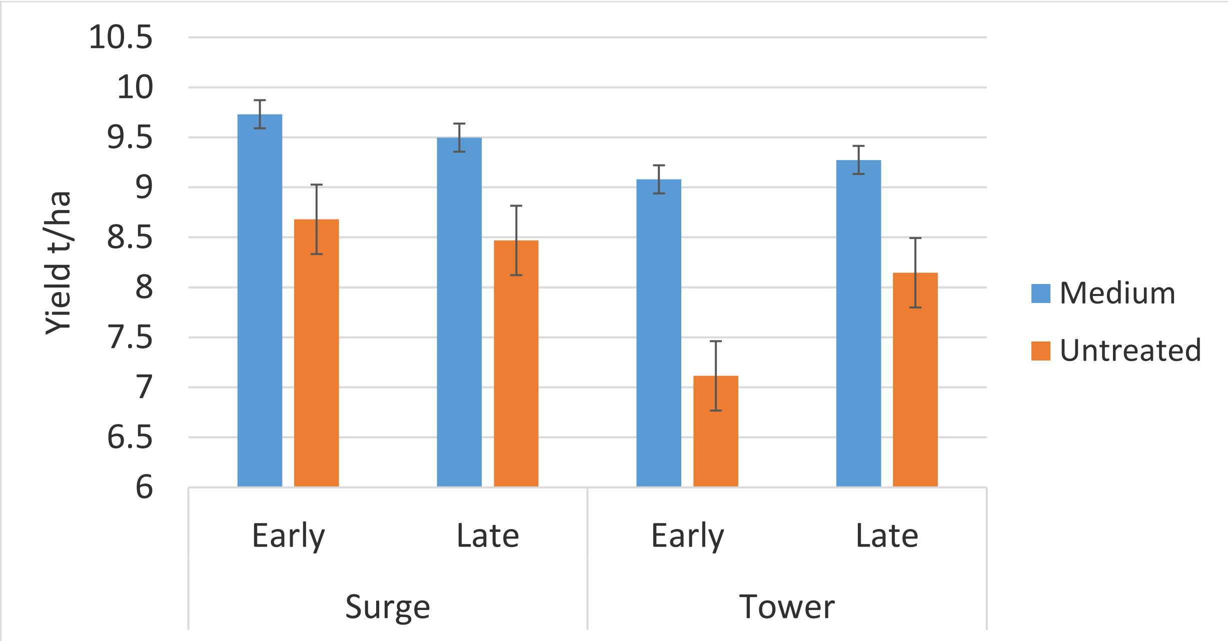

Figure 4. Yield response in winter barley sowing date trials

For the resistant variety Surge treatment had a much greater influence on yield than sowing date. The same was true for the susceptible variety KWS Tower. For this variety there was a distinct advantage on sowing the crop late.

DISCUSSION

Results from the seed treatment trial indicate little reduction in disease control from a novel seed treatment alone (Figure 1). Given the disease pressure on barley crops this is no surprise and very few crops are left untreated in Scotland. Both winter and spring crops receive an average of 2.5 fungicide sprays during the cropping season (SASA, 2018). Yield results were variable with a combination of Elicitor 1 + Elicitor 2 then reduced fungicides giving the highest yield after a full fungicide programme (Figure 1). This type of result has been reported previously in barley trials (Walters *et al.*, 2014) and suggests a possible incorporation of elicitors into disease control programmes. Elicitor 2 was chosen as a suitable seed treatment for the integrated trials. A big response was observed in these trials following foliar sprays (Figure 2). There were no significant differences between the treatments over the trials carried out in 2019. The laminarin seed treatment plots yielded slightly higher than the untreated but this was not statistically significant (9.134 t/ha compared to 9.047 t/ha). These trials will be repeated over a second cropping season. The analysis does not include fungicide costs and that should also be considered when comparing programmes. The removal of a chemical seed treatment from a programme offers a cost benefit an environmental benefit and helps protect existing chemistry.

The sowing date trial revealed some interesting differences in yield results. Delaying the sowing date in the resistant winter barley cv Surge had little effect on yield but there was a significant increase in yield in the delayed crops of the susceptible line cv. KWS Tower. Green leaf area was increased in late sown crops as the early sown crops had significantly higher levels of tan spot (*Pyrenophora tritici-repentis*) and ramularia leaf spot. Recent epidemiology studies have revealed a late season movement of these fungal spores from barley crops (Havis *et al.,* 2012; Havis, unpublished). The early sown crops may be acting as a green bridge for these pathogens. The results from these trials indicate that the adoption of IPM measures will necessitate an increased number of management decisions for barley growers.

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OPPORTUNITIES FOR RATIONALISING FUNGICIDE INPUTS IN THE MANAGEMENT OF SPRING BARLEY DISEASE

I J Bingham[1], N D Havis[1] and F J Burnett[2]
[1]*Agriculture Horticulture and Engineering Sciences Department, SRUC, King's Buildings, West Mains Road, Edinburgh, EH9 3JG*
[2]*Head of Connect for Impact, SRUC, King's Buildings, West Mains Road, Edinburgh, EH9 3JG*
E-mail: ian.bingham@sruc.ac.uk

Summary: Fungicide timing experiments were conducted over multiple sites and years on two spring barley varieties differing in resistance to *Rhynchosporium commune* to investigate opportunities for reducing fungicide inputs in disease management programmes. In the majority of site-season combinations, yield was maximised with a single application made at booting. At only one site and in one year was there a significant benefit of applying an additional treatment at the start of stem extension. The results demonstrate that where field inspection reveals little or no visible rhynchosporium in the crop at the start of stem extension, a variety with good resistance is being grown and a period of heavy or sustained rainfall after the start of stem extension is not forecast, the risk of rhynchosporium epidemics developing is likely to be low and a single fungicide application at booting may be sufficient to manage disease and maximise yield.

INTRODUCTION

Management of foliar disease in UK grown spring barley is usually based on a two spray fungicide programme. The first application made at the start of stem extension (referred to here as the T1 timing) is primarily designed to control disease during stem extension and protect the development of grain sink capacity, whilst the second (T2), made during booting, is designed to protect the canopy from disease during grain filling. The main disease threat during stem extension is rhynchosporium leaf blotch and T1 applications are often made prophylactically on the grounds that rhynchosporium is difficult to control once epidemics become established. However, with the current trend towards drier spring weather and more integrated approaches to disease management, including the use of more resistant varieties, the risk of rhynchosporium may be diminishing. We have been investigating opportunities for omitting T1 applications when the risk of rhynchosporium is low.

The rationale for our approach is as follows. Rhynchosporium is a polycyclic disease. The primary inoculum for infection may come from *R. commune* conidia on stubble from the previous crop or from seed borne infection (Zhan et al. 2008). Secondary spread can occur through splash dispersed conidia leading to disease in the upper canopy. The period between infection and the development of sporulating lesions can be long; typically two to three weeks under UK conditions, although it is recognised that sporulation can sometimes occur before symptoms appear (Zhan et al., 2008). This time period broadly corresponds to the period between the start of stem extension and booting in spring barley. We hypothesised that if there was no visible disease observed in the crop at the start of stem extension, and negligible amounts of asymptomatic *R. commune* detected in the leaves, a T1 application may be omitted and any later disease threat dealt with via the T2 application.

To test the hypothesis we investigated the yield response of spring barley crops to fungicide timing at three to five sites in each of three years. The sites were selected to differ in their rhynchosporium risk. The risk of disease was also varied by using two varieties of contrasting resistance to rhynchosporium.

MATERIALS AND METHODS

Experiments were conducted at SRUC's spring cereal trial sites between 2017 and 2019. Sites common to all years were Boghall farm (Edinburgh), Cauldshiel (East Lothian) and Drumalbin (Lanarkshire) (Fig. 1). In 2018 there was an additional site at Broadleys farm (Perth and Kinross), whilst in 2019 the additional sites were Balruddery (Dundee) and Kirkton farm (Aberdeenshire). At the majority of sites the previous crop was spring barley, but at Cauldshiel it was winter wheat and at Drumalbin it was grass. Cereal straw from the previous crop was baled and the stubble ploughed.

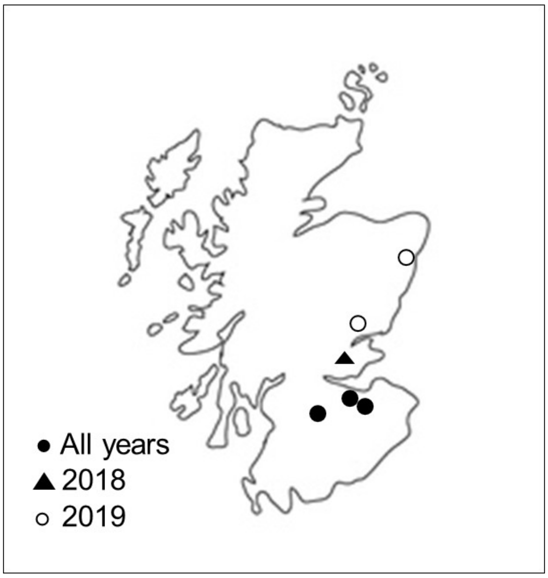

Figure 1. Location of trial sites

Experiments were laid out in a split-plot factorial design with two factors (variety and fungicide timing) and four replicate blocks. Variety was randomised within main plots and fungicide timing within sub-plots. Plots 10 x 2m were sown at a seed rate of 360 viable seeds between the middle of March and the first week of April. Spring barley (*Hordeum vulgare*) varieties were Concerto (rhynchosporium resistance score 4) and Fairing (resistance score 8 at the start of the project) (AHDB, 2019). Five fungicide timing treatments were imposed; untreated (controls), T1 only (application at Zadoks growth stage 30/31), T2 only (application at GS 45/49), T1 followed by a T2, and a late T1 followed by a standard T2 (referred to here as T1.5+T2 where the first application was made approximately ten to fourteen days after GS30/31). The treatment applied at T1 or T1.5 was bixafen + prothioconazole (Siltra Xpro @ 0.4 l ha^{-1}) and at T2 it was bixafen + prothioconazole (Siltra Xpro @ 0.4 l ha^{-1}) plus chlorothalonil (Bravo @ 1.0 l ha^{-1}). NPK, S and Mn fertilizer was applied according to recommendations for malting barley crops. Herbicide was according to standard farm practice. No growth regulator was applied.

The severity of rhynchosporium was assessed on untreated plots at GS 30/31 using whole plant scores in 2017 and on ten randomly sampled plants by leaf layer in 2018 and 2019. Sampled plants were then divided into the top four leaf layers and stored at -20^{o}C for determination of *R.*

commune by qPCR (Fountaine et al., 2010). The youngest fully unfolded leaf of three plants in each of four plots was tagged and the number of leaves between the tagged leaf and flag leaf counted during booting to establish leaf identity at the T1 spray timing. The severity of all diseases on all plots was assessed again at booting/ear emergence and in 2019 for a further time at mid-grain fill (GS75/77). Plots were combine harvested for determination of grain yield. Grain moisture content was determined after oven drying and used to adjust yields to 85% dry matter content.

RESULTS

Rainfall

April of 2017 (year 1) was an usually dry month at all sites with <20% or between 20-30% of the long term (1981-2010) average rainfall for the area (Anon 2019). This was followed by a drier than average May in the east (Edinburgh and E Lothian). By contrast, June was extremely wet in all regions, whilst July and August had average rainfall.

In year 2, April was wetter than average in Edinburgh, E Lothian and Perth and Kinross, but all sites in May received only 30-50% of the average rainfall. June and July were also drier than average at most sites. Year 3 was a more typical year for rainfall. It was average or a little below average in April (depending on the site), but in May, June and July it was average or above average. August was very wet in most areas except the NE.

Yield response to fungicide treatment

There were significant yield responses to fungicide treatment at all sites in 2017 ($P<0.01$), in two of the four sites in 2018 ($P<0.05$) and four of the five sites in 2019 ($P<0.001$). In all but one of those cases, the T2 application on its own gave a significant yield increase relative to untreated controls. At only one site in one year (Lanark 2019) was the yield following the T1+T2 application significantly ($P<0.05$) greater than the T2 on its own (Fig 2). This was observed in the rhynchosporium susceptible variety Concerto and not the more resistant variety Fairing.

When data from different sites, years and varieties were combined and analysed using site-year as a random effect, the average yield response to the T1 treatment on its own was 0.21 t ha^{-1}, whilst that to the T2 application was 0.56 t ha^{-1} (Table 1).

Disease severity

In the majority of site-seasons visible rhynchosporium lesions were either absent at GS30/31 or observed at very low severities (0.01-0.38%, averaged over the top four fully unfolded main shoot leaves). At Lanark in 2019 the severity was 0.55% in the relatively resistant variety Fairing and 5.39% in the susceptible Concerto. In spite of the presence of lesions on lower leaves of Fairing at the start of stem extension, rhynchosporium failed to spread into the upper canopy (leaves 1-3) during grain filling, even in untreated plots (Table 2). By contrast, rhynchosporium spread into the upper canopy of untreated Concerto reaching severities of 17-33% at GS78 depending on the leaf layer. A T2 application on its own restricted the epidemic, but the T1+T2 treatment reduced the severity on each of the top three leaves still further ($P<0.05$). At the time of writing qPCR data were not available.

Table 1. Effects of fungicide timing on yield (t ha^{-1} @ 85% DM). A cross site-year anova of 12 experiments with variety and fungicide treatments analysed as fixed effects and site-year as a random effect.

	Yield t ha^{-1}			
Fungicide	Concerto	Fairing	Mean	Response
Untreated	6.00	6.49	6.24	
T1	6.22	6.68	6.45	0.21
T2	6.54	7.05	6.80	0.56
T1 + T2	6.62	7.01	6.81	0.57
T1.5 +T2	6.62	7.03	6.83	0.58
Variety (V)		<0.001		
Fungicide (F)		<0.001		
V*F		0.799		
LSD Fung		0.120		
LSD V*F		0.169		
residual df		376		

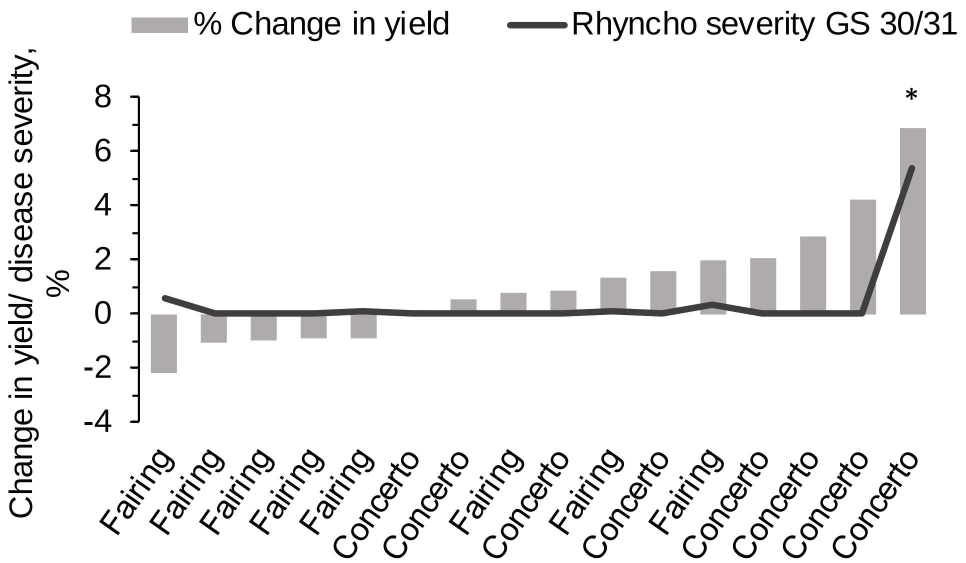

Figure 2. Yield benefit from the T1 component in a two spray programme (calculated as the difference in yield between the T1+T2 treatment and the T2 treatment, expressed as a % of the T2 treatment) plotted alongside the severity of rhynchosporium at GS30/31. * indicates the only case where the two spray programme gave a significant (P<0.05) increase in yield over a T2 application on its own. Results have been arranged in ascending order of change in yield. Analysis includes only those site-years where an overall yield response to fungicide was found.

Table 2. Severity (%) of rhynchosporium during crop growth by leaf layer at Lanark 2019. ELN; eventual leaf number. L4 was the tagged youngest fully unfolded leaf at GS 30/31. Two way anova was undertaken for each leaf layer and growth stage; for each the variety x fungicide interaction was significant (P<0.05). LSD values (P=0.05) from the V x F interaction are shown for comparison of fungicide treatments at a given GS and leaf layer. Shaded blocks are leaf layers that had senesced at the time of assessment.

Concerto	GS 30/31	GS 51				GS 78			
ELN	Untreated	Untreated	T2	T1+T2	LSD	Untreated	T2	T1+T2	LSD
L1 (flag)	xx	1.0	1.5	0.0	0.8	16.5	3.0	0.5	1.8
L2	xx	3.3	3.3	0.0	1.2	25.5	5.8	1.3	2.4
L3	0.0	5.5	5.3	1.5	1.9	36.3	11.8	2.5	4.2
L4	0.0	8.0	7.0	2.5	2.5				
L5	0.3	12.3	10.0	5.3	3.4				
L6	4.5								
L7	16.8								

Fairing	GS 30/31	GS 51				GS 78			
ELN	Untreated	Untreated	T2	T1+T2	LSD	Untreated	T2	T1+T2	LSD
L1 (flag)	xx	0.0	0.0	0.0	0.8	0.0	0.0	0.0	1.8
L2	xx	0.0	0.0	0.0	1.2	0.0	0.0	0.0	2.4
L3	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0	4.2
L4	0.0	0.5	0.3	0.3	2.5				
L5	0.1	1.8	1.5	2.0	3.4				
L6	0.3								
L7	1.8								

DISCUSSION

Our results show that the major yield response to fungicide application in spring barley comes from treatments applied at booting (T2 timing) and that only rarely, when there is a high risk of rhynchosporium spreading to the upper canopy, does the addition of an earlier application (T1 timing) result in a further yield increase. A yield response to a T1 application was observed in only one of 12 experiments conducted over multiple sites and years. In 2017 and 2018 the dry weather leading up to stem extension would be expected to limit initial disease development accounting for the absence or negligible visible disease observed at GS30/31. In 2018 it was also dry during stem extension minimising the risk of any splash dispersal of spores into the upper canopy. However, even under the more typical rainfall patterns of 2019 a severe rhynchosporium epidemic, requiring a two spray programme for its control, was observed at just one (high risk) site.

Disease located in the upper leaf layers of barley has the greatest impact on canopy light interception and photosynthesis (Bingham and Topp, 2009). Our results highlight the value of varietal resistance to rhynchosporium for restricting the spread of epidemics into the upper canopy. Thus, in untreated plots grown under the same weather conditions at Lanark in 2019, rhynchosporium failed to develop on the uppermost three leaves of the resistant variety Fairing, but reached high severity in the susceptible variety Concerto.

We suggest that fungicide treatments to spring barley at the start of stem extension may be omitted from disease management programmes if there is a low risk of rhynchosporium epidemics developing. Low risk situations would be where there is little or no rhynchosporium visible in the crop at the start of stem extension, where varieties with good resistance to rhynchosporium are being grown and where a period of reasonably settled weather without heavy or sustained rainfall is forecast for the following two weeks.

ACKNOWLEDGEMENTS

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CONTROLLING RAMULARIA LEAF SPOT POST CHLOROTHALONIL

N D Havis, K Gorniak, J M Stanisz-Migal, H Creissen and F J Burnett
Carbon, Crop and Soil Group, SRUC Edinburgh, West Mains Road, Edinburgh EH9 3JG
E-mail: neil.havis@sruc.ac.uk

Summary: Ramularia leaf spot is major pathogen of barley crops across the temperate regions of the world, reducing yield and quality in grain. The disease is caused by the fungus *Ramularia collo-cygni.* Research into the development of robust varietal resistance is only just underway and control relies heavily on the use of fungicides. Recently, resistance to the major fungicide groups used in barley has appeared and control relied heavily on the use of the multisite fungicide chlorothalonil. However, this product has lost its approval in the EU and will cease to be an option after May 2020. This paper describes some initial work looking at alternatives to chlorothalonil in a barley disease control programme.

INTRODUCTION

Barley is the second most important cereal crop grown in the UK and production reached a new peak in 2019 of over 8.1 million tonnes (DEFRA, 2019). Ramularia leaf spot (RLS) caused by the fungus *Ramularia collo-cygni* has been categorised as a major disease of barley crops in the UK for over 10 years (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}. The vast majority of symptoms appear in the crop post-flowering, although reports from Ireland in 2019 suggested earlier symptoms were seen in winter barley crops (S. Kildea pers. com.). In general, fungicides are applied as protectants prior to the appearance of the majority of symptoms. Resistance issues to the azole and succinate dehydrogenase groups appeared in the fungus in 2014 and spread across a number of countries. This increased the reliance on chlorothalonil (Bravo®) (chlor) to give effective control of RLS in the UK (Havis *et al.,* 2018). The removal of approval for chlorothalonil by the European Union has accelerated the search for durable and effective RLS control treatments.
Although some non-chemical treatments have been evaluated as seed treatments to control RLS there is limited data on the efficacy of some on chemical treatments as foliar sprays (Havis *et al*., 2012). Biological control measures and elicitors have also been studied for activity against RLS, with limited success (AHDB, 2014; Walters *et al*., 2012). A series of trials were initiated to investigate options to chlorothalonil in control programmes and isolates were tested for their sensitivity to alternative chemistry.

MATERIALS AND METHODS

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Fungicide Efficacy trials

A replicated field trial was undertaken at Boghall farm, Midlothian in 2019. Spring barley (cv. Laureate) 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 (pro) Proline, chlorothalonil (chlor) and mefentrifluconazole + fluxapyroxad (mef +(flux)) Revystar XE™. The fungicides were applied at twice the recommended rate, full recommended rate, half of the full rate and a quarter of the full rate. Fungicide treatments were applied at GS49 (Zadocks, 1974). Ramularia leaf spot levels were assessed in the top three leaf layers twice between GS75 and 85.

Alternative chemistry trials

A winter barley trial (cv. California) was set up at Boghall farm in 2019. Seed was sown in 10m x 2m plots on the site and a range of different treatments applied (see Table 1). Treatments included pro, pyraclostrobin (pyr) Comet™, wettable sulphur (w sulph) Kumulus DF®, chlor, spiroxamine + prothioconazole (spir + pro) Helix ®, 2-(trichloromethylsulfanyl)isoindole-1,3-dione (folp) Folpet®, liquid mancozeb (liq man) Laminator Flo®, cyprodinil (cyp) Kayak ®, cyflufenamid (cyf) Cyflamid ®, proquinazid (proq) Talius® and fluxapyrxad (flux) Imtrex®. Plots were assessed for foliar disease throughout the growing season and the trial was harvested. Yields were expressed as t/ha at 85% dry matter.

Table 1. Treatment detail for winter and spring barley trials in 2019 Dose rates per hectare are in brackets

Treatment	GS 31 (T1)	GS 49 (T2)
1	pro (0.5+ + pyr (0.5)	untreated
2	pro (0.5+ + pyr (0.5)	chlor (0.75)
3	pro (0.5+ + pyr (0.5)	pro (0.72)
4	pro (0.5+ + pyr (0.5)	spir + pro (0.7)
5	pro (0.5+ + pyr (0.5)	pyr (1.25)
6	pro (0.5+ + pyr (0.5)	folp (1.5)
7	pro (0.5+ + pyr (0.5)	liq man (1.0)
8	pro (0.5+ + pyr (0.5)	cypr (1.0)
9	pro (0.5) + pyr (0.5) + w sulph (10kg)	untreated
10	pro (0.5+ + pyr (0.5)	cyf (0.7)
11	pro (0.5+ + pyr (0.5)	proq (0.25)
12	pro (0.5+ + pyr (0.5)	fux 1.0

A spring barley trial (cv. Laureate) was carried at Drumalbin farm, Lanark in 2019. Seed was sown in 10m x 2m plots on the site and the treatments from the winter barley trial (Table 1) were replicated. Plots were assessed for foliar disease throughout the growing season and the trial was harvested. Yields were expressed as t/ha at 85% dry matter.

Sensitivity assays

In order to establish the sensitivity of the fungus to existing and alternative chemistry sensitivity assays were carried out. *R. collo-cygni* isolates were produced from infected leaf samples from SRUC trial sites in 2018. 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.

Fungicide Performance trial

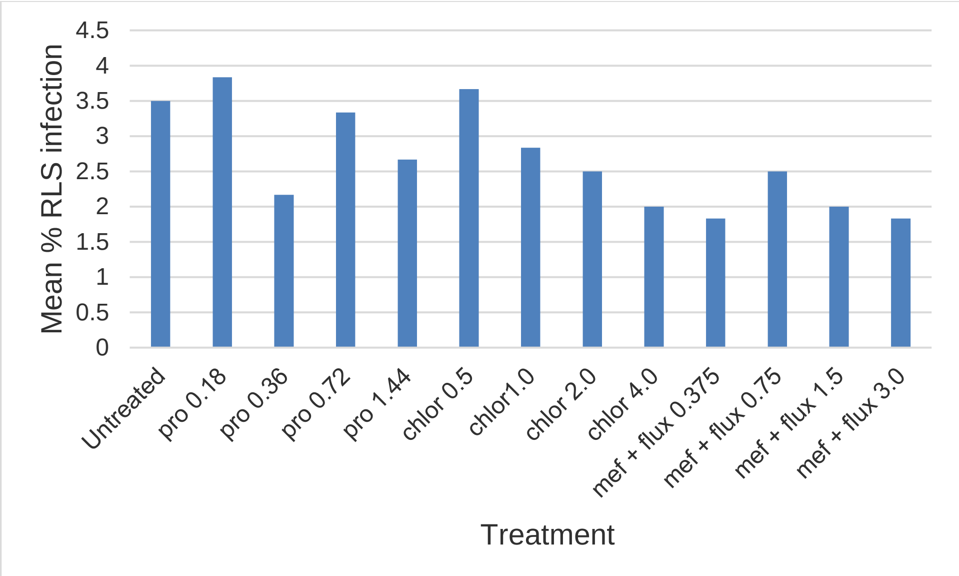

Figure 1. Control of Ramularia leaf spot in spring barley (cv. Laureate) at Boghall trial site (GS 83 – 08/Aug/19). LSD (P=0.05) 1.868

The late season assessment indicated that pro at 0.36 l/ha was giving some reduction in RLS symptoms. Chlor gave the most effective control of RLS in the crop and increasing the dose showed a step reduction in symptoms. The new fungicide, mef+flux also showed activity against RLS in this trial.

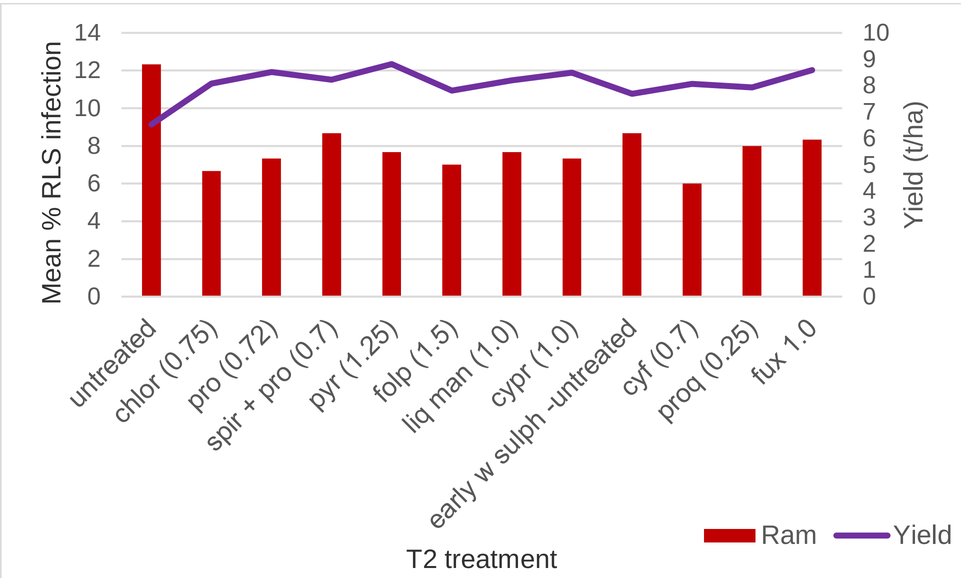

Figure 2. Levels of RLS and grain yield in winter barley trial at Boghall farm, 2019. Ramularia LSD (P=0.05) 2.64; Yield LSD (P=0.05) 0.54

The winter barley trial at Boghall had a number of diseases present including powery mildew (*Blumeria graminis*) rhynchosporium scald (*Rhynchosporium commune)*, net blotch (*Pyrenophora teres*) and tan spot (*Pyrenophora tritic repentis*). RLS levels reached 12% in the untreated crop late in the season. The most effective fungicides against RLS were chlor and cyf. All of the treatments in the trial gave significant reduction in RLS levels (FPr =0.01). Yield increases for the treatments against the control were also statistically significant for this trial (FPr <0.001)

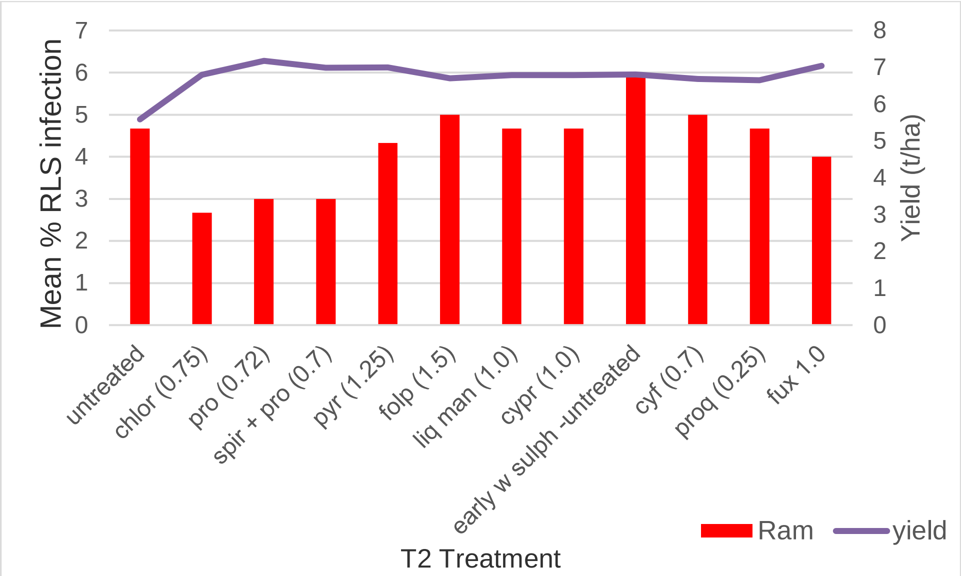

Figure 3. Levels of Ramularia leaf spot (RLS) in spring barley trial at Lanark, 2019. Ramularia LSD (P=0.05) 2.38; Yield LSD (P=0.05) 0.506

The trial also had high levels of *R. commune* despite the early use of a cover spray at T1. As with the w barley trial the chlor again gave the greatest reduction in RLS symptoms. However, none of the control achieved in this trial was significantly different to the untreated. Yield increases were significant (FPr <0.001). This is most likely due to control of *R. commune* in the trial.

Sensitivity testing indicates a range of values for the fungicides tested. The isolates were most sensitive to chlor. Although the mean figures for flux and folpet were similar to chlor there was a wide range in the values obtained, indicating variability in the population. The azole epoxy had the lowest sensitivity values.

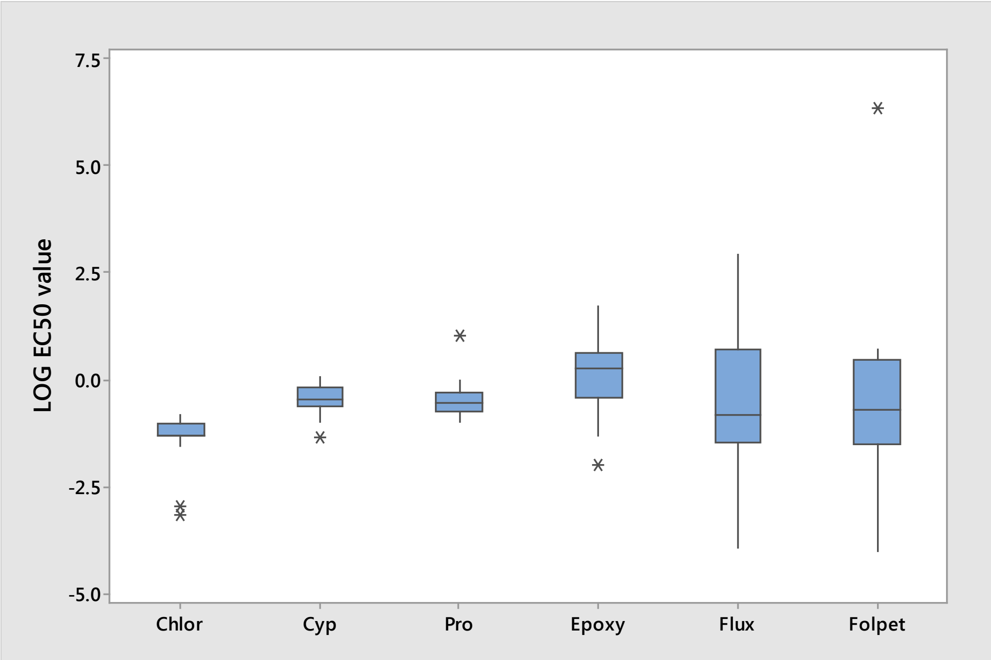

Figure 4. Sensitivity of *Ramularia collo-cygni* isolates to fungicides (2018).

DISCUSSION

Ramularia leaf spot emerged as a major pathogen of barley in the last 15 years (Havis *et al.,* 2015) and control has been based in many countries on the use of chlorothalonil. The removal of its approval by the EU has presented barley growers with a dilemma. A recent report from Ireland indicated that the replacement of chlorothalonil with another multisite, folpet, would still mean losses of 10% could be expected on moderate to susceptible varieties in a moderate disease year (Kildea *et al*., 2019). This will have an impact on the profitable production of barley in many countries. The trials undertaken in Scotland in 2019 indicate that the new azole mefentrifluconazole does have good activity against RLS in trials (Fig 1). The careful management of new products against RLS in the absence of a strong multisite represents a challenge to growers and researchers alike. *R collo-cygni* has been shown to develop resistance to azole and sdhi chemistry even in the presence of chlorothalonil so ideally new modes of action are required to help in an anti-resistance strategy. Several mutations in the target genes of sdhi fungicides and the target gene of azoles Cyp51 have been recorded across many countries (Rehfus *et al*., 2019). This has led to decreases in fungicide sensitivity since 2014. Previous reports indicated the significant loss in sensitivity on *R collo-cygni* in Scotland (Havis *et al*., 2018). Testing of isolates from 2018 indicated that the azole and sdhi fungicides have a reduced efficacy against *R. collo-cygni* (Figure 4). However two of the alternative fungicides examined in field trials (cyp and folpet) did show some activity against *R. collo-cygni* in lab tests. They may be able to give some control of RLS in field situations. This suggestion was supported by data from field trials in 2019, in particular the winter barley trial at Boghall. All of the alternatives showed significant control of RLS (Figure 2). This trial also indicated that the azole and shdi fungicides retained some activity against RLS in contrast to the results obtained in 2017 which were reported previously (Havis *et al*., 2018). This change in the fungal population and increased sensitivity has been observed across Europe (Andreas Mehl, pers. com.). The warm dry summer could have affected fungal populations and reduced the population of mutated isolates. This phenomenon of increased sensitivity has also been seen in isolates of *Zymospetoria tritici* in 2019 (Bart Fraaije, pers. Com.). It remains to be seen whether these shifts are permanent or transitory.

Ramularia leaf spot symptom expression is known to be related to stress in the crop (Havis et al, 2015). A trial was undertaken evaluating the potential of biostimulants and crop additives to reduce stress in the crop and disease symptoms. However, no disease was observed in spring barley at the trial. The trial will be repeated in 2020.

Future long-term control of RLS requires the development of robust varietal resistance, uptake of integrated pest management methods and the availability of effective fungicides to manage disease levels and produce high quality barley crops.

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THE EFFECTIVENESS OF BARLEY RESISTANCE TO RHYNCHOSPORIUM IN THE FIELD

A Avrova, J-N Thauvin, M Coulter and M Looseley
The James Hutton Institute, Invergowrie, Dundee DD2 5DA
Email: anna.avrova@hutton.ac.uk

Summary: Rhynchosporium is by far the most destructive and economically important disease of both spring and winter barley in the UK. The disease is primarily controlled through the application of fungicides. However, high fungicide costs, combined with the evolution of fungicide insensitivity, make this type of control an expensive requirement for farmers. One of the most effective and sustainable ways of providing protection against fungal infection is through varietal resistance. However, due to the pathogen's high genetic variability, developing cultivars with long lasting resistance is challenging. Over 50% of new UK spring barley cultivars carry *Rrs1*, highlighting its continuing contribution to host resistance in current elite spring barley germplasm. Until recently, *Rrs1* has remained effective against natural *R. commune* populations, but it is important to stay a pace ahead of the pathogen through identification of new resistances. Recently, we have fine mapped another major resistance gene, *Rrs18*. To characterise the effectiveness of different resistance genes to *R. commune* in the field we evaluated barley cultivar with *Rrs18* alongside lines containing *Rrs1, Rrs2, Rrs13* and a combination of *Rrs1* and *Rrs18*. This research will help varietal resistance to remain a key part of the integrated disease management.

INTRODUCTION

Rhynchosporium or leaf scald is by far the most destructive and economically important disease of both spring and winter barley in the UK. It is caused by a fungal pathogen *Rhynchosporium commune*. The disease is primarily controlled through the application of fungicides, however, high fungicide costs, combined with the evolution of fungicide insensitivity, make this type of control an expensive requirement for farmers (reviewed in Avrova and Knogge 2012; Walters et al., 2012). Despite routine fungicide applications, *R. commune* still costs the UK economy £10.8 million per year (King *et al*., 2013). This level of losses calls for a sustainable way to control *R. commune* infection. Therefore, effective cultivar resistance to this damaging disease has long been an important breeding target. However, due to the pathogen's high genetic variability (Zaffarano *et al*., 2006), one of the biggest challenges is developing cultivars with resistance that would last.

A few years ago we showed that over 50% of new UK spring barley cultivars carry a major resistance gene *Rrs1*, highlighting the significant and continuing contribution of this gene to host resistance in current elite spring barley germplasm (Looseley *et al*., 2018). Until recently, *Rrs1* has remained effective against natural *R. commune* populations, but it is important to stay a pace ahead of the pathogen through identification of new resistances. Recently we have fine mapped another major resistance gene, *Rrs18*, towards the telomere of chromosome 6HS when compared to *Rrs13* (Coulter *et al*., 2019).

The aim of this work was to characterise the effectiveness of different resistance genes to *R. commune* in the field. We evaluated barley lines with *Rrs18* alongside lines containing *Rrs1*, *Rrs2*, *Rrs13* and a combination of *Rrs1* and *Rrs18*. This research will help varietal resistance to remain a key part of integrated disease management.

MATERIALS AND METHODS

Plant Material

Spring barley cultivars, known to contain different major resistance genes against rhynchosporium, as well as susceptible cultivars used in the field trials are listed in Table 1.

Table 1. Barley cultivars used in the field trials

Barley cultivar/line	**Ressitance gene**	**Reference**
Belgravia, Beryllium, Brahms, Propino, Westminster	*Rrs1Rh4*	Looseley *et al*., 2020
Atlas	*Rrs2*	Hanemann *et al.,* 2009
BCline 30	*Rrs13*	Abbott *et al*., 1992
Steptoe	*Rrs18*	Coulter *et al.,* 2018
CIho3515	*Rrs1* and *Rrs18*	Coulter *et al.,* 2018
Alexis, Concerto, Morex, Optic and Waggon	No *R* genes	

Disease Nursery Trials

Spring barley cultivars were tested in field trials at a Rhynchosporium disease nursery at the James Hutton Institute, Dundee, Scotland. Disease assessments were conducted over the course of 2 growing seasons: 2016 and 2019. In 2016 the trial was sown in spring, while in 2019 the trial was sown in late autumn to increase the chance of rhynchosporium infection development. For each of the trials, two replicates were sown using a randomized row-column design. Trials were sown as 1.5m^2 plots using an approximate sowing rate of 145 seeds/m^2. Continuous growing of barley in the disease nursery had resulted in considerable build-up of inoculum so that natural infection occurred and was encouraged by application of overhead irrigation on alternate days. Visible disease symptoms were assessed according to the method described by Looseley et al (2015). Briefly, plots were scored on a 1-9 scale, where 1 represented complete absence of disease symptoms, and 9 - a complete coverage of the non-senescent leaf area by lesions. Disease symptoms were assessed 3 times per season. For each trial, line means and standard deviations for the final disease score were estimated using Excel.

RESULTS

The assessment of 11 spring barley cultivars for resistance to rhynchosporium at a Rhynchosporium disease nursery in 2016 showed that while rhynchosporium scores were significantly lower on BCline 30 with *Rrs13*, Steptoe with *Rrs18* and CIho3515 with *Rrs1* and *Rrs18* compared to susceptible cultivars Alexis, Concerto, Morex, Optic and Waggon, none of the 3 cultivars with *Rrs1*, Belgravia, Propino and Westminster, showed a significant level of resistance to the natural *R. commune* population (Figure 1).
Following an unusually warm summer of 2018 that prevented scoring for rhynchosporium, to increase the chance of rhynchosporium infection development the second trial was sown in late autumn of 2018 and assessed for resistance to rhynchosporium over summer of 2019. As expected this led to overall high levels of infection allowing to see the effect of different resistance genes even clearer than in 2016 trial. In 2018/19 two cultivars containing *Rrs1*, Beryllium and Brahms, and Atlas, containing *Rrs2*, were added to the trial (Figure 2). This time

while cultivars Beryllium, Brahms and Propino did not show any resistance to rhynchosporium, cultivars Belgravia and Westminster were significantly more resistant to the natural *R. commune* population than susceptible cultivars (Figure 2).

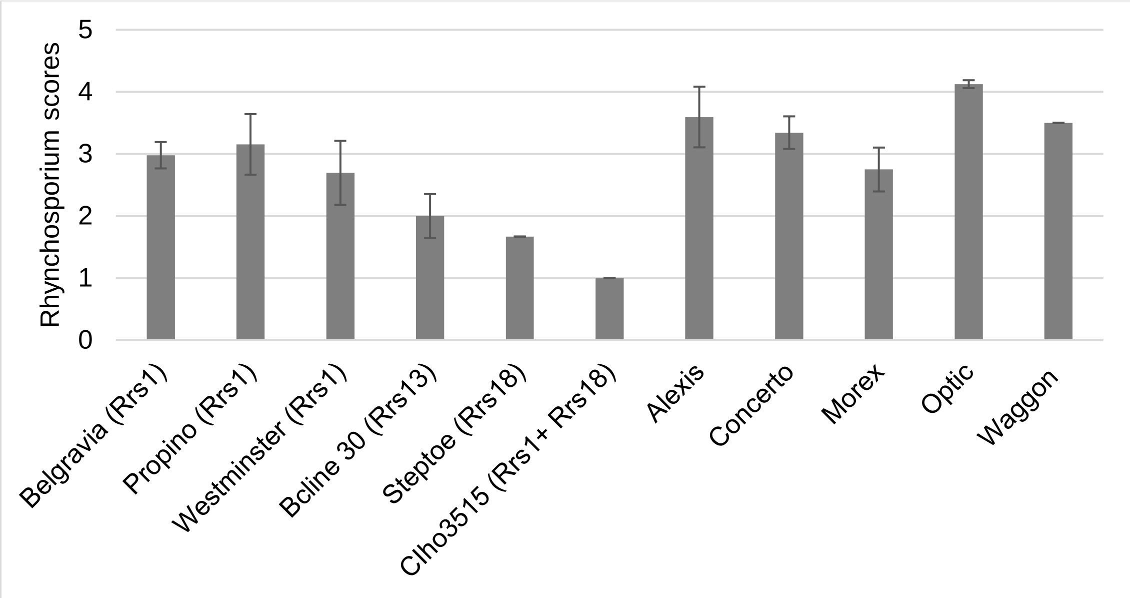

Figure 1. Barley cultivar resistance to rhynchosporium in the Rhynchosporium disease nursery, James Hutton Institute, in 2016. Spring sown field trial.

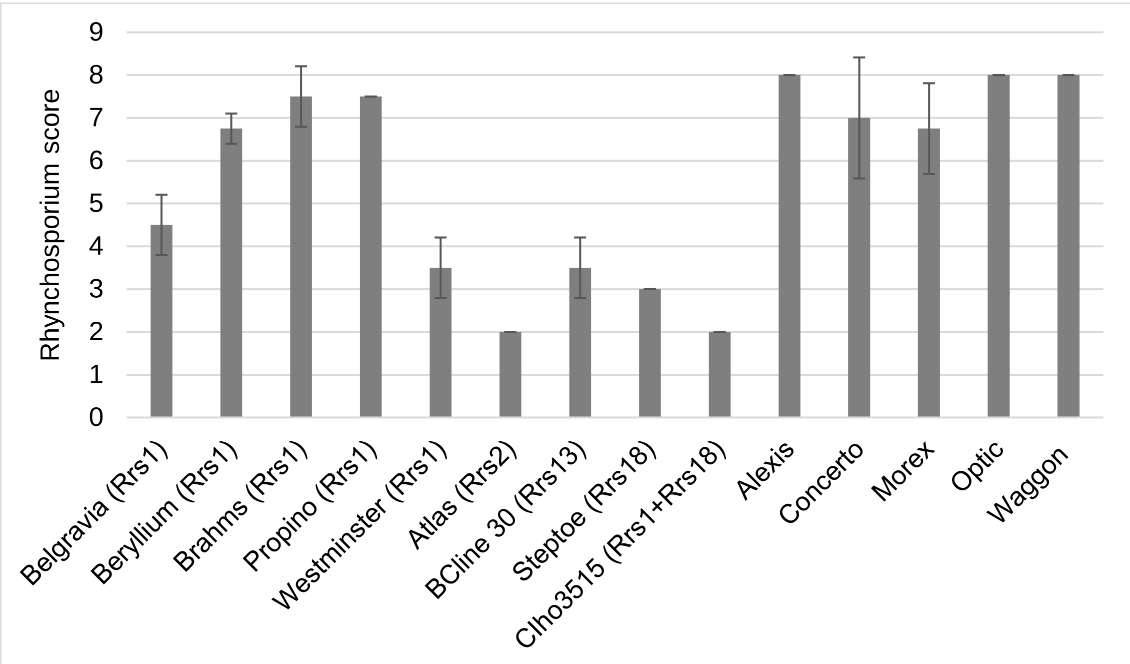

Figure 2. Barley cultivar resistance to rhynchosporium in the Rhynchosporium disease nursery, James Hutton Institute, in 2019. Winter sown field trial.

DISCUSSION

Previous field trials performed in Rhynchosporium disease nursery on a collection of 660 lines of spring barley showed a consistently high effect of *Rrs1* in 2013, 2014 and 2015 (Looseley *et al*., 2018). The breakdown of *Rrs1* resistance in 2016 and 2019 is consistent with the evolution of *R. commune* population exposed to *Rrs1* over several years. While around 12% of spring barley lines used in 2013, 2014 and 2015 contained *Rrs1*, only 3 barley cultivars contained *Rrs2*, and none had *Rrs13* or *Rrs18*. This explains why *Rrs2*, *Rrs13* and *Rrs18* were effective in 2016 and 2019 trials while the effect of *Rrs1* was at best inconsistent.

A combination of *Rrs1* and *Rrs18* also resulted in the consistently high resistance effect. These results support the importance of pyramiding of complementary resistance genes in order to increase their longevity in the field and achieve more durable resistance.

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QUANTIFYING THE EFFECTS OF ASYMPTOMATIC AND SYMPTOM EXPRESSING PHASES OF *RAMULARIA COLLO-CYNGI* INFECTION ON PHOTOSYNTHESIS AND YIELD FORMATION OF SPRING BARLEY

C Burrell[1], N D Havis[1], S H Spoel[2] and I J Bingham[1]
[1]*Agriculture Horticulture and Engineering Sciences Department, SRUC, King's Buildings, West Mains Road, Edinburgh, EH9 3JG*
[2]*University of Edinburgh, School of Biological Sciences, Institute of Molecular Plant Sciences, SRUC, King's Buildings, Daniel Rutherford Building Max Born Crescent, Edinburgh, EH9 3BF*
E-mail: ian.bingham@sruc.ac.uk

Summary: Controlled environment and field experiments were conducted to determine the relative effects of asymptomatic and symptom expressing phases of *Ramularia collo-cygni* infection on photosynthesis and yield formation in spring barley. In young plants inoculated with mycelial suspensions of *R collo-cygni*, infection had no effect on photosynthesis, as measured by chlorophyll florescence imaging, prior to visible symptom development or in areas of leaves out-with the visible lesion. As a result, when symptom severity was low, there was no overall effect on the rate of CO_2 uptake per unit leaf area. Under natural infection in the field, again there was no effect on photosynthesis (maximum quantum yield, Fv/Fm) until after visible symptom development. Grain yields predicted from the difference in post-anthesis light interception between fungicide-treated plants and untreated plants with ramularia leaf spot were comparable with the measured yield response to fungicide. Collectively these results suggest that effects of ramularia on yield are associated primarily with symptom development and not the asymptomatic phase of infection. We conclude that prevention of symptom expression should be the major target for management of this disease.

INTRODUCTION

Ramularia leaf spot (RLS) is a major disease of barley crops that is becoming increasingly difficult to control. Resistance of the causal organism, *Ramularia collo-cygni*, to several fungicide groups is now widespread and the most effective fungicide, chlorothalonil, is being withdrawn from use. There is an urgent need to develop new methods for controlling the disease, including the introduction of resistance into the host plant. Progress will be quicker if the search could be targeted at the phases of the fungal lifecycle that are most damaging to crop yield formation. Currently, how *R collo-cygni* reduces yield of barley is not known with any certainty.

It has been established that *R collo-cygni* can grow systemically through the plant from inoculum within the seed without producing visible symptoms (Havis *et al*., 2014). The symptomless phase of the life cycle has been likened to that of an endophyte fungus. Under field conditions the transition to necrotrophy and development of visible symptoms typically occurs after flowering in the crop. Infection from airborne spores, released during the growing season, has also been demonstrated, with hyphae from germinated spores entering the leaf through open stomata (Stabentheiner *et al*., 2009).

Yield formation in non-diseased barley crops grown in cool temperate climates such as the UK is considered to be predominantly sink-limited. It is limited by the number of grains produced and their storage capacity rather than the supply of carbon assimilates for starch deposition during grain filling (Bingham et al. 2019). Grain sink capacity is established prior to flowering through processes governing tiller, ear and spikelet development, processes which are sensitive to reductions in carbon assimilation. It is possible that *R collo-cygni* reduces barley yield through effects of asymptomatic infection on pre-anthesis photosynthetic activity and the development of grain sink capacity. Alternatively yield reductions may be associated with reductions in photosynthetic activity during grain filling resulting from lesion development and loss of green leaf area.

The objective of experiments reported here was to investigate the relative effects of asymptomatic and symptom expressing phases of *R collo-cygni* infection on photosynthesis and barley yield formation. Leaf photosynthetic activity was measured in controlled environment experiments on seedlings inoculated with suspensions of fungal mycelia. Measurements were made before and after symptom development using Infra-Red Gas Analysis (IRGA), chlorophyll fluorescence analysis and chlorophyll fluorescence imaging. In field experiments crop growth analysis was used to test the hypothesis that increases in post-anthesis light interception, resulting from the prevention of symptom expression, can account for yield responses to fungicide.

MATERIALS AND METHODS

Controlled environment experiments

Experiments were conducted on seedlings of spring barley (*Hordeum vulgare* L) cv Concerto or Fairing. Seeds were germinated at room temperature on moist filter paper in Petri-dishes and two to three day-old seedlings transplanted into pots of Levington M3 high nutrient pot and bedding compost (ICL, Suffolk, UK). Plants were grown in a controlled environment chamber at 18^{o}C day, 12^{o}C night, 90% relative humidity (RH). Fluorescent lamps provided an irradiance of around 230 µmol m^{-2} s^{-1} at initial plant height over a 16h photoperiod. Pots were inspected daily and watered as required to keep the compost moist, but not waterlogged. After the 2nd leaf had fully emerged (Zadoks GS12; Tottman and Broad, 1987), plants were sprayed with 0.5 ml of mycelial suspension prepared from cultures of *R. collo-cygni* isolate DK05 Rcc001 in potato dextrose broth (PDB) (Makepeace *et al*., 2008). Additional plants sprayed with water or PDB served as separate controls. After treatment, plants were placed in a propagator within the growth chamber for 5 days to maintain high RH to encourage infection, the first two of which were in darkness. Plants were then removed from the propagators and arranged in randomised blocks within the growth chamber. Leaves were inspected daily and the appearance and severity of ramularia leaf spot symptoms assessed. Simultaneous IRGA and chlorophyll fluorescence measurements were made on the second leaf (youngest fully emerged at the time of inoculation) using a LI-6400XT Portable Photosynthesis System with the 6400-40 Leaf Chamber Fluorometer (LI-COR Biosciences, Lincoln, USA) at a leaf temperature of 18^{o}C and atmospheric CO_2 concentration of 400 ppm. Measurements were made at 0 (dark respiration), 230 (growth irradiance) and 1250 (light saturation) µmol m^{-2} s^{-1} PAR. Chlorophyll fluorescence imaging was conducted separately but on the same leaves using an IMAGING-PAM M-Series Chlorophyll Fluorescence System (Walz, Effeltrich, Germany).

Field experiment

A field experiment was conducted on spring barley cv Concerto in 2017 at Boghall farm, SRUC, Edinburgh. The experiment was laid out in a randomised block design of two core treatments with four replicates. Plots were drilled on 29th March at a seed rate of 360 seeds m^{-2} in plots measuring 10 x 2 m. NPK fertilizer, Mn and herbicide was applied according to local practice for a malting barley crop. Treatments were designed to vary the severity of ramularia leaf spot epidemics. The core treatments were: 1) untreated (no inoculation or fungicide); and 2) full fungicide treatment (bixafen + prothioconazole [Siltra Xpro 0.4 l ha^{-1}] at GS30 followed by prothioconazole [Proline 0.4 l ha^{-1}] plus chlorothalonil [Bravo 1.0 l ha^{-1}] at GS 45). An additional treatment with inoculation of *R collo-cygni* at GS332/33 failed to increase the severity of ramularia leaf spot over that observed in untreated plants and so the data are not presented here. Crop growth analysis and estimation of light interception by healthy canopy tissue was as described by Bingham *et al*. (2019). Commencing at flag leaf emergence, measurements of disease severity, % green leaf area and chlorophyll fluorescence were made approximately weekly on leaf 2 (leaf below flag leaf). Leaves were also sampled for determination of *R. collo-cygni* DNA by qPCR.

RESULTS

In the controlled environment experiments, RLS symptoms were observed around 16 days after inoculation (dai) but symptom severity increased only a small amount between 16 and 27 dai. Thus, visible disease severity was low in these experiments. Analysis of *R collo-cygni* DNA within leaf tissue by qPCR confirmed that there was significant infection 6 dai on inoculated plants, but not on the PDB or water controls (data not shown).

Rates of dark respiration were comparable over the course of the experiment and did not differ significantly ($P>0.05$) between inoculated plants and controls (Table 1). Rates of net CO_2 fixation measured at both the growth irradiance and at saturating irradiance, declined with leaf age. However, before and after the appearance of visible ramularia symptoms there was no significant effect of treatment on the rate ($P>0.05$). The operating efficiency of PSII photochemistry (Φ PSII) also declined with leaf age (Table 2). As with CO_2 uptake, there was no effect of ramularia inoculation on Φ PSII either before or after symptom development when compared to the controls.

Table 1. Ramularia leaf spot severity (%) and net CO_2 flux (µmol CO_2 m^{-2} s^{-1}) with days after inoculation and measurement irradiance (µmol PAR m^{-2} s^{-1}) for plants inoculated with *R collo-cygni* (ramularia) and their PDB and water controls.

		Ramularia		PDB control		Water control		P values
Days after inoculation	PAR	CO_2 Flux	Severity	CO_2 Flux	Severity	CO_2 Flux	Severity	CO_2 Flux
6	0	-1.4	0.0	-1.8	0.0	-1.2	0.0	0.68
6	230	11.0		9.7		11.0		0.24
6	1250	16.6		15.0		17.0		0.22
16	0	-1.7	0.8	-1.0	0.0	-0.7	0.0	0.30
16	230	8.6		9.5		9.8		0.59
16	1250	12.0		13.5		13.7		0.51
27	0	-0.8	1.4	-0.9	0.0	-1.0	0.0	0.51
27	230	4.4		4.8		5.2		0.54
27	1250	5.7		6.2		6.5		0.75

Table 2. Operating efficiency of PSII photochemistry (Φ PSII) with days after inoculation and measurement irradiance (μmol PAR m^{-2} s^{-1}) for plants inoculated with *R collo-cygni* (ramularia) and their PDB and water controls.

Days after inoculation	PAR	ϕPSII			P values
		Ramularia	PDB	Water	
6	230	0.56	0.56	0.58	0.48
6	1250	0.19	0.18	0.19	0.83
16	230	0.50	0.52	0.50	0.79
16	1250	0.13	0.14	0.14	0.70
27	230	0.34	0.35	0.36	0.86
27	1250	0.07	0.07	0.07	0.92

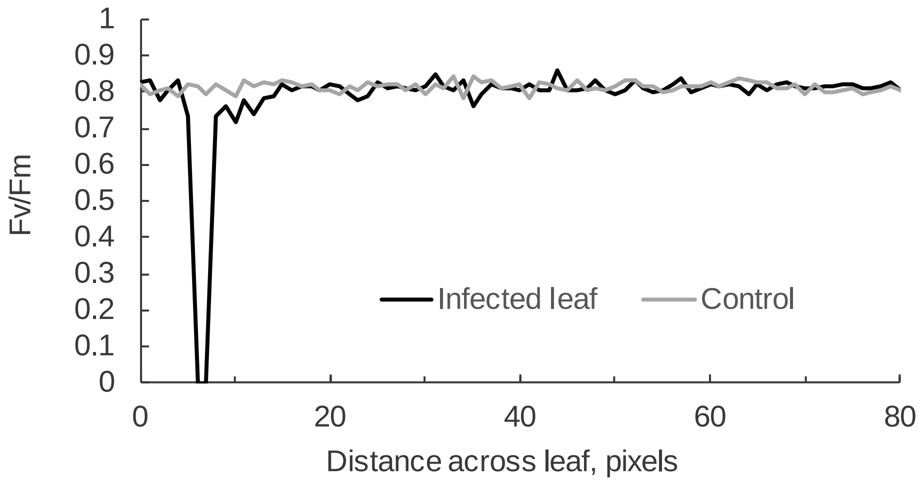

Figure 1. Maximum efficiency of PSII photochemistry (Fv/Fm) of dark-adapted leaves measured in transects across infected and control leaves. A large reduction is seen at the site of a mature lesion in infected leaves, but no difference between non-symptom expressing areas of the infected leaf and controls.

Measurements of the maximum efficiency of PSII photochemistry (Fv/Fm) taken from transects across chlorophyll fluorescence images showed that photosynthetic capacity was inhibited within visible lesions, but not in asymptomatic regions of infected leaves.

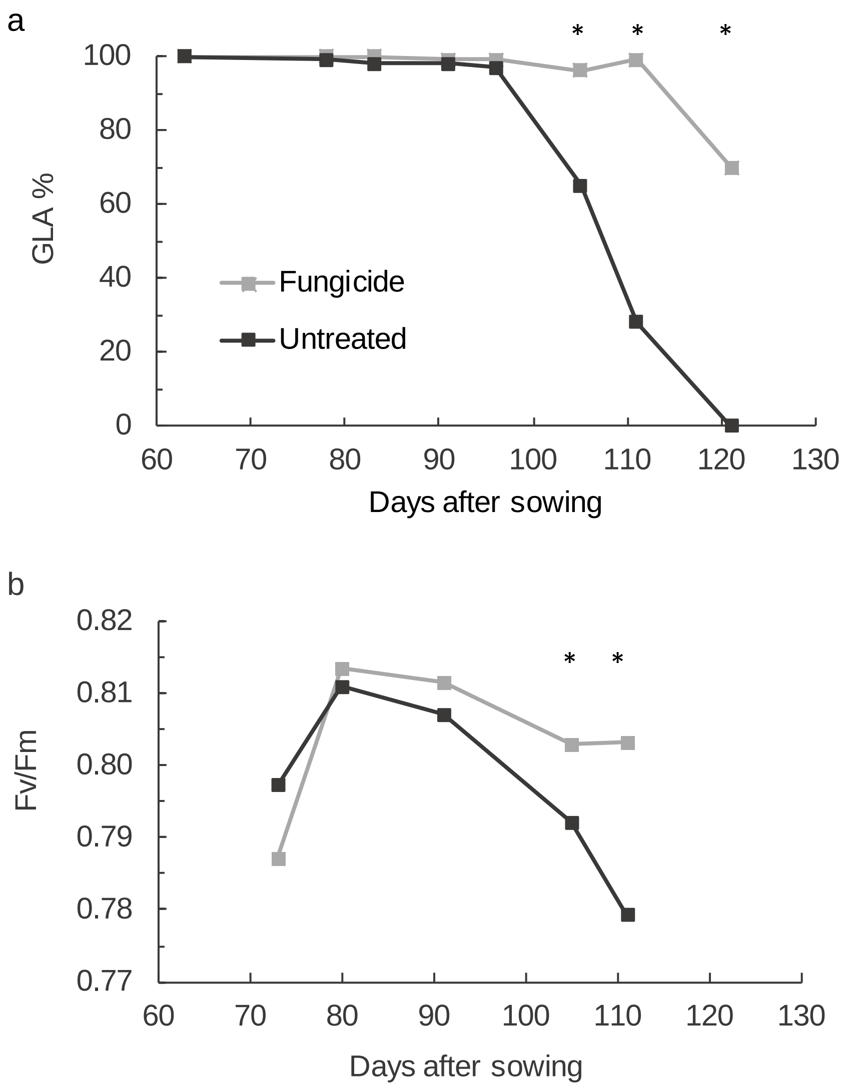

Figure 2. a) Green leaf area % and b) maximum photochemical efficiency of PSII (Fv/Fm) measured on leaf 2 (leaf below flag leaf) of untreated plots infected with *R collo-cygni* and plots treated with fungicide.
* indicates significant difference between fungicide-treated and untreated leaves at $P<0.05$.

In the field experiment, ramularia leaf spot was the only disease of significance that developed on untreated plots. Symptoms developed between 96 and 105 days after sowing leading to the initial loss of green leaf area (Fig 2). Fungicide treatment gave effective control of the disease. The maximum photochemical efficiency (Fv/Fm) was reduced in leaves of diseased compared to treated plants, but only after visible symptoms developed. Grain yield of untreated and treated plots was predicted from measured values of post-anthesis healthy area light interception and fixed common values of radiation use efficiency (RUE) and utilisation of soluble sugar reserves. The predicted yield increase in fungicide treated plots relative to untreated controls was comparable to the measured yield response (1.8 t ha^{-1}). This suggests that the yield response can be explained by an increase in light interception following treatment without needing to invoke effects of disease, or their control by fungicide, on RUE and utilization of storage reserves in grain filling.

DISCUSSION

There was no evidence from the current experiments that photosynthetic activity was reduced in inoculated leaves during the asymptomatic phase of infection compared to that of non-infected leaves. Even after symptoms developed in controlled environment experiments, rates of net CO_2 fixation and the photochemical efficiency were not reduced when averaged over the measurement area. The apparent lack of effect of disease in the presence of visible symptoms is likely to be the result of the low disease severities observed because there was clear evidence from chlorophyll fluorescence images of a reduction in activity within necrotic lesions. These results suggest that for there to be a significant effect of disease on whole leaf photosynthetic activity, visible symptoms must develop into mature lesions and to collectively form sufficiently high severities. Results from field experiments are consistent with this conclusion. The yield response to fungicide could be explained through its effects on healthy tissue area and enhanced PAR interception during grain filling. These effects are likely to be associated with both the prevention of the formation of necrotic lesions and physiological effects of fungicide on green leaf area (Bingham *et al*., 2019). Our results suggest that the prevention of symptom development is the key to managing ramularia leaf spot in barley.

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ROLE OF DEMONSTRATION FARMS IN ACCELERATING POSITIVE CHANGE ON FARM

C Hodge and E Pope
Agriculture and Horticulture Development Board, Stoneleigh Park, Kenilworth, CV8 2TL
E-mail: Claire.Hodge@ahdb.org.uk

Summary: The integration of research and practice is driving forward agricultural Knowledge Exchange (KE). Academic researchers in isolation no longer create improvements in agricultural management practices, but instead the creation and effective implementation of new techniques is an iterative process between multiple stakeholders. Demonstration farms accelerate positive change on farm by facilitating social interactions to increase learning and uptake of changes in behaviour.

The Agriculture and Horticulture Development Board (AHDB) Cereals & Oilseeds KE Monitor Farm Programme brings together groups of like-minded arable farmers to share critical performance data and develop and assess strategies for sustainable business improvement. The role of AHDB as an independent organisation is to provide a unique programme which farmers own and operate to draw on local experience and collaboration to improve farm production and profits. The programme engages with a variety of stakeholders across the agricultural sector, to integrate research and practice, and encourage and facilitate business improvement and the adoption of new technology, practices and innovations.

In 2019, 98% of respondents agreed that the Monitor Farm meetings had been a good use of their time, and 73% of them agreed that it had improved their business decision making. 92% of respondents agreed that they valued the opportunity to share experience and openly discuss issues in a non-commercial environment. 90% of respondents agreed that the meeting topics have been relevant to their business and 77% of respondents agreed that the meetings have helped them identify ways to improve their business. 85% of respondents agreed that the project has improved their technical knowledge.

INTRODUCTION

Knowledge exchange (KE) is an established process which occurs during the meeting of two or more parties (Jacobs 2013). It is defined as “the iterative cycle of sharing ideas, research results, expertise or skills between interested parties that enables the creation, transfer, adoption and exploitation of new knowledge in order to develop new products, processes or services and influence public policy” (Lockett et al. 2008).

Traditionally, the producers of knowledge have been universities (Jacobs 2013) but this assumption is changing; knowledge is produced collaboratively by a variety of stakeholders (Fazey et al. 2014) whereby each individual involved in the process has their own viewpoints, ideas, and motivations (Contandriopoulos et al. 2010). Knowledge exchange, and the use of knowledge, is influenced by its relevance, credibility and accessibility (Contandriopoulos et al. 2010) and therefore researchers are increasingly required to demonstrate economic and social impacts, increase knowledge sharing and ensure that research delivers relevant, valid and practice solutions (Fazey et al. 2014). Bruce (2016) identified the importance of effective KE for the benefits of the participants, in this instance farmers and agronomists, but also to provide feedback to the research community.

There is, and always will be, a need for explicit knowledge which is based on "facts, observations, classification, measurement and cataloguing in addition to principles, rules and ideas of science and technology" (Klerkx and Proctor 2013). In health care, for example, KE is underpinned by a scientific evidence base (Contandriopoulos et al. 2010). Alternatively, tacit knowledge is based on the implementation of skills at a practical level (Klerkx and Proctor 2013). In order to do this, improved access to information is needed through farmer-to-farmer networks in addition to technology platforms and tools to complement these meetings (Bruce 2016). The Beef and Lamb New Zealand's Monitor Farm Programme has been helping farmers, supported by industry experts, to improve their businesses (Beef and Lamb New Zealand 2016).

Rural and agricultural extension services which are commonplace across the world focus on the transfer of knowledge from researchers to farmers. The aim of these services is to support the education and development of farmer's decision making and the uptake new techniques and technologies to overcome challenges, increase efficiency, productivity and agricultural development. Bowyer (2015) reported that knowledge transfer within agriculture does not take into account the complexities of influencing behaviour change and often focuses on the "specific problem to which a solution is needed".

Effective knowledge exchange is complicated, and the multi-actor interactions require facilitation (Jacobs 2013). Within a KE network there will be actors of perceived differential status which facilitators need to recognise and manage to avoid bias in the interaction of group members (Thomas-Hunt et al. 2003). Greater information sharing occurs between parties where pre-existing relationships, and therefore trust, already exists and is reinforced in a cyclical process of continued communication (Gruenfeld et al. 1996; Contandriopoulos et al. 2010). There is an increasing contribution from rural social scientists (Blackstock et al. 2010) within the agricultural industry where the role of social interactions to increase learning and uptake of changes in behaviour have been reported (Dwyer et al. 2017).

The evaluation of KE, to determine its "value, significance, worth or condition" (Chapman et al. 2007) is lacking but is increasingly becoming a focus of funding bodies (Jacobs 2013). The uptake of research is complex, as it can take place and form over a range of temporal and spatial scales (Beyer and Trice 1982). Measurable changes in participants are therefore necessary to evaluate KE strategies (Jacobs 2013), involving quantitative assessment of repeated behaviour, which can be a direct change in action or a confirmation of existing practice (Contandriopoulos et al. 2010). Beyer and Trice (1982) reported on methods of data collection which included participant observation, case studies, interviews and surveys.

The Monitor Farm Programme

The Agriculture and Horticulture Development Board (AHDB) is a UK statutory levy board, established in 2008 from dissolved predecessor levy boards. The AHDB is funded by farmers, growers and others in the supply chain, and is independent of both commercial industry and of Government. The purpose of AHDB is to "*equip levy payers with independent, evidence-based information and tools to grow, become more competitive and sustainable*" achieved by delivering extensive research and development programmes (AHDB 2016a). The AHDB covers six sectors, namely: Pork, Dairy, Beef & Lamb, Horticulture, Cereals & Oilseeds, and Potatoes.

The AHDB Cereals & Oilseeds research and knowledge exchange strategy was formulated following analysis and consultation, and will run until 2020 (AHDB 2015). The top 5 business challenges in the strategy are: cost/price pressures; pesticide availability; weed management; weather volatility; and legislative impacts. In accordance with the strategy, levy funds will be spent on:

1. Informing on-farm activities to increase productivity
2. Improving business opportunities through understanding product quality and making the most of market potential
3. Preparing the industry by assessing future challenges and conducting activity in response to these challenges.

The AHDB Cereals & Oilseeds knowledge exchange programme on which this report is based, is known as the Monitor Farm programme (AHDB 2016b) and was initiated in the UK in Scotland in May 2011. Currently, nine monitor farms exist in Scotland funded by £1.25 million secured from the Scottish Government and European Union's Knowledge Transfer and Innovation Fund. Quality Meat Scotland (QMS) and AHDB Cereals & Oilseeds run the project in collaboration between 2016 and 2020. In England & Wales the Monitor Farm programme operates on an annual phasing where new farms are launched each year for a term of three years, as shown in Table 1. The distribution of Monitor Farms is shown in Figure 1.

Table 1. England and Wales Monitor Farm phases, dates and number of farms launched between 2014 and 2019

Phase	Duration of programme	Number of farms
1	April 2014 – March 2017	8
2	October 2014 – September 2017	7
3	April 2015 – March 2018	8
4	April 2017 – March 2020	6
5	April 2018 – March 2021	6
6	June 2019 – June 2022	7
	Total	**42**

Figure 1. Distribution of host farms in the Monitor Farm programme launched between 2015 and 2019.

The Monitor Farm programme brings together groups of like-minded farmers to share, discuss and evaluate critical performance information to encourage and facilitate business improvement. The farmers direct the content of the programme, resulting in issues and solutions being relevant to the local area, and has demonstrated the importance of integrating research and practice to best drive sustainable business improvement activities. The engagement of stakeholders across the agricultural industry including those involved in research, development, and implementation, ensures that farmers are challenged and supported to learn and interact. Key messages and technical communications are transferred to the wider agricultural industry through effective KT which utilises a variety of written, verbal and social media channels.

The integration of research through the participatory model that has been implemented has generated similar initiatives, known as Strategic Farms, in other crop and livestock sectors. These provide a platform for the demonstration of research for more technically produced and high value specialist crops. The evaluation of KE impact allows farmers to measure their own business improvement, as well as the assessment and continual development of the overall programme.

Measuring impact

A paper-based and online survey is conducted every year. In total, 1233 survey responses were received between 2015 and 2019. The number of respondents per year is shown in Figure 2.

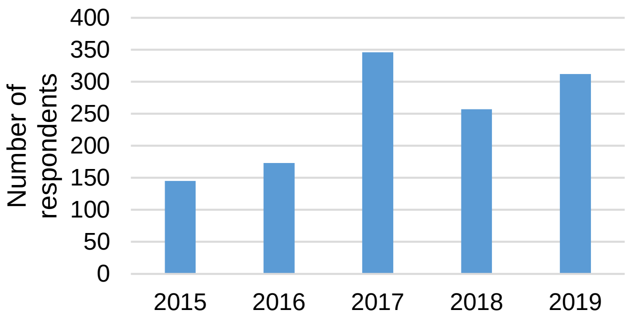

Figure 2. Number of respondents to the annual Monitor Farm survey between 2015 and 2019.

The survey determined the participant's level of involvement in the programme, duration of farming experience and current farming system. A series of three-level Likert Scale questions were asked to determine opinion on the impact of the Monitor Farm programme on time use, business decision making, sharing experiences, business relevance and improvements, and technical knowledge. Respondents were asked to identify the most important aspects of the programme and changes to their business management. The AHDB operates across six sectors, with separate but collaborative research and knowledge exchange teams. Respondents were therefore asked about changes to their current and future involvement and opinion of AHDB. Data were collected from the surveys and combined. Written responses were analysed by identifying the most frequently occurring words.

RESULTS & DISCUSSION

A series of three-level Likert Scale questions (agree, neither agree nor disagree, disagree) were asked to determine opinion on the impact of the Monitor Farm programme (Figure 3).

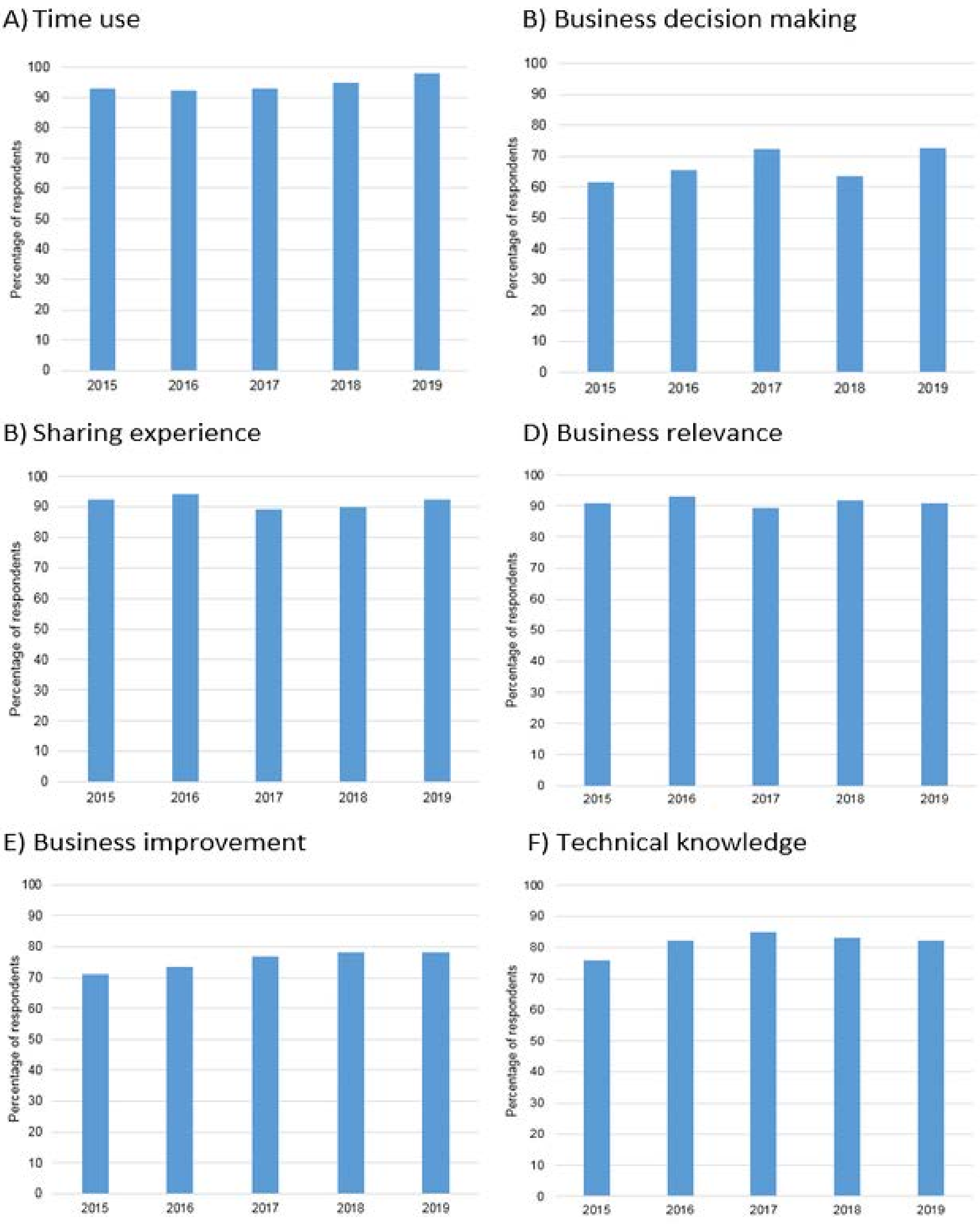

Figure 3. The impact of the Monitor Farm programme, 2015-2019. Percentage of respondents who agree that: A) **a**ttending the Monitor Farm meetings has been a good use of my time; B) the project has improved their business decision making; C) they value the opportunity to share experience and openly discuss issues in a non-commercial environment; D) the meeting topics have been business relevant; E) meetings have helped them identify ways to improve my business; F) the project has improved my technical knowledge

In 2019, 98% of respondents agreed that the Monitor Farm meetings had been a good use of their time, and 73% of them agreed that it had improved their business decision making. 92% of respondents agreed that they valued the opportunity to share experience and openly discuss issues in a non-commercial environment. Similarly, Bowyer (2015) reported on knowledge transfer in UK agriculture, and stated that farmers favour peer-to-peer learning in an independent setting, rather than where it is associated with a commercial company and thus an invested interest in sales. Some farmers, identified as managers, did value the input of commercial organisations (Bowyer 2015) which highlights the importance of meeting the multiple interests and requirements of different actors in a KE environment. The interaction of different actors in the Monitor Farm programme is valuable for access to multiple viewpoints and experiences, but requires facilitation to ensure that the information is delivered in an appropriate format, as the way in which experts perceive the appropriate presentation of information does not always align with that of the farmers (Franz et al. 2009).

Ninety percent of respondents agreed that the meeting topics have been relevant to their business and 77% of respondents agreed that the meetings have helped them identify ways to improve their business. 85% of respondents agreed that the project has improved their technical knowledge.

Respondents were asked to identify the three most important aspects of the programme from a pre-determined list (Table 2). In 2019, the top three most important aspects were farmer led agenda, locally relevant and independent.

Table 2. The most important aspects of the programme.

	2015	**2016**	**2017**	**2018**	**2019**
1	Farmer-led agenda	Farmer-led agenda	Farmer-led agenda	Farmer-led agenda	Farmer-led agenda
2	Locally relevant	Locally relevant	Locally relevant	Independent	Locally relevant
3	Opportunity to share experience with other farmers	Independent	Independent	Locally relevant	Independent

Figure 4 shows that in 2015 68% of respondents were involved with AHDB Cereals & Oilseeds before attending their first Monitor Farm meeting. In 2019, 60% of respondents were involved with AHDB Cereals & Oilseeds before attending their first Monitor Farm meeting. Bowyer (2015) reported on knowledge transfer in UK agriculture and reported that farmers felt that levy board information, although good was not widely used. The second group, defined as larger operators or managers, did consider the levy board as a key players in knowledge transfer. Similar to the farmers, however, many growers and managers were not making use of levy board information. The result presented here demonstrate the value of the Monitor Farm programme in reaching members of the industry who are not already interacting with AHDB.

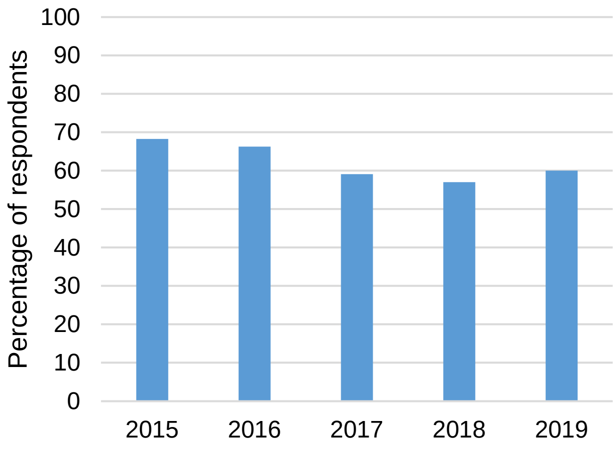

Figure 4. Involvement with AHDB Cereals & Oilseeds before attending first Monitor Farm meeting.

Respondents were asked to identify any changes that they have made as a result of attending the Monitor Farm meetings. The most commonly occurring words were determined by frequency of words used in the responses, with Cropping (43%), Cost (35%), Look (29%), Soil (26%), Cover crops (24%) and Benchmarks (18%) being the top six cited changes.

In 2019, 80% of respondents felt the meetings had improved their opinion of Cereals & Oilseeds, with 86% of them agreeing that it had improved their knowledge of how AHDB spends the levy. This could result in improved use of levy board information within these groups but also within the wider agricultural community.

CONCLUSIONS

A key learning outcome of the Monitor Farm programme thus far is that meetings with a farmer led agenda, that are independent and locally relevant are a good use of time and improve business decision making. Farmers, agronomists, traders and many stakeholders in the UK agricultural landscape value the opportunity to share and discuss experiences to identify business improvement opportunities and practices and improve technical knowledge. Individual changes to businesses may not be quantifiable in terms of cost benefit through this survey but they can be identified across a range of business aspects including soil management, crop protection, marketing, and financial and cost management.

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WHERE DO SOCIAL-MEDIA USERS GET THEIR PLANT HEALTH INFORMATION? RESULTS FROM THE TWITTERSPHERE

J E Meador and H Creissen
SRUC W Mains Rd, Edinburgh EH9 3JG
E-mail: elliot.meador@sruc.ac.uk

Summary: Social media is widely becoming a platform for farmers and plant health stakeholders to share and obtain information about plants. This may likely increase in the future as more and more stakeholders become comfortable with using social media as a part of their everyday lives. This research uses data collected from plant stakeholders on Twitter based primarily in Scotland between 1 January 2019 and 1 January 2020. This analysis reveals that many stakeholders are actively engaging in discussions around plant health, including around new pathogens and best practices in plant health. However, most discussions occur in tightly clustered networks. Active engagement with key stakeholders in plant health (farmers, gardeners and media) may be an effective approach at ensure accurate information is reaching those who need it *(see Figure 4)*.

INTRODUCTION

Active stakeholder engagement is a critical component of research into plant health in the UK (Osterrieder, 2013). Stakeholder and the wider public's perception of plant health issues does not always align with the scientific community's. This misalignment can prove disastrous if the public reaction to disease and plant health is based on inaccurate information.

In order to better understand and map stakeholder and public understanding of plant health issues, project team members at SRUC have been mining social media data from plant health stakeholders in the UK since 1 January 2019.

MATERIALS AND METHODS

Data is retrieved daily from Twitter's application programming interfaces (API) using a method called *network jumping*, which was developed specifically to identify stakeholders in the plant health system in Scotland. Network jumping works by first identifying trusted and key stakeholders on a given topic. These users' tweets are pulled, and we then identify the users who are captured within each tweet (called *mentioning*). Finally, we then pull in those new users' tweets into the database. This process is repeated every day. The starter-stakeholders used in this study are:

- @FASSCot - Official Twitter handle of ***Scotland's Farm Advisory Service***
- @ScotGovSASA - Official Twitter handle of ***SASA (Science & Advice for Scottish Agriculture)***
- @PlantHealthScot - Official Twitter handle of ***Scotland's Plant Health Centre***
- @NFUStweets - Official Twitter handle of ***NFU Scotland (National Farmers Union)***

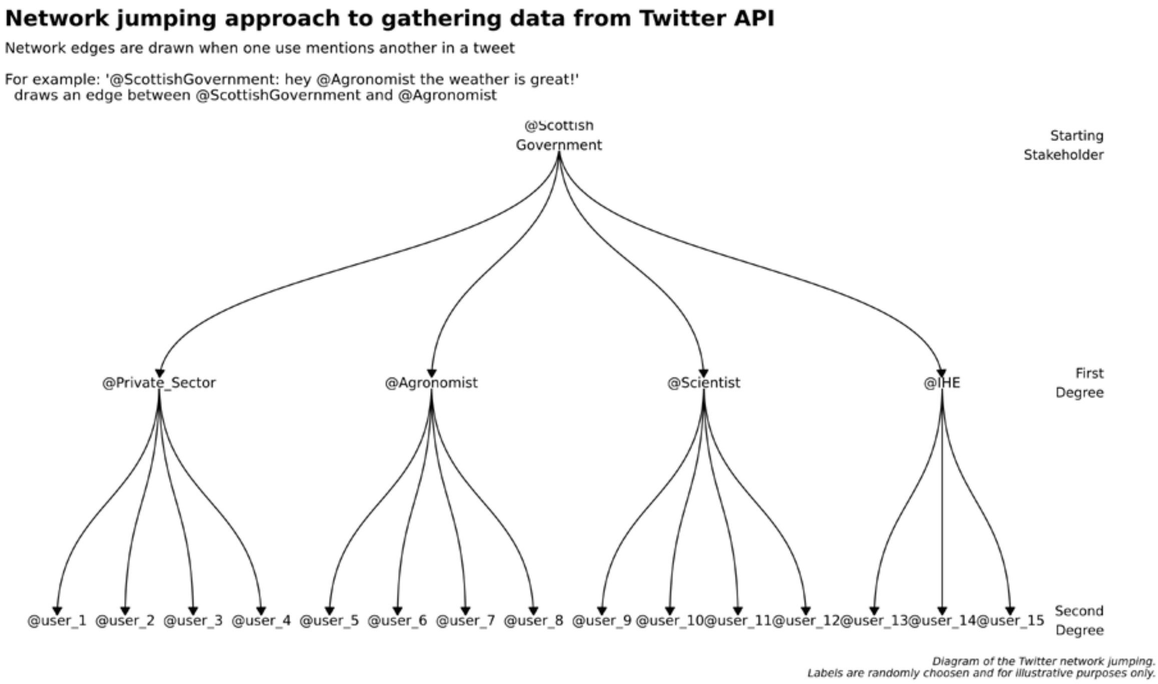

Figure 1. Overview of network jumping approach

A conceptual illustration of the data collection method used in this study is shown in Figure 1. The database is constructed as a social network, which allows us to identify key areas of interest within conversations occurring between stakeholders. Farmers and scientists associated with agriculture were the most prominent users discussing plant health related issues. Currently, the database has 453,450 tweets, which contain:

- information on the topic in the form of 280-character micro-blogs;
- images and photographs shared by users (there are 8,782);
- 882 diffent *url's* containing shared reports and websites pertaining to plant health stakeholders;
- 15,290 different hashtags.

Categorising users and messages

Data collected from Twitter's API often includes biographical information that is submitted by the user. Users also have the option to allow Twitter access to their location, though this must be opted into by the user (it rarely is). We can use these data to help categorise users based on several criteria including general location, key messages contained in text data and conversations between users.

Each tweet that connects two users is pulled along with characteristics about shared media (urls, images and video). This data can be analysed using text and sentiment analysis. One approach to quickly analyse what users are discussing is counting words. Key phrases around plant pests were collected from the Scottish Government and DEFRA websites. These words are extracted from the text data and analysed for content.

RESULTS

The following paragraphs present results from analysis on user categorisation, frequency of key-term use and network analysis on discussions containing key terms.

User-location

Only 2.79% of users have enabled Twitter to publish their location. Figure 2 illustrates the number of users from each country. The vast majority of users (>10,000) are from the UK. Most of the users from outside of the UK are located in Europe. There are 129 represented in the database.

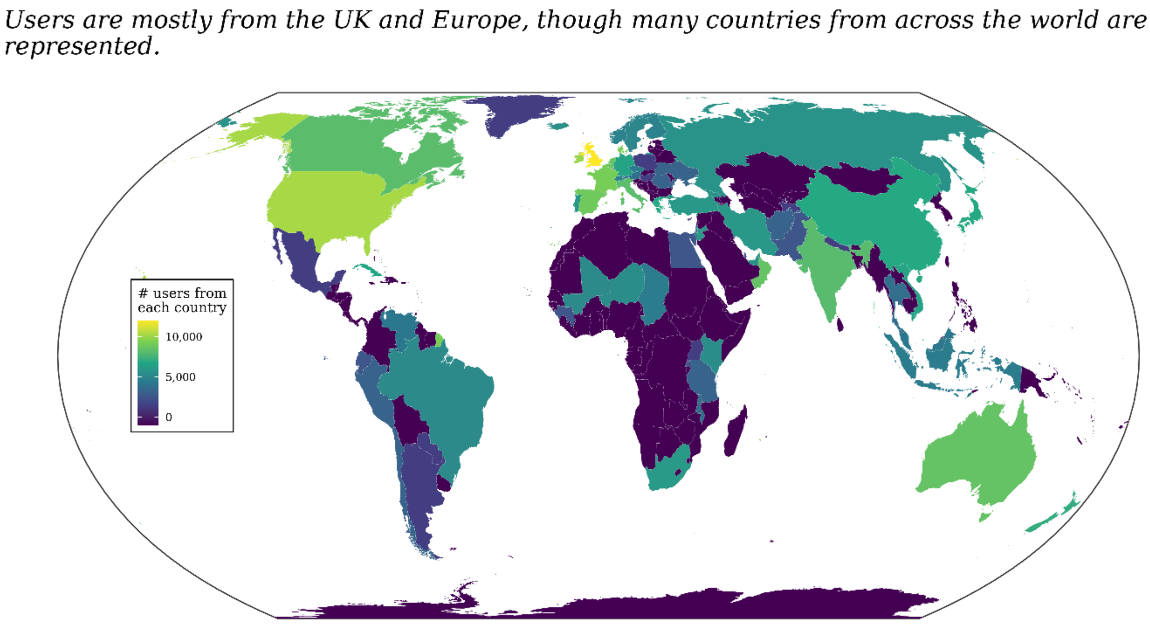

Figure 2. User country location

Relative frequency of key terms

Each tweet that connects two users is pulled along with characteristics about shared media (urls, images and video). This data can be analysed using the text and sentiment analysis. One approach to quickly analysis what users are discussing is counting words. Key phrases around plant pests were collected from the Scottish Government and DEFRA. The term *xylella* appears the most by far, and it is followed by the generic terms *fly*, *bug*, *aphid* and *nematode*. *Ashdieback* and *dieback* are also mentioned frequently. This would suggest that these pests are quite concerning for users in the database.

Terms related to plant pests are gathered from two general sources: Scottish Government and DEFRA. The Scottish Government's Plant pests and disease guide website (https://www.gov.scot/publications/plant-pests-and-disease-guide/pages/overview/) identified six search terms related to plant pests. DEFRA's website on *notifiable* pests from *its pest and disease factsheets* was scraped and key terms were aggregated into a list. In total 44 individual terms were gathered and used to search within tweets. DEFRA's website can be found at https://planthealthportal.defra.gov.uk/pests-and-diseases/pest-and-disease-factsheets/notifiable-pests/

One drawback to using these search terms is that they are for the most part scientific terms, and these may not be the ideal search terms as users may choose to use a colloquial term in online discussions.

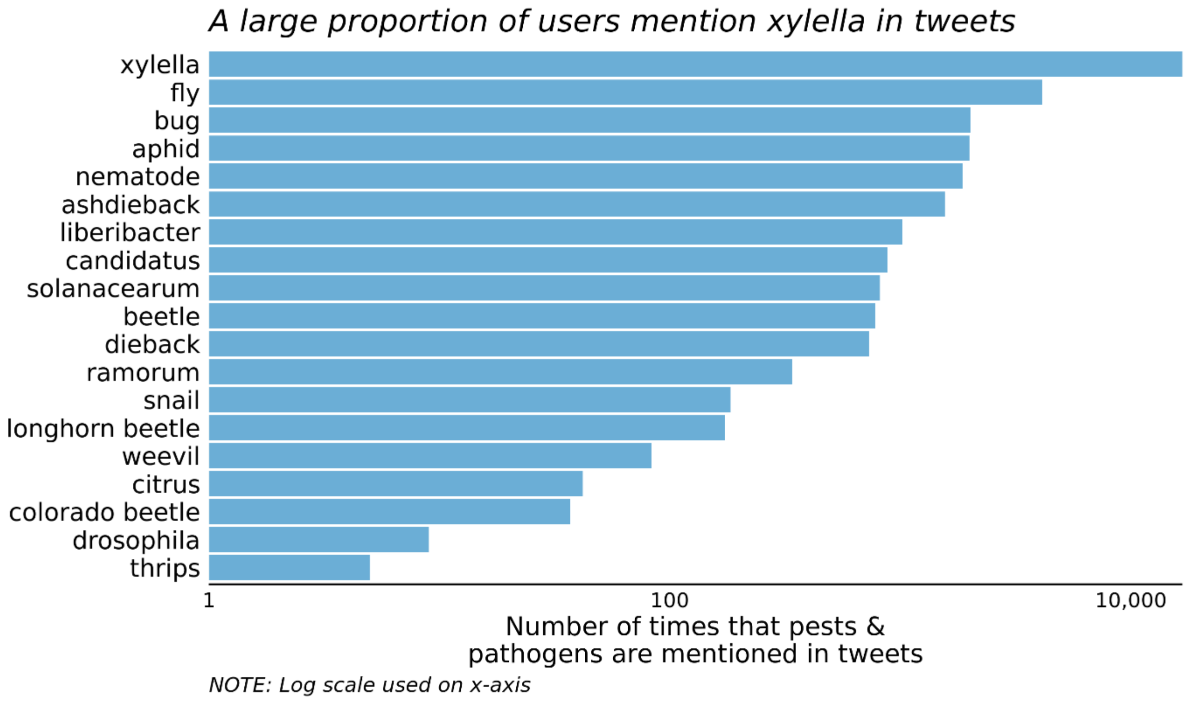

Figure 3. Frequency of key terms

Proportion of discussions of key terms

An important component of understanding how accurate information is conveyed in social media is the proportion of conversations that mention key terms. Understandably, not all online discussions between users will be on the same topic. Users who engage one another a variety of users on a topic will have a different impact than those who engage with the same user over and over again. Figure 4 illustrates the proportion of all discussions between two users that include a key term.

In Figure 4 each node represents a user that has engaged in discussion that mentions *at least* one key word having to do with plant pests. Some node pairings have many discussions about plant pests. The edge colour represents this, with darker green edges indicating much discussion around plant pests. Likewise, some users discuss plant pests more than others entirely. This is represented in node colour, with darker green nodes devoting more of their online discussions to plant pests. There appears to be a very high concentration of users discussing plant pests together, surrounded by more casual discussions.

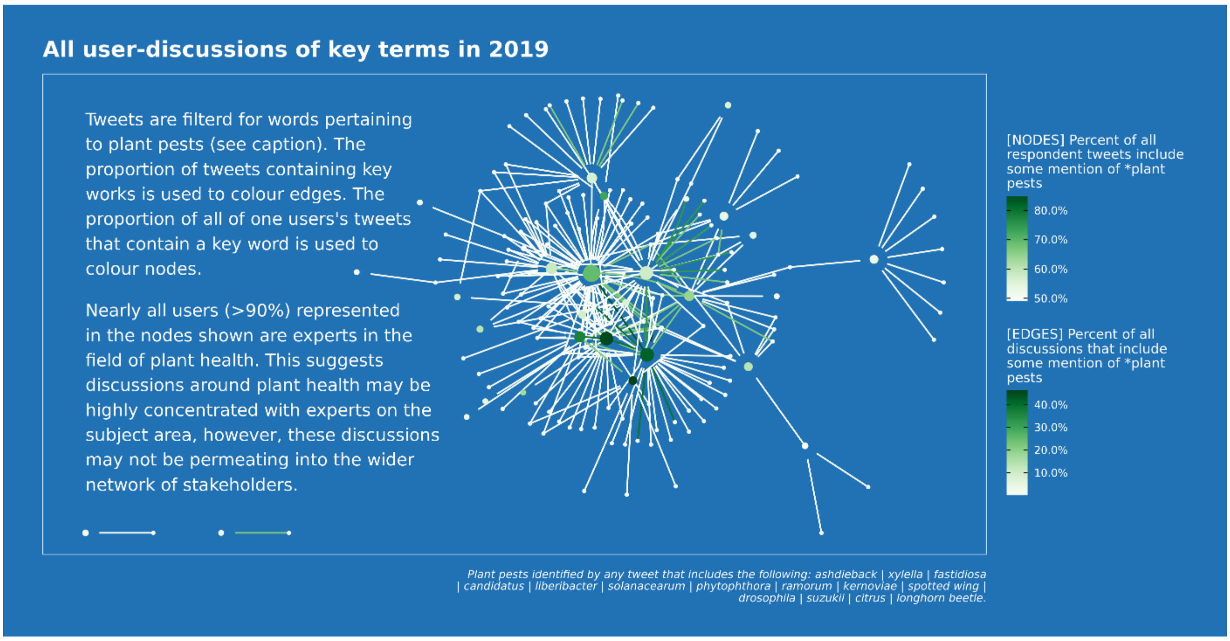

Figure 4. Proportion of all discussions of key terms

DISCUSSION

Key Take away

Just under a thousand (n = 968) discussions are identified that include one of the key terms. This accounts for about 0.21% of all tweets captured in the database. It is difficult to gauge the impact of these discussions as there is an unknown number of users who might have witnessed these discussions in their own timeline. Twitter's algorithms decide who sees what, as not all users will view *all* discussions of users they follow.

In general, online discussions of information that is important to plant health appear to be happening amongst a knowledgeable group of experts (indicated by dark green nodes). Many stakeholders receive this information peripherally, that is, they may passively view other users' discussions on the subject. However, close inspection reveals that key stakeholders are for the most part having discussions with one another or having discussions with similar the same users. This is more easily seen in Figure 5, which shows the core network from Figure 4.

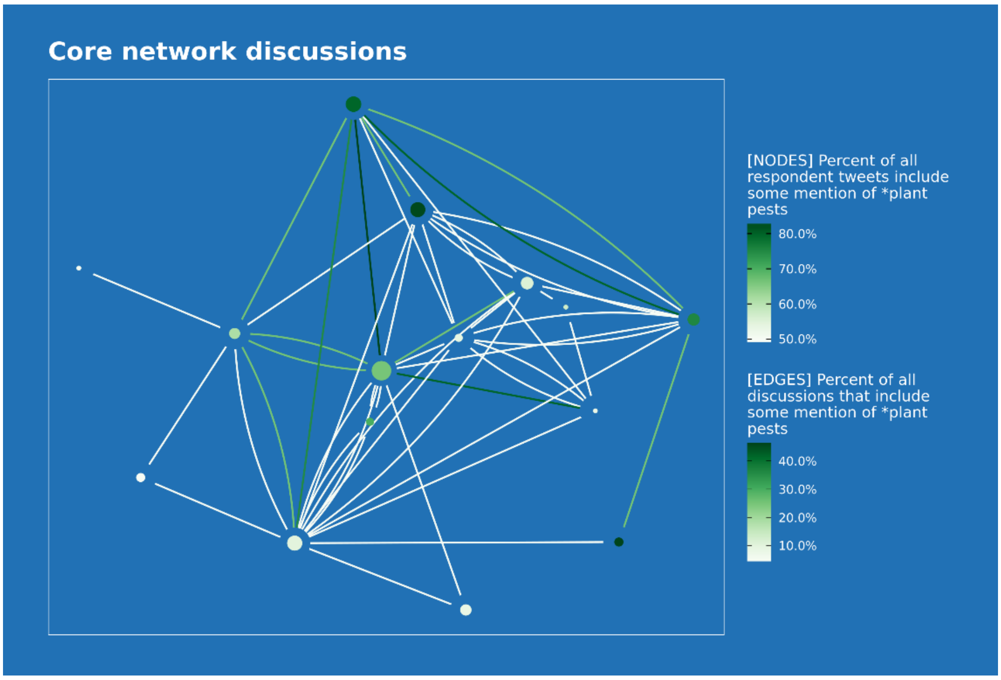

Figure 5. Zoomed image of core discussions

Targeted engagement with a more diverse group of stakeholders online should help spread information more quickly and will increase the number of users who view it by several orders of magnitude. Potential stakeholders are those with a large following and who have earned the trust of their stakeholder groups. More research is needed to identify those users.

Experts in plant health should begin having meaningful discussions with users who they might not normally engage with. Meaningful engagement is characterised by having discussions that promote the building of reciprocal trust between experts and users. This is often referred to as social capital (Lyon, 2000) in literature on peer-to-peer relationships in agricultural systems. The development of high levels of social capital has been shown to positively influence the way in which information diffuses in agricultural systems where people interact person-to-person (Meador & O'Brien, 2019). Whilst not yet researched empirically, it is not too much of a stretch to imagine that a similar approach would work online. Therefore, we recommend that experts in the field of plant health in Northern Britain work to create strong relationships with users outside of their field of expertise and who might identify as farmers, gardeners, sellers or other plant-user.

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FENPICOXAMID PROVIDES A NEW TARGET SITE FOR CONTROL OF *ZYMOSEPTORIA TRITICI* (SEPTORIA TRITICI BLOTCH) IN WHEAT.

A Leader[1], G Kemmitt[2] and D Young[3]
[1]*Corteva agriscience, CPC2 Capital Park, Fulbourn, Cambridge, CB21 5XE*
[2]*Corteva agriscience, 3B Park Square, Milton Park, Abingdon, Oxfordshire, OX14 4RN*
[3]*Corteva agriscience,* 9330 Zionsville Rd, Indianapolis, IN 46268
E-mail: andy.leader@corteva.com

Summary: Future regulatory restrictions, resistance and erosion in efficacy of many active ingredients used today has created an urgent need for new modes of action for control of *Zymoseptoria tritici*. Fenpicoxamid (Inatreq™[1] active fungicide) belongs to a new chemical class of fungicide (picolinamide) and acts at the Qi (quinone inside) site of the cytochrome bc_1 complex in mitochondria which is a new target site in cereals. A pre-launch baseline established between 2011-2018 based on 3848 European isolates of *Z. tritici* found the MEC50 distribution pattern falls within the range <0.01 to 0.19 mg/L for 99.2% of the population and 67% of the samples are between 0.01 and 0.039 mg/L The sampling of 674 UK isolates mirrored the European baseline pattern. Although fenpicoxamid has a novel mode of action in cereals with no target site-based cross-resistance with current fungicides, a robust resistance management strategy will be critical to maintain long-term effectiveness of fenpicoxamid in the field.

INTRODUCTION

Controlling cereal diseases is essential for all arable growers to maintain yield, but this task is becoming more complex and will continue to be so throughout the next decade. *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 Europe (EU) (Fones et al, 2015). In the UK, yield losses of around 5 – 10% (ADHB, 2016) are typically seen in wheat varieties with a higher disease rating even when treated with fungicides. Effective control of Septoria tritici blotch will be especially challenging with several important fungicides (chlorothalonil, epoxiconazole, cyproconazole, propiconazole, tebuconazole amongst others) expected to be restricted or removed from the EU wheat grower's toolbox due to increased regulatory scrutiny. The loss of the multisite active ingredient chlorothalonil will be especially problematic as it is the foundation to many cereal disease programmes. 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 (Lorsbach and Sparks, 2016) for a new active ingredient to reach the market from time of initial discovery. In addition to widespread resistance to strobilurin fungicides, an erosion in efficacy of triazole fungicides, and increased detection of isolates with resistance to the more recently introduced succinate dehydrogenase inhibitor (SDHI) fungicides has created an urgent need for new modes of action for crop protection products and renewed management strategies

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

for disease control in cereal crops including use of resistant varieties and decision support systems.

Fenpicoxamid is derived from the natural product UK-2A, which is produced through fermentation of a *Streptomyces* species (Leader et al 2018). Fenpicoxamid 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 mitochondria at the Q_i site of the respiratory cyt bc_1 complex. Fenpicoxamid is classified in FRAC group C4#21. The Q_i ubiquinone binding site is distinct from the Q_o site targeted by the strobilurin class of fungicides (Young et al, 2018). There is no target site-based cross resistance between fenpicoxamid and strobilurin, triazole and SDHI fungicides as reported by Owen et al, 2017. Fenpicoxamid will be the first new target site to be introduced to the cereal fungicide market in nearly two decades and comes at a time when new fungicide solutions will be critical to manage *Z. tritici* resistance issues with current chemistries. Studies in the field and laboratory have shown favourable activity of fenpicoxamid against UK and Irish *Z. tritici* isolates which are resistant to other chemistries (Jackson et al 2018). However, implementation of a sound resistance management strategy will be critical to maintain long-term effectiveness of fenpicoxamid.

Work was initiated in 2011 to establish a pre-launch sensitivity baseline for fenpicoxamid which is required as a component of the EU registration dossier application as well as a reference point for future sensitivity monitoring post launch (2020 onwards). In addition, this is critically important work due to the fact fenpicoxamid is a single site inhibitor, and *Z. tritici* is considered a medium risk pathogen by FRAC. Baseline sensitivity testing of European *Z. tritici* isolates was conducted between 2011 and 2018 to characterize sensitivity of existing populations to fenpicoxamid. Exceptions were 2013 and 2015 when no testing was undertaken. In addition to establishing a baseline across Europe and the UK, focused sampling was also conducted at the Corteva research station near Warwick, UK where fenpicoxamid has been applied multiple times per season since 2011. This site is used to specifically test new fungicide candidate molecules and established cereal fungicides on highly susceptible varieties, often inoculated with *Z. tritici* isolates and irrigated to present optimal conditions for disease epidemics. The Wellesbourne site is an ideal site to observe any shifts in baseline where fenpicoxamid has been exposed for multiple years to challenging *Z. tritici* epidemics. In this paper we present baseline data for Europe and the UK and compare the UK baseline to the monitoring data for the Wellesbourne location over the past 8 years.

METHODS AND MATERIALS

Leaf samples were collected from a range of sites from thirteen European countries during the 2011-2018 seasons to obtain representative data from a pan European *Z. tritici* population. Twenty to thirty randomized leaf samples bearing typical *Z. tritici* lesions with active pycnidia were collected from each sampling site and shipped to EpiLogic, Germany. Each sample was used to generate 5 separate isolates by transferring cirrhi from single pycnidia onto yeast-malt agar (2 % agar, 1.5 % yeast extract, 1 % malt extract) plates. Plates were grown for 5 days at 18°C under continuous UV followed by a further multiplication step (same conditions) in order to yield enough spores for the bio-assay. The sensitivity of each isolate was determined using a fungicide/nutrient broth dilution assay in 96-well microtitre plates (Pijls et al.,1994). As test treatments, concentrations of 0.0, 0.0025, 0.010, 0.040, 0.156, 0.625, 2.500, 10 mg/L fenpicoxamid as GF-2810 (fenpicoxamid 133 g ai/L SC) were added to microtitre plates. Plates were incubated for six days (18C, darkness, 70 % RH), after which the adsorption/optical density of mycelial growth is measured automatically in a 96-well plate photometer (wavelength: λ = 405 nm). Dose-response of the test isolate at each fungicide concentration was scored relative to the respective untreated control. EC50 values were calculated from the scoring data of each isolate by Probit analysis (Weber, 1980). Five isolates were generated from each leaf sample, The

EC50 values generated were averaged using the geometric mean to give a MEC50 value for each sample. The MEC50 values for each sample were in turn then averaged to give an overall country MEC50. The MEC50 data was grouped in to frequency classes and is presented in Figure 1.

RESULTS

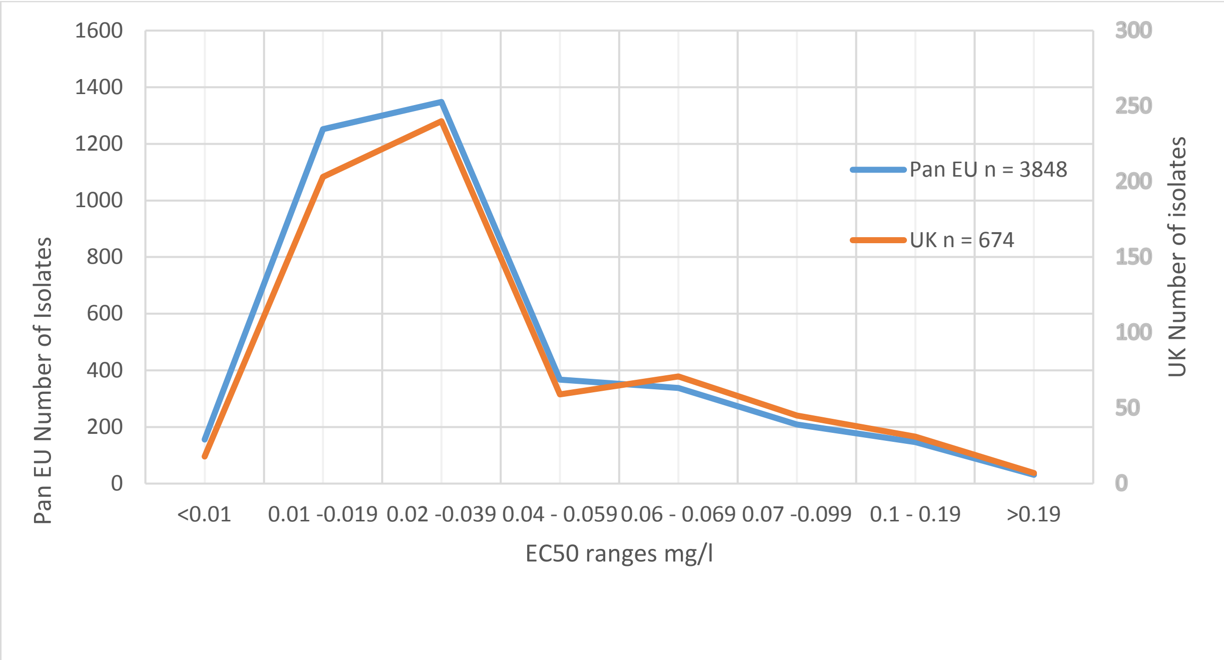

Figure 1. Fenpicoxamid *Z. tritici* sensitivity baselines, Pan EU and UK 2011-2018 (Corteva internal reports 2011-2018)

Table 1. The MEC50 (mg/L) values from 2011 to 2018 for UK and Wellesbourne

Year	Wellesbourne MEC50 (arithmetic)	UK MEC50 (arithmetic)
2011	0.025 (n=10)	0.032 (n=50)
2012	0.065 (n=5)	0.053 (n=67)
2014	0.032 (=10)	0.033 (n=77)
2016	0.013 (n=15)	0.032 (n=90)
2017	0.033 (n=32)	0.053 (n=154)
2018	0.05 (n=55)	0.042 (n=190)

The results in Figure 1 summaries MEC50 data (mg/L) grouped according to frequency classes taken from 3848 European samples and 674 UK samples between 2011 and 2018. Table 1 shows the MEC50 values for 674 *Z. tritici* samples taken from the UK between 2011 and 2018 and the MEC50 values for samples from the Wellesbourne site over the same period.

DISCUSSION

The MEC50 data (mg/L) between 2011 and 2018 when grouped according to frequency classes showed the distribution pattern from 3848 European samples was mirrored by the 674 UK samples. Looking across the MEC 50 values for a substantial sample size of 3848 EU isolates it is evident that 3817 isolates (99.19%) fall within the range <0.01 to 0.19 mg/L and 67% of the samples are between 0.01 and 0.039 mg/l. It is suggested that this range be used to set the upper limits of the baseline distribution with careful analysis of future post-launch monitoring program data to track any appearance of a significant frequency of isolates with EC50 values >0.2mg/L.

When MEC50 data (mg/L) from 674 UK samples is compared to 128 samples taken from the Wellesbourne site between 2011 and 2018, the values are similar for each year. No shift in sensitivity across the sample years was observed when fenpicoxamid was applied to the same location over 8 years, with multiple applications per year and subjected to high disease pressure. Fenpicoxamid offers an additional fungicide solution for cereal growers as a novel chemistry and a new target site, with no cross-resistance to existing modes of action . However, fenpicoxamid is a single target site inhibitor, and as such it will need to be used within the framework of an effective resistance risk management strategy. Fenpicoxamid should never be applied alone to ensure that it will remain effective long-term in the UK and resistance risk is minimized. Fenpicoxamid is recommended only for use in mixture with other effective modes of action against *Z. tritic*i at a robust dose and with a maximum of one application of fenpicoxamid-containing products per season.

ACKNOWLEDGEMENTS

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IMPACT OF ENHANCED FORMULATION TECHNOLOGY OF FENPICOXAMID i-Q4 ON SPRAY DEPOSITION AND FIELD EFFICACY FOR THE CONTROL OF *ZYMOSEPTORIA TRITICI* (WHEAT LEAF BLOTCH) WHEN APPLIED AT DIFFERENT WATER VOLUMES AND FORWARD SPEEDS

J Fraser[1], N Foster[2], D Hopkins[2], S Jackson[1], G Kemmitt[1] and A Leader[1]
[1]*Corteva agriscience, CPC2 Capital Park, Fulbourn, Cambridge, CB21 5XE*
[2]*Corteva agriscience, 67410 Drusenheim, France*
Email: John.Fraser@corteva.com

Summary: The biological activity of an active substance can be significantly influenced by the formulation. Advances in formulation were key to the development of fenpicoxamid (Inatreq[TM2] Active) as the first cereal fungicide to be developed from a new picolinamide class of chemistry assigned to FRAC group C4-21, (QiI, quinone inside inhibitor). It controls a range of cereal pathogens including *Zymoseptoria tritici, Puccinia striiformis* and *Puccinia triticina.*

Laboratory, glasshouse and field tests evaluated the impact of formulation on the efficacy of fenpicoxamid. Tests confirmed that fenpicoxamid formulated as an emulsifiable concentrate (EC) in the i-Q4 matrix significantly improved intrinsic characteristics of retention, coverage, penetration and uptake compared to a suspension concentrate formulation. These formulation characteristics of i-Q4 significantly enhanced efficacy of fenpicoxamid and studies clearly showed that nozzle type, forward speed or water volume did not impact spray deposition.

INTRODUCTION

The biological activity of a crop protection molecule can be influenced significantly by the formulation used to deliver the active substance to its target. Key factors determining the design of a formulation include the solubility characteristics of the active ingredient, cost of manufacture and the intended use (Kah *et al*., 2013). The efficacy of a crop protection product is generally a function of the intrinsic properties of the active substances such as their toxicity to the target, plant movement, penetration capacity and their mode of action (Wang *et al*., 2007)

Fenpicoxamid is a new active substance originally developed by Dow AgroSciences, now Corteva agriscience 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 nearly two decades and comes at a time when new fungicides solutions will be critical to manage *Z. tritici* resistance issues with current chemistries.

Early development work was carried out evaluating a suspension concentrate (SC) formulation that provided a high level of protectant control but with limited curative activity and pest spectrum. In 2014, a new formulation matrix was developed that overcame inherent challenges

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

faced with the molecule, namely molecular size and insolubility in water. This new i-Q4 formulation was based on a unique and patented emulsifiable concentrate (EC) matrix which was observed to behave in a different way to the standard SC or conventional EC formulations in terms of greatly enhanced curative activity, broadened pest spectrum, by offering improved retention, coverage, penetration and uptake on the cereal leaf.

This paper summarises laboratory, glasshouse and field testing undertaken between 2014 and 2019 that sought to understand how and why the improvements in formulation from the i-Q4 EC matrix were so profound.

MATERIALS AND METHODS

Trial design

Seven replicated field trials were conducted in winter wheat to GEP standards in in Maritime Europe in 2014 to evaluate the efficacy of fenpicoxamid formulations (GF-2925 130 g ai/L SC and GF-3311 66.7 g ai/L i-Q4 EC) 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 200 litres/ha. Each treatment was applied twice to the same plot at GS 31-32 of the crop, (leaf 3 emerged) and again at GS 37-39 of the crop, (leaf 1 just visible to leaf 1 unrolled) (Zadoks *et al*, 1974). Trials were placed on susceptible varieties in locations where *Z. tritici* is an issue.

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 2.

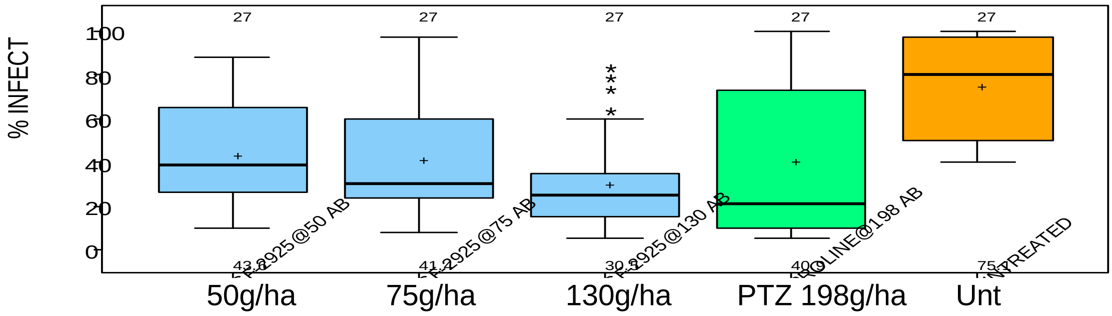

Figure 1. % control of *Z. tritici* on leaf 2 with fenpicoxamid (GF-2925) SC at 50, 75 and 130 g as/ha, 5-6 weeks after application in comparison to prothioconazole. 7 trials

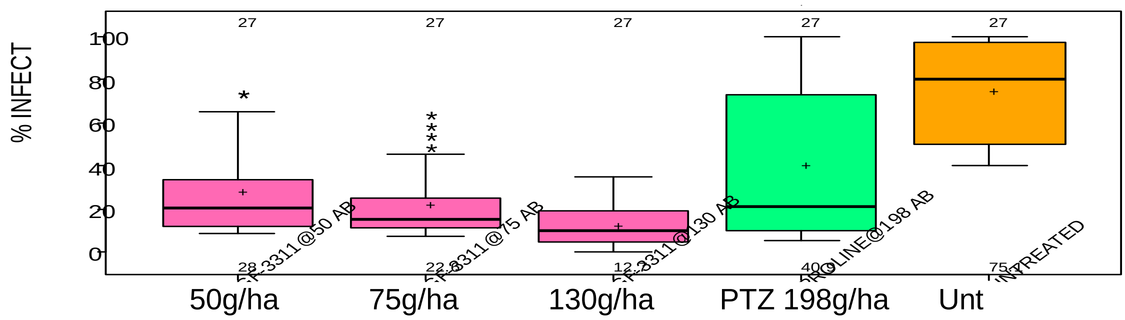

Figure 2. % control of *Z. tritici* on leaf 2 with fenpicoxamid (GF-3311) i-Q4 at 50, 75 and 130 g as/ha, 5-6 weeks after application in comparison to prothioconazole. 7 trials

These data from 2014 clearly showed that there was a positive impact of formulation between the original SC formulation of fenpicoxamid (coded GF-2925, 130 g as/L) and one of the early EC formulations of fenpicoxamid (coded GF-3311, 66.7 g as/L) utilising the i-Q4 matrix.

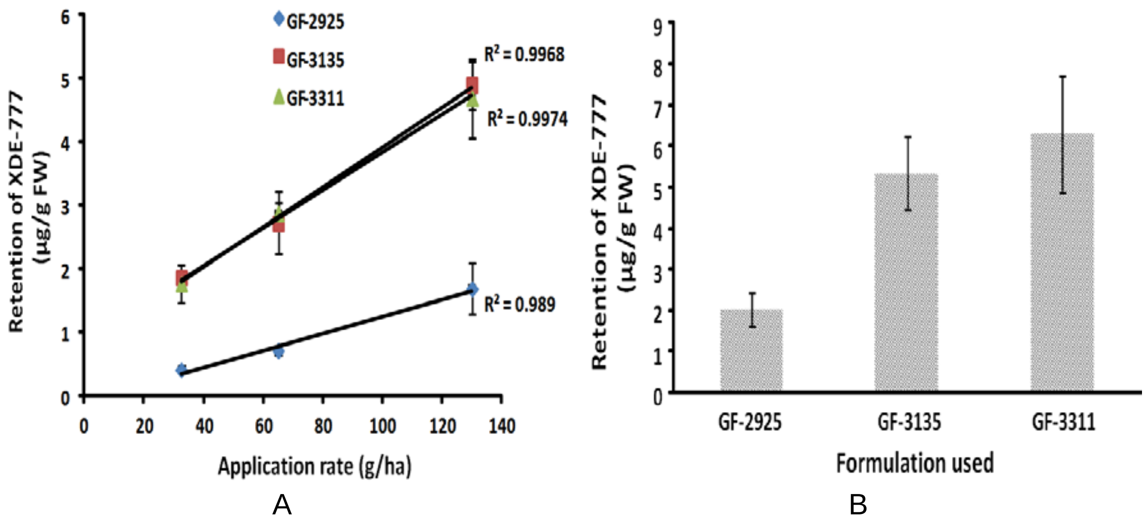

Figure 3. A) Laboratory testing evaluated retention of fenpicoxamid in three different formulations GF-2925, SC, GF-3135, (50 g as/L fenpicoxamid, EC i-Q4) and GF-3311, EC i-Q4) on wheat plants after spraying at three different rates (32.5, 65, and 130 g as/ha). Bars represent standard deviations of means of 4 replicates. B) Retention of fenpicoxamid in three different formulations (GF-2925, GF-3135, and GF-3311) on wheat plants after spray at a rate of 130 g/ha. Bars represent standard deviations of means of 12 replicates in three separate experiments.

It is likely that the immediate spreading of fenpicoxamid on the leaf surface in GF-3135 and GF-3311 (i-Q4 matrix) contributed to the higher retention as mentioned above. It can be reasonably assumed that fenpicoxamid in GF-3135 and GF-3311 immediately disperses when droplets contact wheat leaf surfaces, thus minimising 'bounce off' and increasing retention.

The MALDI (Matrix Assisted Laser Desorption Ionization) image in Figure 4 shows the intensity of signals corresponding to masses *m/z* 614.176 (615) and *m/z* 636.677 (637) of fenpicoxamid proton ($M + H^+$) and sodium ($M + Na^+$) adducts, respectively. The colour bar shows intensity of signal that indicates the relative abundance of the selected ions (0-100%).

Since more fenpicoxamid was retained on the leaf surface with i-Q4 based formulations (Figures 3A and 3B), it was questioned whether leaf surface coverage of fenpicoxamid i-Q4 could also be increased. MALDI imaging analysis showed intense signals of fenpicoxamid at *m/z* 614.176 ($M + H^+$) and 636.677 ($M + Na^+$) on the leaf treated with GF-3135 and GF-3311, while the signals of fenpicoxamid on the leaf treated with GF-2925 (SC) were much weaker. The fenpicoxamid signals at *m/z* 614.176 and 636.677 in the application of GF-3311 were more intense than the signals obtained from samples treated with GF-3135 (Figure 4).

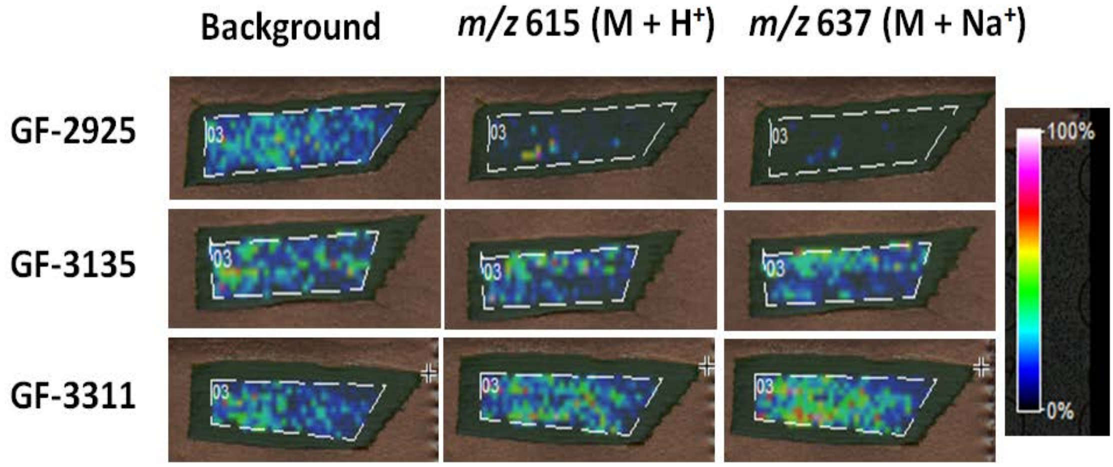

Figure 4. MALDI imaging analysis of fenpicoxamid in three different formulations (GF-2925, GF-3135, and GF-3311) on wheat leaf surfaces after spray at a rate of 130 g/ha.

Characterisation of GF-3308 (50 g as/L fenpicoxamid, i-Q4) and visualisation of deposits on leaf surfaces

A series of tests were undertaken between 2016 and 2019 by Silsoe Spray Applications Unit Ltd, UK to quantify the effect of formulation on the characteristics of sprays produced by agricultural nozzles and to provide qualitative information through photographic images on the characteristics of spray deposits on both real and artificial surfaces using the final commercial formulation of GF-3308 (50 g as/L fenpicoxamid, i-Q4).

Initially in 2016 two nozzles were used; (i) a conventional flat fan nozzle, FF110 03 (Hypro EU Ltd) at 3.0 bar; (ii) an air-induction nozzle, AIXR 03 (Spraying Systems Ltd) at 3.0 bar. This was broadened in subsequent years to encompass 75% and 90% drift reducing nozzles, forward speeds between 8 and 14 km/h, water volumes between 80 and 200 L/ha and evaluation of the effect of variety.

Imaging of sprays

Leaves were removed from a plant and secured to a horizontal surface underneath A track sprayer with a three-nozzle boom (0.5 m nozzle spacing) was set up in the Silsoe wind tunnel. Three flag leaves from field-grown winter wheat leaves (obtained locally) were secured horizontally onto a rigid surface and placed below the centre nozzle. A fluorescent tracer was added to the spray liquid to give a good visual contrast between the spray and the plant surface. Each sprayed set of leaves and plant were photographed at a range of times after application, to identify an appropriate protocol, since fluorescence reduces as the spray dries. This poses a challenge with formulations that spread well, since (a) they produce a thin film which fluoresces less than a concentrated droplet, and (b) they dry quickly. Photographs were therefore taken at 30 seconds after application.

Initial tests evaluated standard flat fan nozzles (Figure 5) to understand the behaviour of the standard SC of fenpicoxamid(GF-2925) in comparison to the final i-Q4 formulation matrix GF-3308. Application was simulated using a forward speed of 12 km/h and a water volume of 120 L/ha. The pictures in Figure 5 clearly show the improved spreading characteristics of the i-Q4 matrix over the standard SC. Application through air Induction nozzles with a 75% drift reduction (LERAP classification of 3 star) also showed that the i-Q4 matrix maintained near complete coverage at 120 L/ha water volume though the forward speed in this test was lower at 8 km/hr (Figure 6). Further testing focused on the use of nozzles capable of 75% and 90% drift reduction (Figure 7) which would result in relatively large droplets hitting the target. In this test, the water volume was reduced to 100 L/ha and the forward speed was moved back to 12 km/h. The result was very high levels of target coverage aided by the I-Q4 matrix moved both along the leaf vein but also across it. Finally, in Figure 8, fenpicoxamid i-Q4 was applied in comparison to benzovindiflupyr + prothioconazole and bixafen + prothioconazole applied at 150 L/ha water volume and 12 km/h through a 3-Star (75%, Teejet TTI 04) nozzle resulting in high coverage with the i-Q4 matrix.

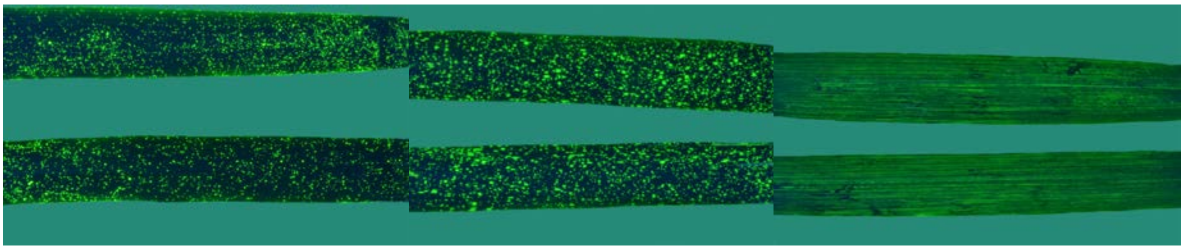

Figure 5: Standard Flat Fan, F110 03 at 3 bar, 12 km/h, 120 L/ha

Water **GF-2925 (SC)** **GF-3308 (i-Q4)**

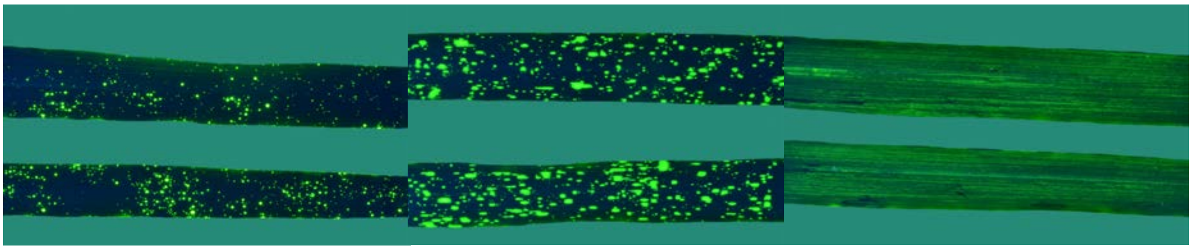

Figure 6: Air Induction, AIXR 03 (‘3-star’ 75% DRT) at 1.4 bar, 8 km/h, 120 L/ha

Water **GF-2925 (SC)** **GF-3308 (i-Q4)**

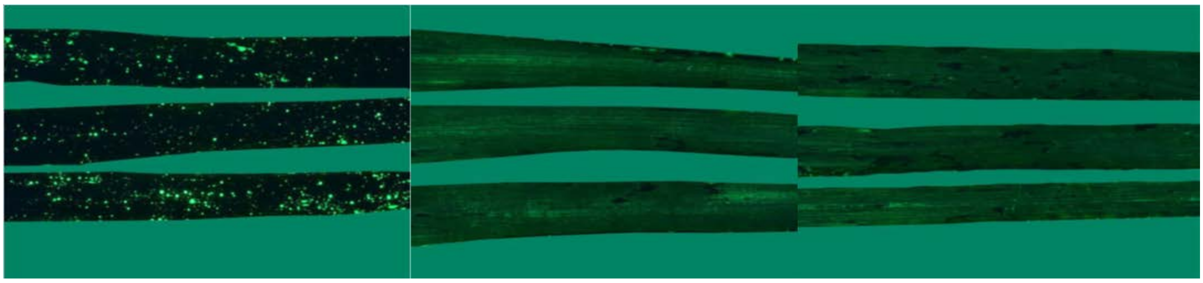

Figure 7: Comparison of GF-3308 (i-Q4) applied at 100 L/ha, 12 km/h, with 3-Star (75% DR Teejet TTI 025) and ‘4-Star’ (90% DR Lechler ID3-05) nozzle, 3.0 and 2.0 bar

Water 90% DR, Lechler ID3 05 **i-Q4, 75% DR, Teejet TTI 025** **i-Q4 90% DR, Lechler ID3 05**

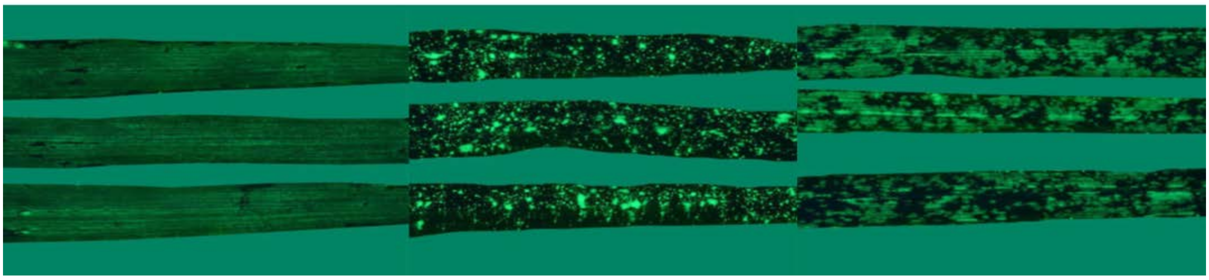

Figure 8: Comparison of GF-3308, benzovindiflupyr + prothioconazole and bixafen + prothioconazole applied at 150 L/ha, 12 km/h, with 3-Star (75%, Teejet TTI 04) nozzle, 2.6 bar

GF-3308 (i-Q4) benzovindiflupyr+prothioconazole bixafen+prothioconazole

DISCUSSION

Intrinsic activity of an active substance can be greatly enhanced through effective delivery to the target area. The work demonstrates clearly that not only did formulation deliver improved efficacy but also that the characteristics of the active substance were changed. The original formulation as a suspension concentrate delivered effective control of *Z. tritici* but mainly through protectant activity, whereas the emulsifiable concentrate and revised delivery i-Q4 matrix provided additional residual activity, curativity and broadened its spectrum. Results show clearly that the retention has been greatly enhanced but additionally that the behaviour of the matrix changes under different conditions. Independent testing demonstrates that regardless of forward speed, nozzle choice, or water volume, the behaviour on the leaf is comparable. It is noted that the formulation matrix termed i-Q4 is not a 'one size fits all' approach but has been recognised through the granting of a patent for Inatreq Active.

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EFFICACY OF A NOVEL AZOLE AGAINST *ZYMOSEPTORIA TRITICI* AND YIELD OF WINTER WHEAT IN SCOTLAND AND IRELAND WHEN APPLIED AT THREE TIMINGS

B Freer[1], A Christie[2], A Gilchrist[2], S Kildea[3], D Kitching[1] and R Bryson[1]
[1] *BASF plc, Cheadle Hulme, Cheadle, Cheshire SK8 6QG*
[2] *Scottish Agronomy Ltd, Arlary Farmhouse, Milnathort, Kinross KY13 9SJ*
[3] *Teagasc, Oak Park, Carlow, Ireland*
E-mail: ben.freer@basf.com

Summary: Mefentrifluconazole is the first Isopropanol-azole which belongs to the triazoles chemical family. It shows outstanding biological performance and conforms to very high Global and European regulatory standards. Over many years, triazole-based fungicides have been the backbone of crop protection programmes on many economically important crops. Mefentrifluconazole, with its unique chemistry, exhibits a very broad level of efficacy against many fungal pest species in arable and speciality crops, turf and on seeds, including isolates which already showed a reduced sensitivity to older triazoles, such as *Zymoseptoria tritici*. This unique ability to control already “shifted” isolates of pathogens demonstrates the important role of Mefentrifluconazole in resistance management (Bryson *et al*., 2018).

The co-formulated emulsifiable concentrate product Revystar XE (100g/l Mefentrifluconazole + 47g/l Fluxapyroxad) has been registered for use in the UK on winter wheat, spring wheat, durum wheat, spelt wheat, winter barley, spring barley, triticale, rye and oats with a maximum dose of 1.5 l/ha and a maximum of two applications to the crop from GS30 - GS 69. It can be applied in 100-300 l/ha of water with a 5m reducible buffer zone. It has a very broad-spectrum of activity against all major cereal diseases, i.e. Septoria, rust species, powdery mildew, Ramularia, Rhynchosporium and net blotch.

Field trials done in winter wheat, by Teagasc and Scottish Agronomy in 2019 have demonstrated its efficacy when application was on time, delayed by 7-10 days or 14 days under contrasting disease pressure and subsequent yield increase.

Treatment comprised of a single application of each product at 80% of the label dose, with no prior or subsequent fungicide being applied.

Disease pressure was particularly high at Oak Park (cv Lumos), Revystar XE showed curativity when applied at the GS 39-41 on leaf 2. At Balgonie (cv KWS Barrel) large differences in green leaf were evident particularly at the GS 39 + 7 day later timing. Longevity of disease control was evident from the green leaf area assessments and the final arbiter, yield.

Whilst Revystar XE demonstrates flexibility in timing compared with older products, the optimum timing for disease control and maximum yield remains GS 39 (full flag leaf emergence). It does, however, highlight that when the optimum timing is compromised due to poor weather conditions, there is less of a yield penalty when it is used.

Disease control All products applied at 80% label dose	**Oak Park on 2 July** **Septoria % Leaf 1**	 **Septoria % Leaf 2**	**Balgonie on 9 July** **GLA % Leaf 1**	 **GLA % Leaf 2**
Untreated	42.6	35.3	38.7	8.0
Revystar XE at GS 39-41	3.3	5.6	73.0	58.4
Ascra Xpro at GS 39-41	6.2	29.8	69.4	43.3
Elatus Era at GS 39-41	5.7	28.1	58.5	27.3
Revystar XE at GS 39 + 7d	5.4	17.2	71.0	39.6
Ascra Xpro at GS 39 + 7d	6.4	22.3	41.5	19.3
Elatus Era at GS 39 + 7d	6.8	28.1	48.1	16.4
Revystar XE at GS 39 + 14d	7.7	30.3	84.8	61.9
Ascra Xpro at GS 39+14d	8.1	29.7	68.1	23.3
Elatus Era at GS 39+14d	14.5	33.1	66.9	23.9
P value	<0.001	<0.001	<0.001	<0.001

Yield All products applied at 80% label dose	**Teagasc** **Yield t/ha at 85% DM**	**Scottish Agronomy** **Yield t/ha at 85% DM**	**Mean** **Yield t/ha at 85% DM**
Untreated	8.28	6.17	7.23
Revystar XE at GS 39-41	10.84	7.72	9.28
Ascra Xpro at GS 39-41	9.84	7.45	8.65
Elatus Era at GS 39-41	10.33	7.05	8.69
Revystar XE at GS 39 + 7d	10.12	7.64	8.88
Ascra Xpro at GS 39 + 7d	9.57	6.77	8.17
Elatus Era at GS 39 + 7d	9.81	6.59	8.20
Revystar XE at GS 39 + 14d	9.96	7.93	8.94
Ascra Xpro at GS 39 + 14d	8.72	7.37	8.04
Elatus Era at GS 39 + 14d	8.67	7.31	7.99
P value	<0.001	<0.001	

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Bryson RJ, Stammler G, Huff A, Strobel D, Meyer L, Moronval, M-H, 2018. Mefentrifluconazole – the first Isopropanol-azole fungicide for the control of *Zymoseptoria tritici* including field isolates with known complex CYP 51 haplotypes. Proceedings of Végéphyl – 12^{e} Conference International sur les maladies des plantes.

STABILISING AMINE UREA IN NITROGEN FERTILISER INCREASES LEAF CHLOROPHYLL CONTENT, TILLER BASE DIAMETER AND ROOT LENGTH OF WHEAT PLANTS

D J Marks, A K Weston and S Wilkinson
Levity Crop Science, Myerscough College and University Centre, Preston, PR3 0RY, UK
E-mail: david@levitycropscience.com

Summary: Technologies for stabilising urea N in fertiliser, and prolonging its availability for plants, have been developed. We have confirmed that stabilised urea amine in fertiliser increases N use efficiency and yield in potato and lettuce through specific and novel effects of this N form on plant physiology and development. The concurrent reduction of N degradation, and loss from the field as gas or soluble nitrate, whilst fortuitous, is only a part of the explanation for the observed increases in plant performance. Here we investigate whether chemically stabilising urea amine N (in a product called 'Elona') in foliar fertiliser applied to pot-grown wheat induces similar effects, compared to those of industry standard N fertilisers. All treatments contain identical amounts of N by weight, equivalent to a rate of 2.5 L/ha stabilised amine nitrogen (SAN) in 100L water, and were applied every 3-4 weeks in March-June 2018, in a greenhouse in Preston, Lancashire, UK. The chlorophyll content of wheat leaves was significantly increased by SAN nutrition 3 and 10 days after the first treatment, initially at 4-5 tiller stage; and tillers were more upright. At 14-15 tiller stage (one plant per pot) tiller bases had an increased diameter. This gave rise to a higher tiller diameter – canopy height ratio. Three weeks later roots of SAN-treated plants were significantly longer, which gave rise to a larger root length – canopy height ratio. We discuss how these attributes relate to specific effects of ureic amine N on plant phenotype, and how they may affect yields in the longer term. We argue that screening germplasm for high yield-linked phenotypic traits may be more effective when wheat is fertilised with stabilised urea amine.

INTRODUCTION

All nitrogen (N) forms (ammonium, urea, nitrite, organic amines) are eventually degraded to nitrate (and gaseous pollutants) within hours to weeks of application (dependent on environmental conditions) unless they are stabilised (see Wilkinson *et al.* 2019a). Thus effects of nitrate on plants have been the focus of much investigation. It was initially understood that non-leguminous plants could only access and assimilate inorganic N forms - ammonium, nitrite, and nitrate - such that when effects of N form have been studied on plant growth, this mainly relates to comparisons between nitrate and ammonium nutrition (e.g. Carlisle *et al*. 2012). These show contrasting effects on many physiological characteristics which influence yield, including in wheat. The effect of other N forms such as urea, on plant development and yielding, have often been overlooked. It is now recognised that plants have evolved to take up organic N forms - urea and amines - and possess highly conserved systems within root cells for doing so (Wang *et al.* 2016). We anticipate that effects of urea on plant traits will have a role in research designed to find novel genetic and/or agronomic routes to increase crop NUE and yield (Cormier *et al*. 2016). This is a growing area of research, particularly as technologies for preventing urea degradation to nitrate and pollutants are becoming available, which can increase crop nutrient use efficiency and/or yield in some cases (e.g. Wang *et al.* 2015). We have found, in several species, that in comparison to conventional ammonium nitrate and/or un-stabilised urea controls, stabilised urea amine nutrition generates plants with a particularly advantageous architectural appearance and physiology, called a ‘phenotype’. It is the attributes - or traits - of this phenotype (Wilkinson *et al.* 2019a, b) that improve plant performance and yield compared to those of the phenotypes typically associated with nitrate nutrition or ammonium nutrition (see Andrews *et al.* 2013).

Where effects of N form on plants have been compared in controlled experimental systems such as hydroponics or agar-filled pouches, plants grown in the presence of ammonium or urea alone can exhibit reduced growth, and generate symptoms of toxicity compared to nitrate nutrition (see Yang *et al.* 2015). When a detrimental effect of ammonium on hydroponic solution pH is corrected, however, ammonium nutrition improves biomass and tillering of wheat in comparison to nitrate (Chen *et al.* 1998). Further increases in biomass and tillering occurred when both N forms were supplied together. Pinton *et al.* (2016) found that different genes were up-regulated when two N forms were present, increasing the efficiency of total N uptake, assimilation and use. When two N forms are supplied in ratios: e.g. 75-25 urea-nitrate compared to 25-75; nutritional effects on plants in experimental systems can still be attributed to the dominant N form (Pompeiano & Patton, 2017), whilst reflecting conditions in the field more accurately. In the latter case both above and below ground biomass was greatest under a ratio of 75-25 urea-nitrate in pot-grown Zoysia grass.

We are gathering field and greenhouse data showing that, when stabilised, the unique phenotype generated by urea amine can increase tuber yields in potato (Marks *et al.* 2018; Wilkinson *et al.* 2019b), and increase flowering in ornamentals (Wilkinson *et al.* 2019a). Phenotypic traits induced by this N form can be some or all of the following: increased root-shoot ratio, increased root development *per se*, reduced shoot extension rate, and increased chlorophyll content during early development. In more mature plants, vegetative biomass increases compared to controls, lateral shoots develop, and chlorophyll content remains high. Here we describe effects of foliar treatments of three N fertilisers, including chemically stabilised amine nitrogen (SAN), on wheat growth and physiology. We aimed to determine whether any of these traits, several of which are known to contribute to and/or proxy for increased yields in the field (Bai *et al.* 2013), occur in pot-grown seedlings of this staple food crop. Experiments were conducted in a greenhouse in Preston, UK, in 2017-2018.

MATERIALS AND METHODS

Triticum aestivum L. cv Anapolis was used in greenhouse trials. Seeds were drilled in modules in December 2017, in J. Arthur Bowers John Innes No. 2 compost (Westland Horticulture Ltd., Co. Tyrone, UK), at a rate of one per 2.5 x 2.5 cm module sub-compartment. Prior to tillering seedlings were transplanted singly to 5 L pots containing the same compost. The pH of this is 5.5-6.0, and it initially provides appropriate macro- and micro-nutrients to all plants. Foliar spray treatments with a range of nitrogenous compounds occurred every 3-4 weeks from the onset of tillering, in a heated and ventilated greenhouse under natural light (PPFD 200-1000 µmol m^2 s^{-1}), in Preston, northern England, UK. Night-time temperature was 12-16°C, and day-time temperature was 16-32°C. Plants were watered by hand to soil capacity as required. Each of the nitrogenous treatments comprised of five replicate wheat plants randomised within an area of 2.5 x 1.0 m^2.

Nitrogen (N) fertiliser treatments were applied at non-limiting concentrations as liquid formulations: a standard N-P-K control, stabilised amine nitrogen (SAN) in a formulation called ‘Elona’ (supplied by Levity Crop Science Ltd., Preston, UK), and a cereal-specific industry standard (IS). SAN was applied at a rate of 2.5 L ha^{-1} in 100 L water. It contains 9.0 % N (by weight), and the control and IS treatments were designed to provide the same amount of N to the plants. The N-P-K analyses for the different treatments are shown in Table 1. All plants were supplemented via the soil with standard N-P-K (control treatment) at 50% recommended strength every 2 weeks, ensuring access to sufficient micro-nutrients and P-K. Main treatments with N fertiliser occurred approximately every 3-4 weeks, as specified in Table 2, at a rate of 20 cm^3 per m^2.

Table 1. N-P-K analysis of foliar N treatments applied to greenhouse-grown *Triticum aestivum* L. cv Anapolis.

Control	SAN (Stabilised Amine Nitrogen)	IS (Industry Standard)
Ammoniacal nitrogen 3.5%	Nitrate nitrogen 3.0%	Ureic nitrogen 14%
Ureic nitrogen 20.5%	Ureic nitrogen 6.0%	
Phosphorus 8.0%		Phosphorus 7.0%
Potassium 16.0%		Potassium 7.0%

Leaf relative chlorophyll content, tiller angle, tiller basal diameter, canopy height and root lengths were measured once or on several occasions at different developmental stages over the course of the experiments (Table 2). Relative chlorophyll content was measured in leaves as an index, with a FieldScout CM 1000 chlorophyll meter (Spectrum Technologies Inc., Illinois, USA). The reflectance of ambient and reflected 700 nm and 840 nm light was measured in a conical viewing area on the adaxial leaf surface 30-180 cm from the light receptor. This comprises of four photodiodes; two for ambient light and two for reflected light from the leaf. Measurement units are calculated as an index of relative chlorophyll content, 0-999 ± 5%. Leaf canopy height and root lengths were hand-measured with a ruler at the times detailed in Table 2. Tiller angle of the three largest tillers, with the soil surface as the horizontal plane, was measured using a protractor. Tiller basal diameter was measured at its widest point with a digital calliper.

Table 2. Chronology of foliar nitrogen (N) application occasions, and measurement activity, during experiments on greenhouse-grown *Triticum aestivum* L. cv Anapolis.

Days from start	Activity	Figure no.
0	N treatment 1	
3	Chlorophyll analysis	1A
5	Tiller angle measured	2
10	Chlorophyll analysis	1B
27	N treatment 2	
36	Tiller diameter measured	3A
36	Canopy height measured	3B
48	N treatment 3	
56	Root length measured	4A
56	Canopy height measured	4B

Means and standard errors of each measurement type per treatment are displayed as bar charts. The significance of the differences between treatments was calculated using a one-tailed *t*-test for two independent means, and where treatments are significantly different from each other (at $p<0.1$), this is denoted by 'a', 'b', or 'c', above the appropriate column on the graphic representations of the data.

RESULTS

Figure 1A shows that leaf chlorophyll content was significantly increased by SAN 3 days after the first foliar N treatment, at 4-5 tiller stage; by 11.6% in comparison to the controls, and by 19% compared to the industry standard (IS) treated plants. Figure 1B shows that the effect of SAN persisted 7 days later. In between the chlorophyll measurements, tillers were more upright in SAN treated plants (Fig 2A), with an increased angle between the soil surface and the three largest tillers per plant (Fig 2B). The increase in angle was 51.5% in comparison to controls, and 50% compared to IS treated plants.

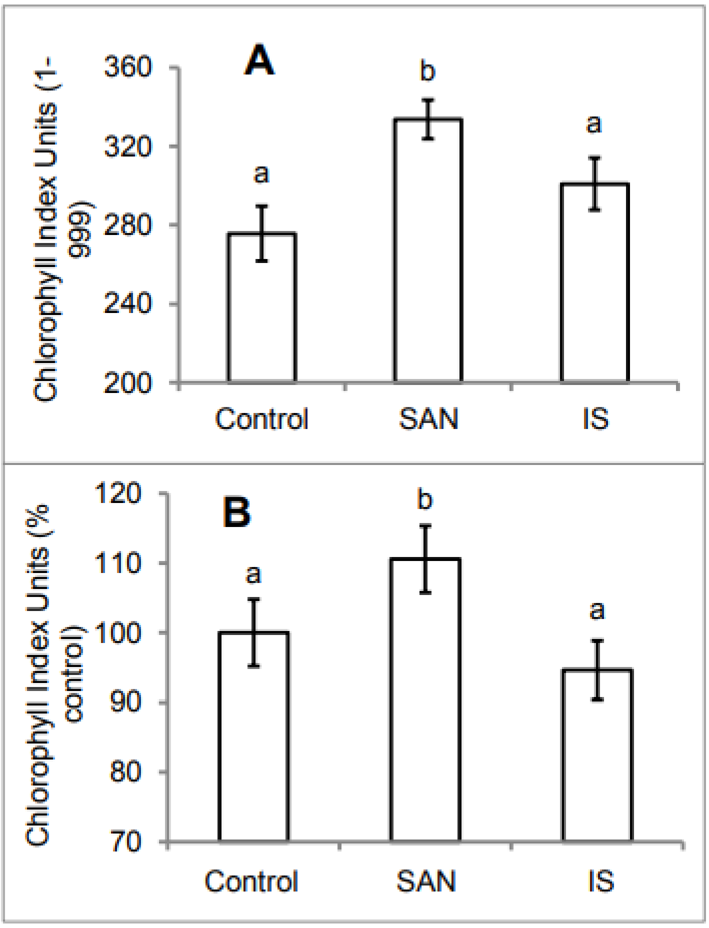

Figure 1. Effect of foliar SAN application on leaf chlorophyll content of wheat plants 3 days after treatment (A), compared to conventionally fertilized control and industry standard (IS) treated plants. Fig 1B shows the effects 7 days later.

At 14-16 tiller stage (more tillers are generated when plants do not experience competition), tiller base diameter was significantly larger in SAN-treated plants (Fig 3A) than in both control and IS treatments. Thus there was a significantly higher tiller base diameter-canopy height ratio (3B), as canopy height was similar among treatments (not shown). Four weeks later, after a total of 3 foliar N fertiliser treatments, root length below the pot was also the highest in SAN treated plants (Fig 4A), as was root length-canopy height ratio (4B), as again canopy height was similar among treatments.

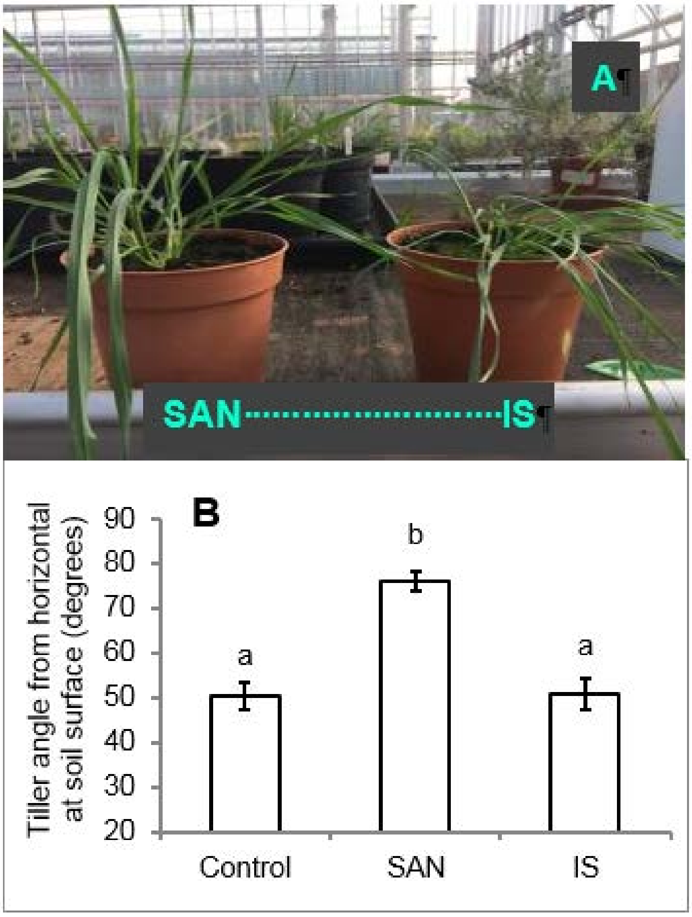

Figure 2. Comparison between the effects of SAN, control and industry standard (IS) foliar N fertilisation treatments on tiller angle.

DISCUSSION

Genes and/or agronomic practises that influence variability in a range of architectural and/or functional traits (termed phenotypes) are being sought, to improve field wheat nitrogen uptake efficiency (N taken up per unit N supplied) and/or nitrogen utilisation efficiency (grain yield per unit N taken up), as well as yield *per se*. Promising traits include root architecture, nutrient uptake and metabolism, photosynthesis and canopy longevity, nitrogen remobilization and wheat grain N accumulation (Cormier *et al.* 2016; Hawkesford 2014, 2017). Several of these characteristics are displayed during pre-anthesis growth of wheat seedlings, and have been linked to yielding of mature plants in the field. These have largely been determined by germplasm screening in a range of experimental and field systems, under a range of conditions including drought, heat, and low and high levels of N. However, these studies are rarely carried out on the basis of the N form(s) of the applied fertiliser (Cormier *et al*. 2016). Here we demonstrate that positive effects on some of these traits can be induced in greenhouse-grown wheat seedlings by a simple change in nutritional N form; these being increased leaf relative chlorophyll content (Figure 1) and increased root length (Figure 4). Furthermore increased wheat tiller basal internode diameter (Figure 3) is closely related to lodging resistance and grain yield (Tripathi *et al.* 2003, Khan *et al.* 2019). Erect tillers (Figure 2) can enhance photosynthesis and dry matter production through greater sunlight capture (Abichou *et al.* 2019).

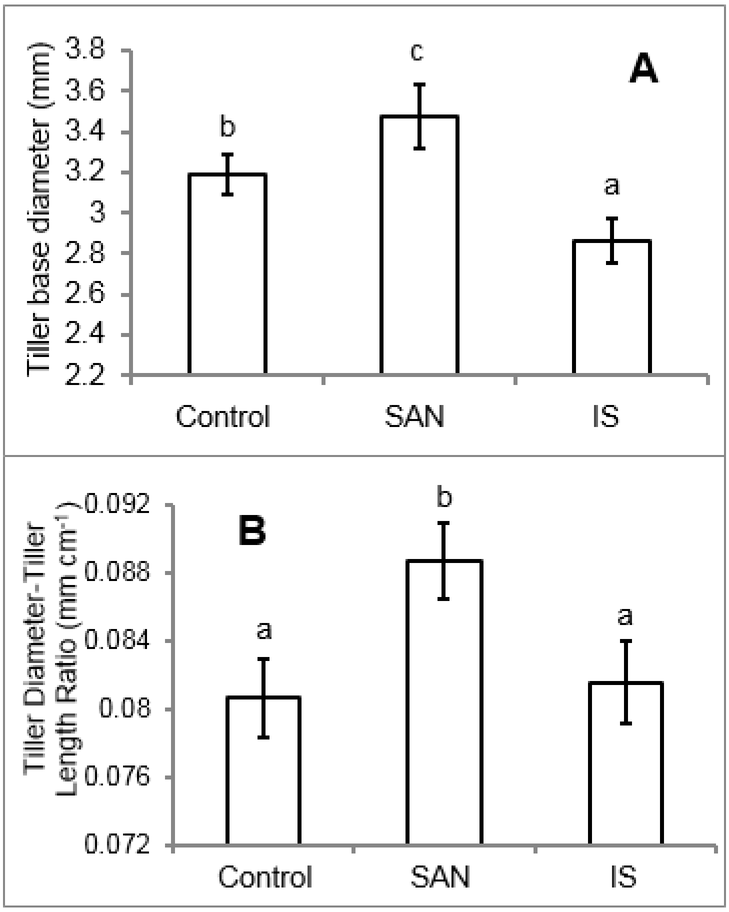

Figure 3. Comparison between the effects of SAN, control and industry standard (IS) foliar N fertilisation treatments on tiller diameter (A) and tiller diameter – canopy height ratio (B).

Improvements in root architecture (lateral root proliferation near the soil surface and at depth) and increases in root biomass have been viewed as promising targets for selection for NUE and yield amongst wheat genotypes (Hawkesford 2014, 2017). However, it has also been demonstrated that root development is largely dependent on genetic differences in above-ground shoot biomass and tillering processes (Allard *et al*. 2013), such that there is an argument that root traits may not be as important as selection targets for improved NUE and grain yield as originally believed. Allard *et al.* (2013) used ammonium nitrate as basal fertiliser in field trials, which can be assumed to have been converted to nitrate, which will have then been the dominant form of N in the soil. Given our research (e.g. Wilkinson *et al.* 2019b), we would maintain that the study by Allard *et al.* (2013) was one based on genetic variation in nitrate use efficiency, rather than one based on wider nitrogen use efficiency *per se*. Nitrate from soil or foliar sources is preferentially allocated to shoots for above ground vegetative growth and tillering during early seedling development, at the expense of root biomass growth (Andrews *et al.* 2013, Wilkinson *et al.* 2019a, b). Compared to ammonium N and ureic amine N, this typically generates a phenotype with a reduced root-shoot ratio.

Screening for root traits would thus have occurred within a narrowed phenotypic range, in which variations in influential vegetative traits would have provided a wider target. Had the authors used stabilised urea as basal or foliar fertiliser, which promotes the generation of the resource use efficient, stress resistant phenotype (characterised by high root-shoot ratio, initially reduced apical dominance, and increased leaf chlorophyll content), we propose that they would have found a wider variation in root traits linked to NUE and/or yield.

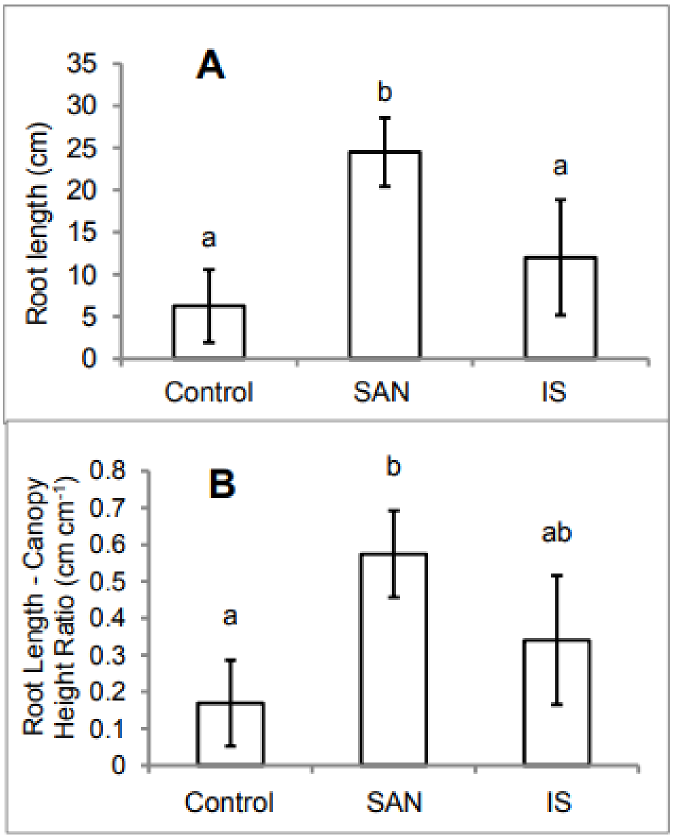

Figure 4. Comparison between the effects of SAN, control and industry standard (IS) foliar N fertilisation treatments on root length (4A) and root length – canopy height ratio (4B).

Urea-induced increases in photosynthesis (Figure 1) and rooting (Figure 4) show promising links to improved yields in other species (potato - Wilkinson *et al.* 2019b, lettuce – Wilkinson *et al.* 2020, manuscript in preparation). Here we show that these traits are also induced in wheat via this simple change in nutritional N form. We expect that wheat crop fertilisation in the field with stabilised urea will increase grain yield via the generation of the specific urea amine-induced phenotype seen here.

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LEGACY EFFECTS ON YIELD OF CEREAL-LEGUME CROPS, CONTINUOUS BARLEY AND SOIL ORGANIC AMENDMENTS ON SUBSEQUENT CEREAL CROPS

A C Newton and D Guy
James Hutton Institute, Invergowrie, Dundee DD2 5DA
E-mail: adrian.newton@hutton.ac.uk

Summary: The principles of rotation are based on the legacy effects of previous crops but these effects are seldom quantified. We show the negative effects of continuous barley on subsequent barley but not wheat, the varied effects or soil amendments on subsequent barley, and some effects of different crops and intercrops on subsequent wheat and barley crops. In particular we highlight positive effects of peas, beans and oats.

INTRODUCTION

Crop rotations are designed to maintain soil fertility and crop health and in general it is accepted that continuous cropping with the same crop species will lead to reduced yields and increased disease issues. Nevertheless, the relatively high value of some main cash crops such as spring barley for malting or winter wheat for milling in some areas mean that break crops are not used as often as they should be for maintaining soil health.

Minimising the negative effects of continuous cropping with a single crop is one strategy, another is interventions that aim to improve the soil structure by adding organic amendments. Commonly these are either animal waste such as slurry or compost such as that from domestic garden waste. Depending on how these affect soil structure, chemistry and biology, the amendments may have varied effects on subsequent crops, particularly under stressed conditions.

Another way to improve soil health and fertility is to grow legumes in the previous crop. Increasingly the value of legumes as a component of an intercrop are being exploited but capturing the nitrogen fixed by the legume in subsequent crops and minimising losses to the environment requires suitable agronomic approaches, particularly tillage.

MATERIALS AND METHODS

Pre-crop treatments are outlined below and subsequent crop responses were measured by overlaying plot trials using standard agronomic protocols except where indicated below. In all cases at least eight current AHDB Recommended List (RL) cultivars of spring barley were used. For two of the trials similar numbers of RL winter wheat and winter barley cultivars were used. For the continuous barley effect trial 2018-19 the winter wheat and winter barley were direct drilled using a custom-built equivalent of our standard 8-row Hege plot drill but using John Deere 750a discs and coulters set up in two banks of four with a row spacing of 15.5cm. The spring barley had minimum tillage treatment then sown with the Hege plot drill. The soil amendment trial was sown in the same way as above with spring barley in both 2017 and 2018. No fungicides were applied to either of these trials. The legume legacy trial 2018-2019 was direct drilled with winter wheat, winter barley and spring barley. In this trial the nitrogen rates were half the standard rates and it had full fungicide treatment programmes. All plots were harvested with a plot combine and either dried to constant moisture then weighed, or for the legume legacy effects, weights recorded by the combine were used. Full trial protocols will be given in subsequent publications with detailed results.

Continuous barley effect trial

An area of 100m x 100m had been sown with barley from 2001 until September 2016. In 2017 three sub-areas were sown with oilseed rape and three sub-areas with barley. In 2018 the three oilseed rape-sown areas were sown with faba beans and the three barley areas were again sown with barley, thus establishing three replicates of “restored rotation” and maintaining three replicates of continuous barley. The replicated plot trial was sown across these two treatments in 2018-19.

Soil amendment effect trial

Since 2004 a field of sandy-loam soil had soil amendment treatments applied comprising ‘Discovery Compost’, ‘slurry’ and an unamended control (Hopkins *et al*., 2016). In 2017 a spring barley plot trial was sown across half the treatment area and in 2018 across the other half, but all treatments and treatment levels were present in 3 replicates in each year.

Legume legacy effect trial

A range of single species and intercrop plots were sown in 100m x 3m beds using an Amazone AD/P Super drill in combination with Amazone KG Power Harrow in September 2017. The monocultures were pea, faba bean, rye, oat and barley. The intercrop mixes were pea+rye, pea+oat, pea+barley, bean+rye, bean+oat and bean+barley and were sown at ratios of 30:70 legume:cereal. The field was treated with the herbicide Glyphosate prior to sowing but no other herbicides and no fungicides were used. Plots were sown in a randomised three replicate split plot design with either no nitrogen or 50% of the normal winter barley rate as the main plot. Plots were cut and allowed to wilt on 9th July then baled, wrapped and weighed on 10th July. The results of this and previous biomass crop trials will be reported elsewhere but in September 2018 the area of the above trial was treated with the herbicide Glyphosate then the plot trial was sown across these treatments to determine the effects on subsequent crops of these pre-crops.

RESULTS

Continuous barley effect trial

All trial plots established well whether direct drilled or conventionally sown. The effect of restored rotation compared with continuous barley was a highly significant yield increase of about 19% and 26% in the yield of winter barley and spring barley ($p<0.001$), but for winter wheat there was no significant effect (Figure 1). There were no significant interactions of cultivars with the rotation treatments within crop type.

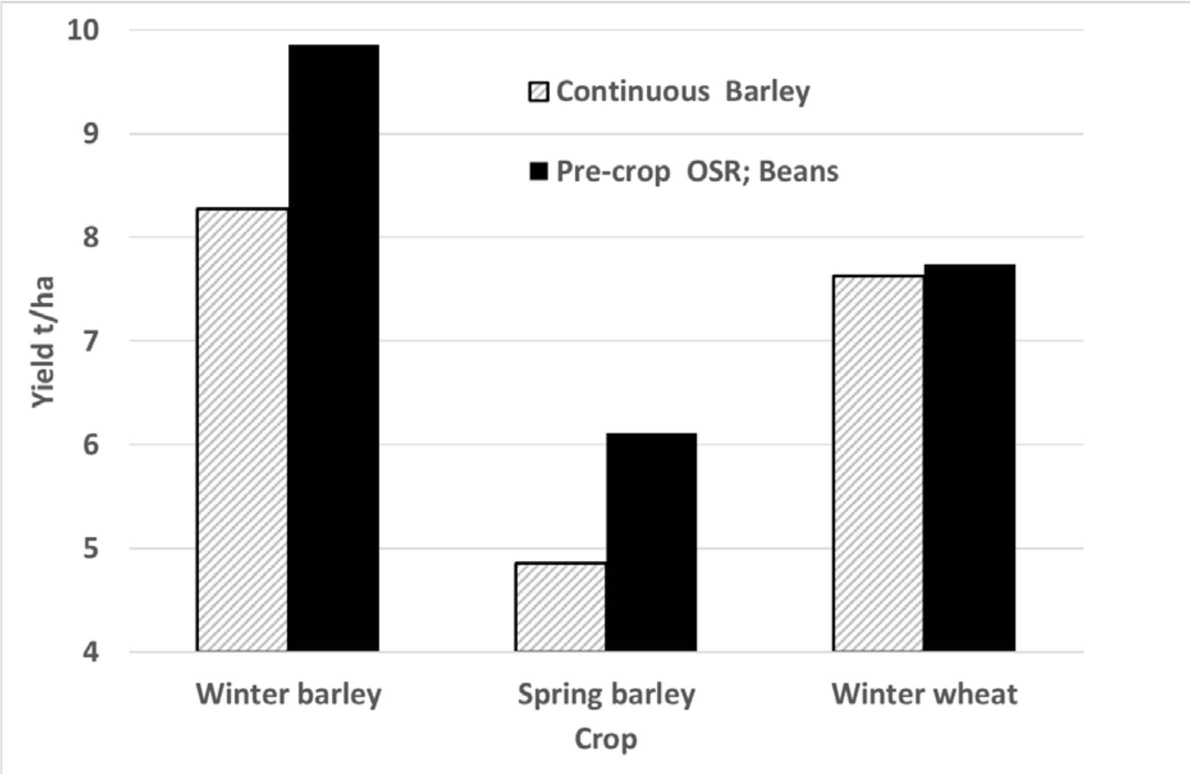

Figure 1. Effect of continuous barley or restored rotation treatments on yield of winter barley, spring barley and winter wheat, LSD = 0.200.

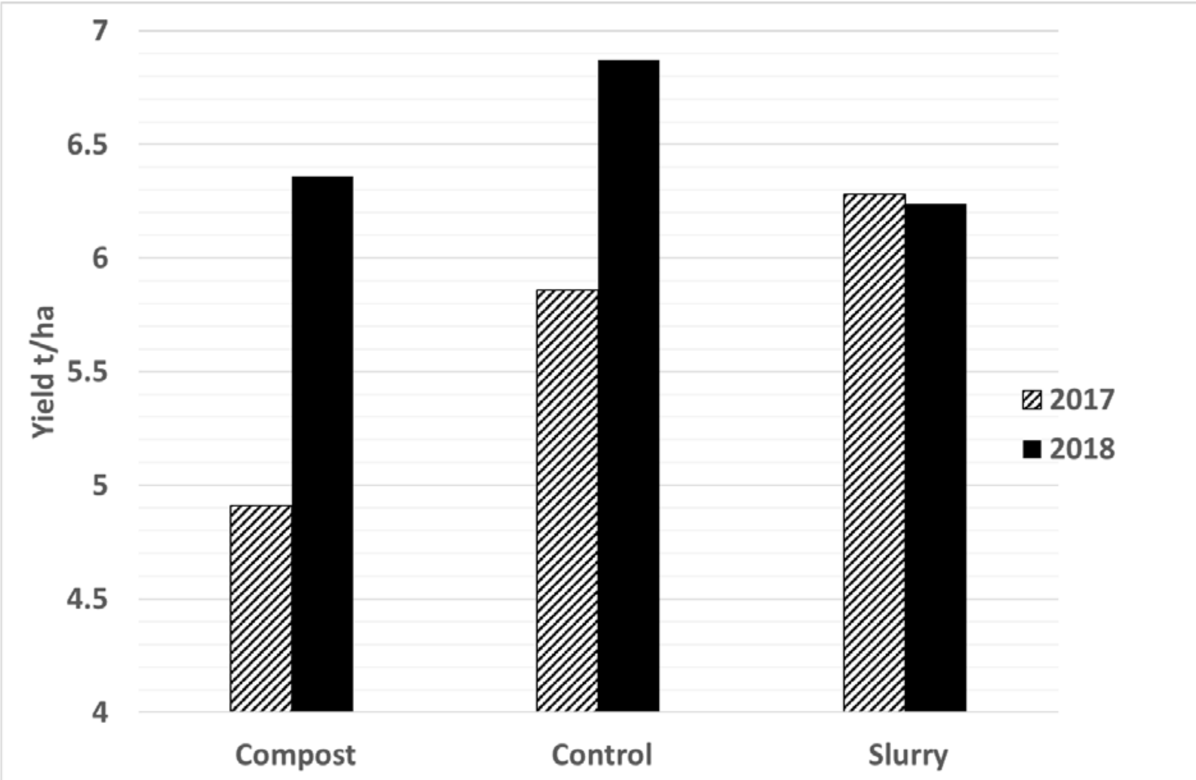

Figure 2. Effect of soil amendment treatment on yield of spring barley in two years, LSD = 0.239.

Soil amendment effect trial

The soil amendments showed a positive effect of slurry and a negative response to compost in 2017. However, in 2018 the compost negative effect was much smaller and the slurry treatment yields were similar (Figure 2). Again, there were no significant interactions between cultivars and soil amendment treatments overall.

Legume legacy effect trial

The legume, cereal and cereal-legume pre-crops showed differential yield responses expressed most strongly in winter wheat, then winter barley and least in spring barley (Figure 3). The pea and bean monocultures were most beneficial, followed by oats then the intercrop of oats and peas and oats with faba beans. The other intercrops had lesser effects and barley and rye as monocrops were least beneficial. Indeed, the lowest yield of all was the effect of rye on winter wheat (thicker line in Figure 3).

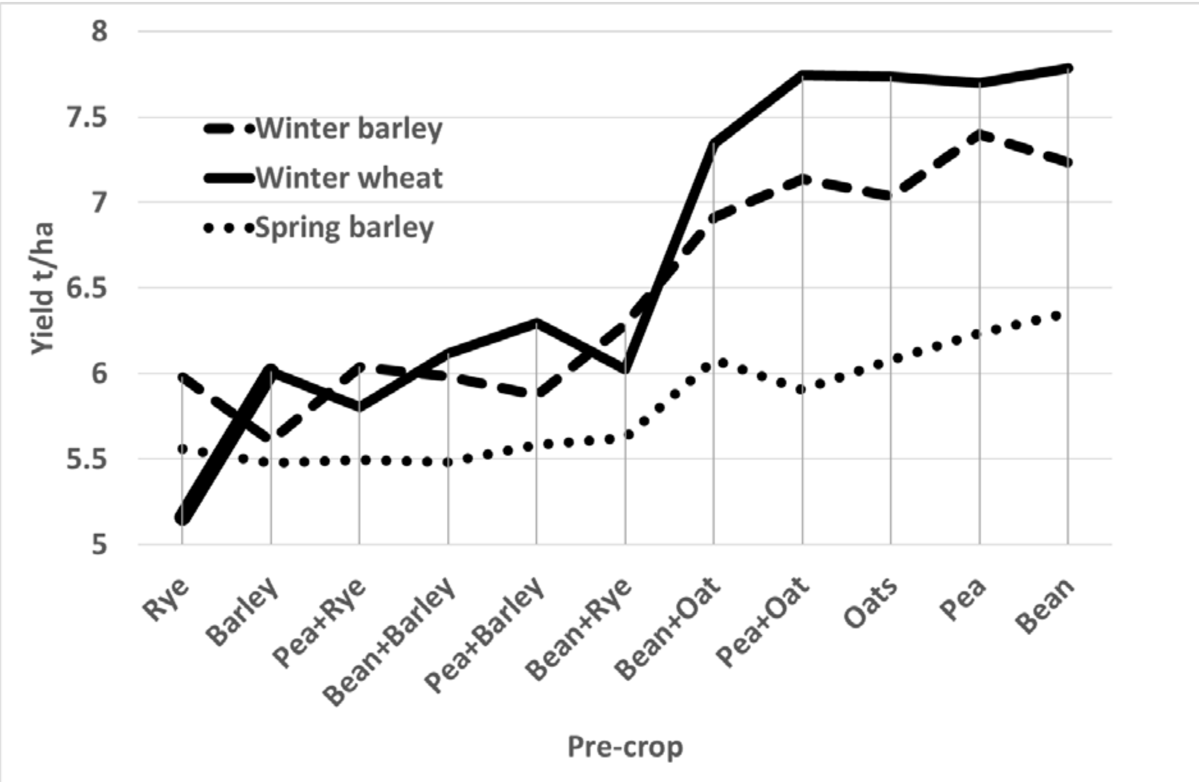

Figure 3. Effect of pre-crop on yields of winter wheat, winter barley and spring barley. Winter wheat and winter barley LSD = 0.273; spring barley LSD = 0.278.

DISCUSSION

Rice is said to be one of the few crops that can be grown continually on the same site due to the unfavourable soil environment for pathogen growth (Adam Price, University of Aberdeen, personal communication). Whilst this probably needs to be validated in trials, for most other crops there is evidence that rotation is necessary to maintain soil health and achieve optimum and sustainable yields. However, the magnitude of potential gains or losses are seldom assessed, and the optimum agronomic interventions and crop sequence options are rarely objectively evaluated.

That barley on a continuous barley site yielded less than after a restored rotation with pre-crops of oilseed rape then faba beans might be expected, though levels of 19% and 26% for the winter and spring crops were large. This is likely to have been enhanced by direct drilling as ploughing might have accelerated losses from microbial activity and breakdown of nutrients in the roots and other organic matter. However, this effect appears to be species specific as the yield of winter wheat was not affected by the treatments. Furthermore, any nutritional effect of the immediately prior crop being faba beans is unlikely as this would have been expected to affect at least both winter crops similarly. Nutrient explanations have been investigated (Clark & Mack, 1972) but the mechanism is likely to be predominantly microbial, possibly root-infecting pathogens. These may have included take-all but there was no evidence of this disease on the wheat. Indeed, negative effects of monoculture soil attributable to the microbial components have been reported, and these affected wheat and oats to a lesser extent than barley (Olsson & Gerhardson, 1992). The negative effect of rye on subsequent winter wheat is likely due to them both being nutrient-demanding crops, perhaps nitrogen in particular.

Soil amendments can have a strong effect on yield also but the effects differ between seasons. That compost amendments can reduce yield might appear surprising but both this effect and the seasonal variation may be attributable to the changes in physical properties of the soil with respect to its ability to retain water and thereby nutrients. It seems likely that this would be highly responsive to abiotic stress levels and these differed substantially between 2017 and 2018, the latter being much warmer and drier than normal.

Specific pre-crops clearly can have substantial effects on subsequent crops in proportion to the nutrient-demanding nature of the crop, wheat being most responsive and spring barley least. These pre-crops form two clear groupings. In the more beneficial group, the pea and bean monocrop effects might be expected as direct nitrogen responses. However, that oats have a similar effect is less expected, but oats with peas or beans would therefore be expected to have a similarly positive effect. The difference between the groupings in yield response were around 30%, 20% and 10% for winter wheat, winter barley and spring barley respectively. Some difference may be attributable to residual nutrient differences left after the previous crops but given the similarity of their biomass yields in the previous trial to those expected (data not shown) this seems unlikely to explain the magnitude of these responses. More likely would be the known allelopathic effects of oats having a strong effect not only on other plant species but also on the microbial composition of the soil (Manns *et al*.,2009).

Clearly crop sequence, the immediately preceding crop in particular, and soil organic amendments can have both detrimental and beneficial effects on subsequent crops. These trials indicate a potential range of around +/- 25% of the yield, greater than the benefits likely to be gained from changing cultivar for example. In particular, the utility of growing oat crops may be under-valued and ways of incorporating more legume crops should be considered. The value of soil amendments need to be carefully evaluated in the context of other soil health and resilience considerations.

ACKNOWLEDGEMENTS

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CROP SPECIES MIXTURES AS PART OF INTEGRATED FARM MANAGEMENT

A J Karley[1], C Mitchell[1], C Hawes[1], M W Young[1], R W Brooker[2], R J Pakeman[2], P P M Iannetta[1], and A C Newton[1]

[1]The James Hutton Institute, Invergowrie, Dundee, DD2 5DA
[2]The James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH
E-mail: Alison.Karley@hutton.ac.uk

Summary: Diversification of crop systems is increasingly recognised to improve the sustainability of crop production. Crop species mixtures can achieve higher productivity with fewer inputs than standard monocultures. However, there is limited information, about which crop species and which cultivars optimise the performance of mixtures at scales relevant to commercial farms. Here, we describe trials that aim to test crop and variety combinations, selected from small plot field trials, for trialling at larger scale under conventional and integrated management at the Institute's Centre for Sustainable Cropping at Balruddery Farm. Initial findings indicated that over-yielding detected in small scale plot trials was not observed consistently at larger scale under conventional treatment, although it might be realised under integrated management. Other positive effects of mixtures included improved soil phosphorus availability and aphid suppression on legumes, but not on barley. Due to the systems trial design, which has limited replication, full robust statistical validation can be conducted only after the second year of the trial.

INTRODUCTION

Species-rich systems often show higher productivity than monocultures, with reduced pest and disease severity, improved resource capture and greater resilience to environmental stress. By understanding the processes promoting productivity in small-scale experimental trials, we can identify the plant traits and mechanisms that promote productivity in crop species mixtures. On-farm validation is a crucial step in knowledge transfer from scientific trials to real-world farming systems. Field validation of diversification approaches at a large scale that is more relevant to commercial settings allows the practicalities of new farming practices to be tested, their benefits and challenges to be demonstrated, and the information to be used by farmers for decision-making. The Horizon 2020-funded project DIVERSify aims to optimise the performance of multi-species cropping systems, or 'plant teams', to promote within-crop diversity by understanding the plant traits and mechanisms that enhance productivity. To achieve this goal, we have adopted a two-step approach, conducting experimental trials at a small plot scale to test multiple combinations of crop species and cultivars, then selecting the best-performing mixtures for testing in large scale trials.

MATERIALS AND METHODS

Experimental trials at small plot scale were conducted in 2017 and 2018 at the James Hutton Institute, UK, to test the performance of commercial cultivars of spring-sown cereal crops (wheat, barley, oat) and legume crops (faba bean, pea, lentil) selected from national recommended lists or other sources to cover a range of morphological, developmental, agronomic, yield and quality characteristics. Cereal and legume species were chosen following consultation with agricultural stakeholders, primarily farmers, both through local contacts and in national workshops conducted in 2017 across Europe (Pearce *et al*., 2018). The best-performing spring barley-pea and spring wheat-faba bean cultivars identified in the 2017 trials (see Karley *et al*., 2017), in terms of over-yielding and other indicators, were tested at large scale in 2018 at the institute's Centre for Sustainable Cropping long-term research platform.

Site information

The Centre for Sustainable Cropping (www.csc.hutton.ac.uk) is a long-term research platform established in 2009 at The James Hutton Institute's Balruddery Farm near Dundee, UK. The farm platform (Fig. 1) comprises a 42 ha block of six fields used to assess the response of the whole arable ecosystem to a change in cropping system based on a suite of ecological, environmental and economic indicators. An integrated management system is compared directly with standard commercial practice across six arable crops in rotation: potato, winter and spring barley, winter oilseed rape, beans and winter wheat. The integrated system aims to maintain crop yields with fewer non-renewable inputs while enhancing arable biodiversity for ecosystem services (Hawes *et al.* 2019). This is achieved through a combination of non-inversion tillage, organic matter amendments, cover cropping, under-sowing and integrated pest management strategies. The harvested crop products have a variety of end uses; cereal and legume crops are typically used for animal feed.

Figure 1. Aerial view of the Centre for Sustainable Cropping field experiment platform at Balruddery Farm.

Trial details

Two trials were conducted in separate fields of the platform, each trial comprising a management treatment (assigned to one half of each field) and two replicate plots of each plant team treatment (crop monocultures or mixture) with treatments assigned at random to plot position. Spring barley (cv. Laureate) and pea (cv. Daytona) were sown in the spring barley field (plots were 3m wide x ~120m long) on 15th April 2018. Spring wheat (cv. Tybalt) and faba bean (cv. Boxer) were sown in the spring bean field (plots were 3m wide x ~200m long) on 16th April 2018 (Fig. 2). Conventional plots were sown with a 3m Amazone AD/P Super drill in combination with Amazone KG Power Harrow, while integrated plots were sown with a John Deere 750A 3m direct drill. Pea-barley mixtures were sown at 40% and 60% of their standard sowing densities (respectively), while wheat-faba bean mixtures were sown at 50% of their standard sowing densities. No crop protection treatments were applied. Fertiliser was applied at sowing as follows: in the barley-pea trial, the conventional treatment received 500 Kg ha^{-1} of 22:4:14 (110 Kg N ha^{-1}, 20 Kg P ha^{-1}, 70 Kg K ha^{-1}) while the integrated treatment received 375 Kg ha^{-1} of 22:4:14 (82.5 Kg N ha^{-1}, 15 Kg P ha^{-1}, 52.5 Kg K ha^{-1}); in the wheat-faba bean trial, the conventional treatment received 200 Kg ha^{-1} of 0:20:30 (0 Kg N ha^{-1}, 40 Kg P ha^{-1}, 60 Kg K ha^{-1}) while the integrated treatment received 150 Kg ha^{-1} of 0:20:30 (0 Kg N ha^{-1}, 30 Kg P ha^{-1}, 45 Kg K ha^{-1}).

Figure 2. Wheat-faba bean trial under conventional management in 2018 at the Centre for Sustainable Cropping at Balruddery Farm, Dundee, UK showing plots (from right to left) of faba bean monoculture, wheat monoculture and wheat-faba bean mixture.

Trials experienced unusually warm dry conditions throughout the growing period (May-July). Trials were monitored for crop development, canopy and individual plant characteristics, soil variables and pest and disease incidence following the Core Partner protocols (Banfield-Zanin *et al*., 2017), with collection and analysis of crop, soil and arthropod samples at specific time points during the season. The barley-pea trial was harvested on 31st August 2018 and the wheat-faba bean trial was harvested on 28th September 2018.

The expected yield was calculated from average yields of monoculture plots of each component using the formula

$$E_i = p_i M_i$$

where p_i is the proportion of species *i* in the mixture (i.e. 0.5, as sowing density was 50% that of monocultures), M_i is the yield of species *i* in monoculture and E_i is its expected yield based on the yield in monoculture (Loreau, 1998).

RESULTS

The wheat-faba bean intercrop showed lower than expected grain yield in the conventional treatment, while over-yielding was detected in one replicate of the integrated treatment (Fig. 3; note that the second replicate plot showed very low yield due to poor crop establishment as a result of high weed pressure). The pea-barley intercrop showed similar yield to expected in the conventional treatment and higher yield than expected in the integrated treatment (not shown).

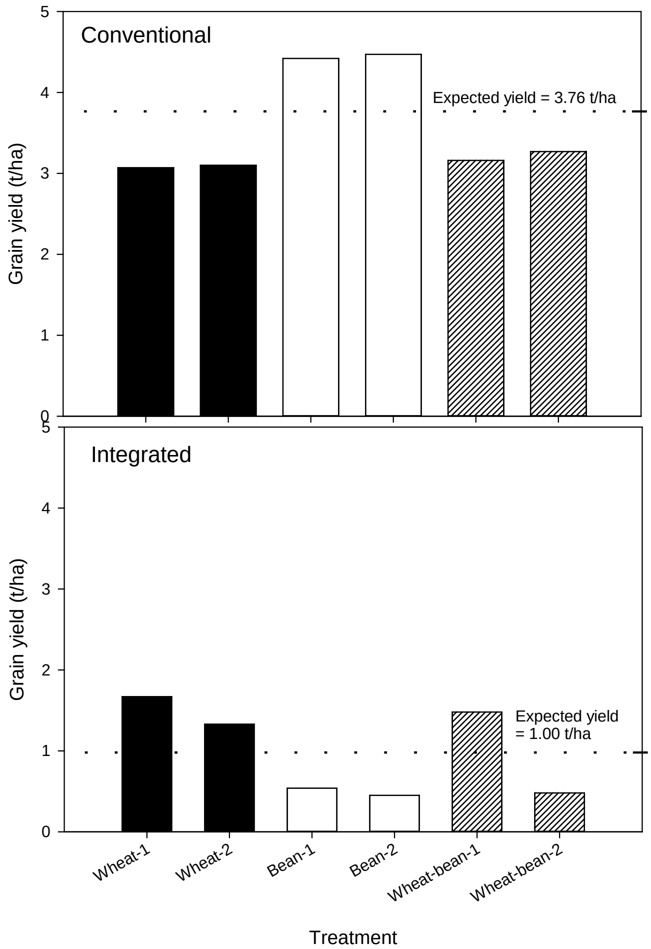

Figure 3. Grain yields of each of two replicate plots of crop monocultures and mixtures of spring cereals and legumes under conventional and integrated management at the James Hutton Institute's CSC platform in 2018. Observed yields of wheat-faba bean are shown; expected yields calculated from monoculture yields (adjusted for reduced sowing density in mixtures) are represented as dashed lines.

Weed cover was lowest under conventional management and in the presence of the cereal crop for wheat-faba bean and barley-pea (not shown). In the conventional management treatment, weed cover was suppressed to a similar extent in wheat-faba bean mixtures and wheat monocultures. Soil phosphorus (P) concentrations were highest under integrated compared with conventional management and with wheat-faba bean mixtures, particularly under conventional management (not shown). Aphid infestation was highest under conventional management and was suppressed on pea in mixtures compared to monocultures under both management regimes (not shown).

DISCUSSION

Over-yielding detected in small scale plot trials of barley-pea and wheat-faba bean plant teams in 2017 was detected at larger scales in 2018 under integrated management but was not observed consistently under conventional treatment. Other plant team effects included improved soil phosphorus availability with wheat-faba bean, particularly under conventional management, and aphid pest suppression on pea in pea-barley compared with monocultures. Continuation of these trials over multiple years will determine the consistency of these findings.

The two-step approach has succeeded in quantifying plant team benefits in research trials and testing the ability to transfer these findings to scales that are relevant to farm settings. Alongside this, we are working with farmers to trial crop species mixtures on their own commercial farms, enabling joint evaluation between farmers and scientists of plant teams in each farm system compared with standard practice. We are also aiming to identify the specific traits of cultivars that result in beneficial interactions at all scales so that cultivar selection will be more predictable and robust. This requires the data from trials such as these at all scales across multiple environments enabling the main principles of intercrop design to be deduced and practical resources to be made available to farmers for cultivar and agronomy decisions to be made.

ACKNOWLEDGEMENTS

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DO WE NEED WEEDS? THE PLACE OF NON-CROP PLANTS IN ARABLE SYSTEMS

R W Brooker[1], A J Karley[2], C Mitchell[2], A C Newton[2] and R J Pakeman[1]
[1] The James Hutton Institute, Craigiebuckler, Aberdeen AB15 8QH
[2] The James Hutton Institute, Invergowrie, Dundee DD2 5DA
Email: rob.brooker@hutton.ac.uk

Summary: Positive biodiversity-ecosystem function (BEF) relationships indicate that declines in agricultural biodiversity could have serious negative impacts on ecosystem functions in farming systems, limiting the development of sustainable management options. Here we consider whether reductions in non-crop arable plants - arable weeds – could result in reduced ecosystem functions, and whether managing and enhancing the diversity of the weed community could have beneficial effects for sustainable crop production. We discuss how small increases in plant diversity, in crop or cultivar mixtures, could have benefits for crop production, and how some of the same underlying mechanisms might be associated with enhanced weed diversity. We also demonstrate using field data how such positive weed-diversity – crop production relationships can occur, but also how they are context-dependent and might need careful management. Finally, we list some key issues that need to be considered if enhanced weed diversity is to be taken forward as a management option.

INTRODUCTION

A major driver of declining global biodiversity is the expansion of intensive agriculture (Brooker 2016). Since the Second World War this trend has been seen widely in the countries of Western Europe (Robinson and Sutherland, 2002), with impacts on a wide range of species. Declines in non-crop plants have resulted from factors including a switch to autumn-germinating cereals, development of competitive cultivars, increased use of inorganic fertilisers and herbicides, changes in crop rotations, and improved seed cleaning (Robinson and Sutherland 2002; Fried *et al,* 2009; Storkey *et al,* 2010). Some of our non-crop arable vascular plants are now amongst the rarest and most threatened plant species in the UK (Byfield and Wilson, 2005). For simplicity we use the term 'arable weeds' to refer to non-crop vascular plants in arable systems. However, although reductions in weed abundance might appear a boon for farming, ecological research indicates that declines in this component of farmland biodiversity may have serious consequences for the development of sustainable farming systems, and it may be that - to some extent at least - we need (some) weeds.

At the heart of this apparent puzzle are biodiversity-ecosystem function (or BEF) relationships. Ecosystem functions regulate the ways in which ecosystems (including crop systems) operate, and the benefits (such as harvestable crop) that we get (Brooker, 2016). Many scientific studies, from a wide range of ecosystems across the globe, have assessed the shape of BEF relationships (Brooker, 2016, and references therein). Although detected relationships depend on context, in general BEF relationships are positive, such that declines in biodiversity result in a loss of function, and asymptotic, such that declines in function accelerate as biodiversity is increasingly lost from the system. Consequently, and based on current understanding, declines in farmland biodiversity including declines in arable weeds could be having negative consequences for natural ecosystem functions in crop systems, limiting our ability to replace external inputs with natural processes and develop sustainable cropping systems. To explore this issue in more detail, here we consider the types of benefits that weeds within arable systems could bring, and how we might develop management strategies to optimise the benefits whilst minimising the negative consequences of having weeds in crop production systems.

DO WE NEED WEEDS? – ADDRESSING KEY QUESTIONS

Is there evidence of positive biodiversity-function relationships in arable systems?

An obvious example of how increased plant diversity can benefit crop production is intercropping, the contiguous growing of two or more crop species in one location (Brooker *et al,* 2015). Benefits include increased availability and efficient use of resources, for example soil N or P (Brooker *et al,* 2015). Intercrops appear to benefit from the asymptotic shape of BEF relationships, whereby small increases in diversity have big impacts on ecosystem functions. However, as with BEF relationships overall, the benefits of enhancing biodiversity through intercropping appear to be context dependent, and assessment of benefits may be dependent on the metric used (Brooker *et al,* 2015).

Another example of benefits from enhanced plant diversity in crop systems comes from cultivar mixtures. Selection for, and widespread use of, elite varieties of key crop species means that many monoculture crops are often (more-or-less) genetically uniform (Newton *et al*, 2009). As with intercropping (where species diversity is enhanced), introducing biodiversity back into crop systems by using crop cultivar mixtures (enhancing genetic diversity) has been shown to have beneficial effects on functions in crop systems (Newton *et al*, 2009) including increased resilience to variable climatic conditions (e.g. (Mason *et al,* 2017; Fletcher *et al,* 2019), enhanced abundance of predatory arthropods (e.g. Johnson *et al*, 2006), and increased disease resistance (Newton *et al*, 2009).

So, taken together, crop and cultivar mixtures demonstrate that positive BEF relationships can operate in crop production. However, as far as we are aware, no-one has explored in detail whether enhancing the diversity of the arable weed community within the crop can have the same positive effects as enhancing crop diversity.

Can increasing weed diversity benefit crop production?

Some of the mechanisms associated with intercropping or cultivar mixtures may also generate positive responses to enhanced weed diversity. Reductions in plant species diversity in arable systems have negative impacts on species at higher trophic levels, including invertebrates and birds (Wilson *et al*, 1999; Hawes *et al*, 2010; Karley *et al*, 2011), and so increasing weed diversity could promote predatory arthropods and reduce pest species. In addition, high weed species diversity may prevent particularly competitive (and perhaps herbicide-tolerant) weed species from dominating a community (Storkey and Neve, 2018).

Below-ground effects may also be important. Soil biodiversity can have positive effects on crop or fodder production, including protection against soil-borne diseases, system resistance to stress and disturbance, and enhanced nutrient-use efficiency (Brussaard *et al*, 2007). The degradation of soils can lead to the loss of predators and biotic regulation, and an increase in pathogens and pests (Sylvain and Wall, 2011). Importantly, enhancing plant diversity can enhance soil diversity (and *vice versa*) (van der Putten *et al,* 2013) and so enhancing arable weed diversity could enhance soil function.

Some field data support the proposition that enhanced weed diversity can have beneficial effects on crop production. Re-analysis of data from Brooker *et al,* (Submitted), in a 2016 study in eastern Scotland exploring the effects of on crop production of barley cultivar and genotype diversity, and the presence of added rare weed species, shows no effects of these factors on crop yield, but does show a positive effect on barley total biomass ($F_{1,52}$ = 6.28, P = 0.016) of total weed species richness (Figure 1). Brooker *et al* (Submitted) show that this effect is driven by changes in the abundance of only a few common weed species and leads to significantly enhanced barley total and grain biomass. However, a critical point to note with all of the above examples, is that these effects rely on or occur when crop species or cultivar mixtures, or weeds and crops, grow intimately together, and so might not occur in the crop if diversity is enhanced in field margins.

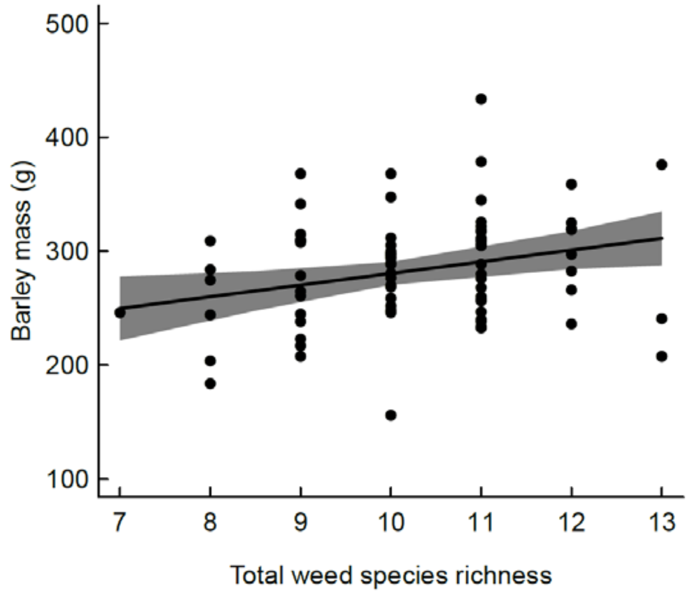

Figure 1. Relationship between total weed species richness and total barley biomass in experimental plots. The solid line indicates the fitted relationship; shaded areas show the 95% confidence limits for the fitted relationships. Analysis based on data from eighty-nine 2 x 1.55 m plots varying in barley cultivar composition, organised in three replicate blocks; analysed using linear mixed effects models, with cultivar composition specified as a random effect and block specified as a fixed effect.

Won't there be negative consequences of higher weed diversity?

To test this for potential negative impacts of higher weed diversity, for example through enhanced weed biomass, we again re-analysed the data from the 2016 study of Brooker *et al*. (Submitted). The expectation might be that increased weed diversity is associated with greater weed biomass. Although we there is a trend in this direction, the effect of weed diversity of weed biomass is non-significant ($F_{1,60}$ = 3.628, P = 0.062; Figure 2a). Furthermore, weed biomass is itself not related to crop biomass ($F_{1,52}$ = 0.156, P = 0.877; Figure 2b). These results indicate that the observed positive relationship between weed diversity and barley mass was not due to greater suppression of weeds by higher yielding barley (Brooker *et al,* Submitted).

However, such responses are not universal. Schöb *et al* (2015) showed that increased weed diversity was strongly positively associated with total weed biomass in greenhouse mesocosm plots. In this study, however, species rich communities were dominated by a single species – *Stellaria media*; in our 2016 field study *Stellaria* was a minor component of the weed community. Similarly, in a 2018 study (unpublished) we attempted to manipulate the diversity of the weed community by sowing weeds as well as crops. We found a strongly negative effect of adding weeds, irrespective of barley sowing density or soil preparation. In this case the study year (2018) was particularly dry, weed development from the seedbank was limited, and only a few weed species (i.e. those sown) dominated the weed community.

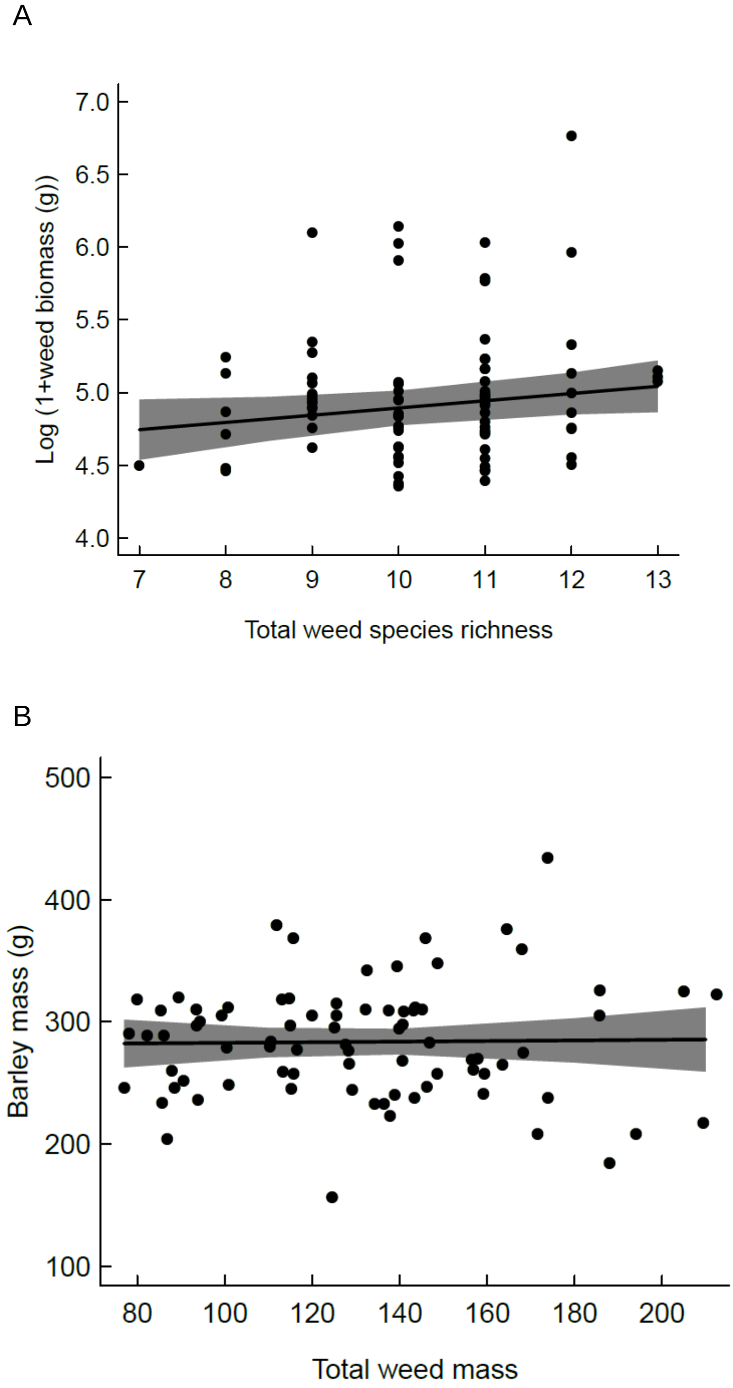

Figure 2. Relationships between a. total weed biomass (g) and weed species richness, and b. total weed mass (g) and barley mass (g) in experimental barley plots. The solid line indicates the fitted relationship; shaded areas show the 95% confidence limits for the fitted relationships. For details of analysis see Figure 1.

More in line with the findings from our 2016 field experiment, Storkey and Neve (2018) showed, in an analysis of data from the Broadbalk winter wheat experiment, that a more species-rich weed community was associated with less yield loss from weed competition. They proposed that this potential benefit of higher weed diversity came from limitation of the abundance of particularly competitive weed species. Furthermore, Schöb *et al* (2015) found that increasing the diversity of barley cultivars in experimental mesocosms limited the effect of the dominant weed.

Overall, therefore, there may be subtle interactions taking place: in some cases a single weed dominates the community and competes strongly with the crop, whereas in others no species becomes dominant and the beneficial effects of higher weed diversity have a net positive impact on crop growth. In order to promote beneficial effects, we may need to ensure a particular level of diversity in the weed community and/or the crop to prevent single weed species running to dominance.

DISCUSSION

We have outlined the concept of biodiversity-ecosystem function relationships, demonstrated how they can operate in arable crop systems (with reference to intercropping and cultivar mixtures), considered whether such effects can arise from enhancing diversity in the arable weed community, and explored potential negative consequences of promoting weed diversity as a component of sustainable crop production. It is clear that it is worthwhile further exploring the proposal that we need weeds as a component of sustainable farming systems. When doing so, some key issues need to be considered:

First, we need to understand better where, when, and why we see any yield benefits from enhancing weed diversity. Second, we need to think about the genetic origin of the weeds. It would seem wise – if managing the weed community becomes part of future sustainable production systems – to get off on the right foot and consider how we might best source and use (and certify) local genotype native plants. Third, even if there is no immediate crop yield benefit from enhancing weed diversity, we must remember the potential wider benefits for biodiversity. If future funding schemes pay farmers for a wider suite of ecosystem services from their land, including nature conservation, perhaps it might be possible to swap some crop yield for nature yield?

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CONTROL OF EYESPOT IN WINTER WHEAT USING RESISTANCE ELICITORS

N D Havis, A Laing, T Yoxall, A Christie and F J Burnett
Carbon, Crop and Soil Group, SRUC Edinburgh, West Mains Road, Edinburgh EH9 3JG
E-mail: Neil.Havis@sruc.ac.uk

Summary: Control of the eyespot fungus (*Oculimacula spp)* in winter cereal crops relies on a combination of varietal resistance and use of chemcials. However, many of the most active fungicides such as azoles and SDHI's are losing their approval. Resistance elicitors and biological fungicides have been shown to control cereal diseases when applied as foliar sprays. This paper reports on a series of trials which investigated the potential of elicitors and biological fungicides to control eyespot as either a seed treatment or an early season foliar spray.

INTRODUCTION

Common eyespot caused by the fungus *Oculimacula acuformis* (R type) and *O. yallundae* (W type) is considered the most important stem based disease in cereal crops in temperate areas (Ray *et al*, 2006). The fungus is thought to be more severe in autumn sown crops. *O. acuformis* and O. *yallundae* have a complicated life cycle and can survive on crop debris, which allows them to infect the crop in both autumn and spring. Infection takes place at the stem base and after penetration of successive leaf sheaths the stem becomes weakened and can twist, bend or even break (Ray *et al*, 2004. The typical honey brown elliptical lesions appear on the base of the stem (Figure 1)

Figure 1. Eyespot lesions on the stem base of wheat plants (image copyright SRUC).

The weakening of plant stems can lead to the lodging in the crop, particularly late in the season after grain filling, when the ear is at its heaviest (Ray *et al*, 2006). Yield losses of up to 50% have been recorded in extreme situations (Scott and Hollins, 1974), but moderate or severe eyespot infections can cause yield loss of 10-30% even in the absence of lodging (AHDB, 2020). Eyespot has been shown to restrict nutrient and water movement in the plant resulting in whiteheads in the crop (AHDB,2020).

Fungicides have been used to control eyespot for many years with azoles and succinate dehydrogenase inhibitors (SDHI's) successfully used (AHDB, 2020). However, new regulations on the use of pesticides is leading to a reduction in the availability of active ingredients which give control of Eyespot. The EU directive 2009/128/EC on the sustainable use of pesticides promotes a holistic approach to disease management and the reduction of fungicide use to reduce disease to levels below those causing economic damage (Chandler *et al*, 2011).

Plants have a range of mechanisms which can act as a defence response. (Walters, 2010). Even plants known to be susceptible to certain pathogens can show enhanced resistance if their defence mechanism is activated. This phenomenon is known as induced resistance (Ton *et al*, 2006). Plant defence systems can be induced directly or "primed" for rapid response to a pathogen challenge. Elicitor molecules or biocontrol agents have been shown to induce resistance. If used early in the growing season, elicitors may be an alternative method of disease control. The use of resistance elicitors in disease control programmes has been investigated in a number of crops (Walters and Foutaine, 2009). This paper reports on experiments and field trials investigating the potential of elicitors and biocontrol agents to control eyespot in wheat.

MATERIALS AND METHODS

Glasshouse experiment

Winter wheat (cv Beluga) was sown in 9 cm diameter pots with Fisons Levington compost. Elicitors, biological agents and fungicides were applied either as a seed treatment or as a growth stage (GS 30,stem elongation) (Zadoks *et al*, 1974) spray. Elicitor treatments used were Regalia® biofungicide (5% extract of *Reynoutria sachalinensis*) (reg), SiTKO-SA® 0-7-17 (Potassium phosphite, Potassium silicate, Potassium hydroxide and salicylic acid) (sitko) and Companion® biofungicide (0.03% *Bacillus subtilis* strain GB03) (b subtilis).The conventional fungicide, Tracker®, a combination of SDHI (boscalid) and triazole (epoxiconazole) (bos+epo), advised for good control of common eyespot (AHDB, 2020), was used to compare against elicitor treatment. Eyespot inoculum from both species were applied to the base of the plant stems at GS 12. Inoculum consisted of infected wheat stems (1.5cm lengths) following the method of Scott *et al*, 1975. At 50 days after inoculation, during tillering phase (GS 24), the main stem and strongest secondary tiller were visually assessed for disease symptoms. A robust, objective method for designation of eyespot symptom severity was devised in the following categorised format: 0 = clear of eyespot disease; 1 = small lesion on first leaf sheath under 25% stem circumference; 2 = lesion penetrated to second leaf sheath and/or lesion covers between 25% and 50% stem circumference; 3 = lesion penetrated to third leaf sheath and/or lesion covers between 50% and 75% of stem circumference; 4 = lesion penetrated to fourth leaf sheath and/or lesion covers over 75% of stem circumference and/or tissues softened. Eyespot Index, expressed as a percentage, was then calculated for each using the devised formula, adapted from Goulds and Polley (1990), shown below:

$$\text{Eyespot Index (\%)} = \frac{N1 + (N2 \times 2) + (N3 \times 3) + (N4 \times 4)}{12} \times \frac{100}{4}$$

Where, N1 = Number of tillers showing category 1 symptoms, and so on. 12 denotes the number of tillers assessed during each assessment procedure. Results were analysed using a Mintab® statistical tool package

Field trials

Winter wheat (cv Beluga) seed lots were sown in 10m x 2m plots, three reps per treatment, (at a rate of 360 seeds/m^{-2}) at a two sites in 2017-2019. An assessment of the eyespot risk at each site was carried out, based on published methods (AHDB 2020) (Table 1). Factors which contribute to risk are region, soil type, previous crop, tillage and sowing date

Table 1. Risk assessment of eyespot trials 2017-19, prior to sowing

Year	Site	Total points	Risk
2017	East Lothian	10	Medium
2018	East Lothian	10	Medium
2018	Aberdeenshire	15	Medium-High
2019	Aberdeenshire	17	Medium-HIgh
2019	Midlothian	15	Medium-High

Fungicides or elicitors were applied as either a seed treatment or as a T1 spray (GS30). The same treatments as the glasshouse experiment were used, along with an experimental biological seed treatment (biol), Laminarin™(Lam) and, Bion® seed treatment (bion) with acibenzolar s-methyl. Eyespot severity was assessed on 25 plants at GS 75 using an index similar to the one described previously, except that no category 4 infections were reported. The trials were also taken to yield. Results were analysed using the Genstat statistics package.

RESULTS

Glasshouse experiment results

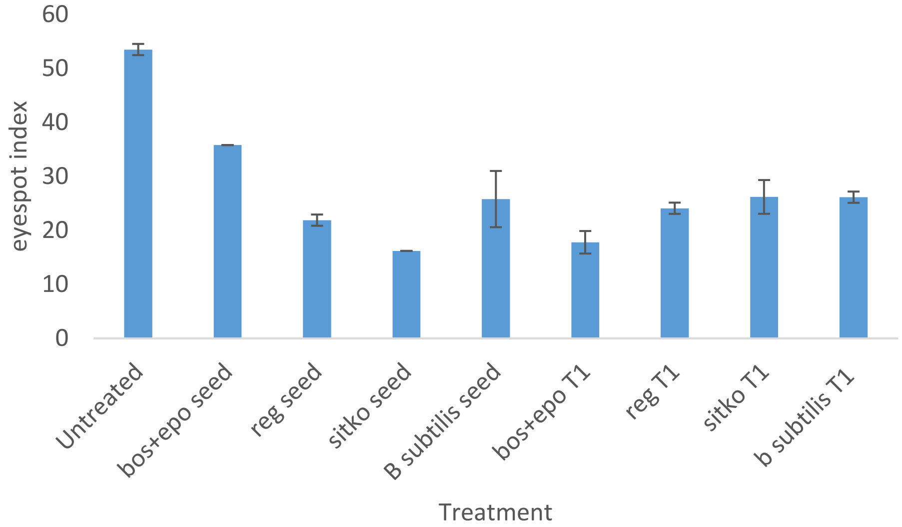

Figure 2. Mean percentage values for eyespot disease index in glasshouse experiment All treatments gave significant control of eyespot at P≤ 0.0001. LSD (P=0.05) 7.74

The sitko seed treatment gave the greatest reduction in eyespot index (17% compared to the untreated control of 53%). Of the T1 treatments, the fungicide spray bos+epo gave the lowest eyespot levels (19%).

Field trial results

The bos+epo seed treatment proved to have a serious deleterious effect on wheat germination. The crop did not establish and this treatment was excluded from the analysis.

Control of eyespot in winter wheat trials

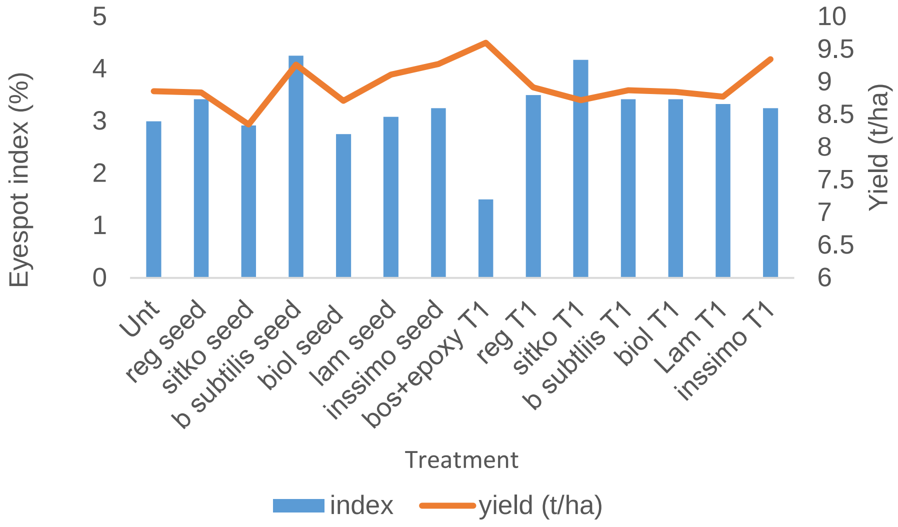

Figure 3. Eyespot index and final yield in field trial, East Lothian 2017.

The lowest eyespot index was recorded by the foliar spray of bos+epo (1.5%) in the 2017 trial. It also produced the highest yield (9.6 t/ha)

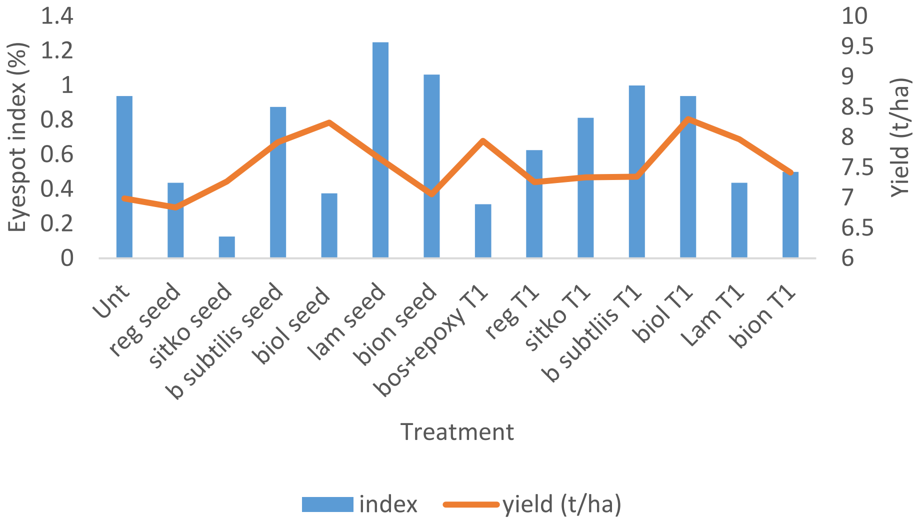

Figure 4. Eyespot index and final yield in field trial, East Lothian 2018.

The lowest eyespot index was produced by the sitko seed treatment in the 2018 trial (0.12%). The bos+epo treatment was the most effective foliar spray in controlling eyespot (0.3%). The biol treatment as a seed treatment and foliar spray gave the highest yield in the trial (8.24 and 8.3 t/ha respectively). A similar pattern was observed in the Aberdeen trial in 2018 (data not shown)

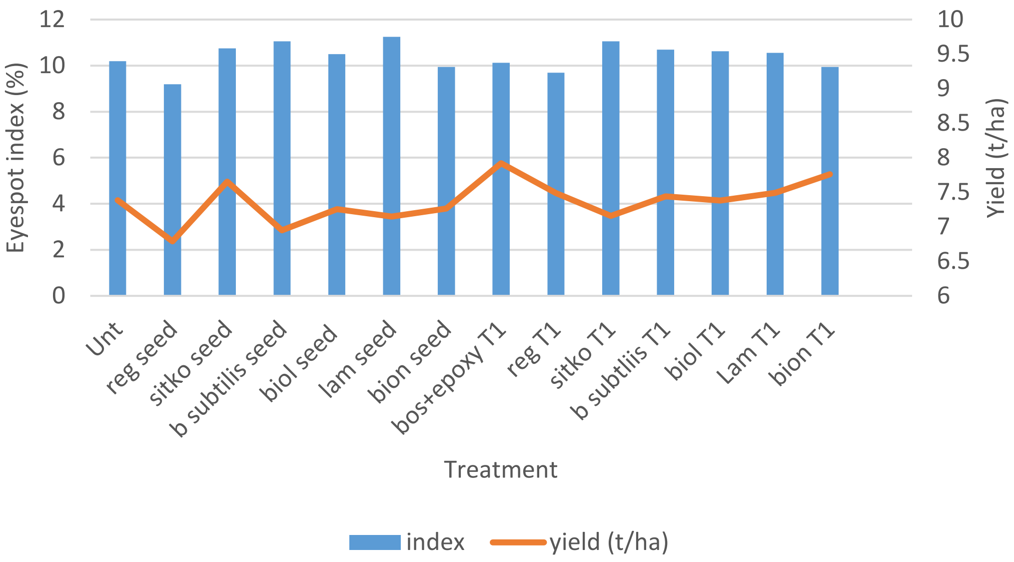

Figure 5. Eyespot index and final yield in field trial, Midlothian 2019.

None of the treatments gave signifcant control of eyespot in 2019 at the Midlothian site. The greatest yield response came from bos+epo spray at T1 tming (7.92 t/ha compared to the untreated 7.38 t/ha). A similar pattern was observed for the Aberdeen trial in 2019 (data not shown).

DISCUSSION

In the glasshouse experiment the fungicide standard (bos+epo) gave significant reduction in eyespot levels compared to the untreated (Figure 1). Disease levels in the inoculated trial were high and the plant stems were seen to be twisting due to the action of the fungus. In elicitor treated samples, several defence response related observations were made. These included morphological changes similar to the effect following treatment of plant growth regulator such as pronounced veinal ribbing, stiffening of the stem and prostrate growth habit, thus conferring a very erect appearance of the leaf canopy and a reduction of apical dominance (Roberts and Hooley, 1988). These observations suggest that plant resources are being diverted towards cell wall deposition increasing defence against the necrotrophic lesion-forming pathogenicity of *Oculimacula* spp. (Blein *et al*, 2009).

Despite the promising results obtained in controlled conditons in a glasshouse no reproducible control from either elicitors or biofungicides was achieved in the field trials. Eyespot severity varied from season to season. The highest Eyespot index percentage of 11% was seen in 2019 and repsonses to treatments were minimal. The fungus is capable of infecting plants throughout the growing season and conditions were conducive to infection in late spring and early summer, temperatures were above 5 °C and there was high humidity in the crop (Rainfall in Apr-Jun was 250mm). The elicitors and biofungicides are known to act of different plant defence pathways e.g. *B. subtilis* has been shown to illict the Induced systemic resistance pathway and control the take all fungus (Liu *et al*, 2009). Effective control of eyespot may require a more effective cocktail of elicitors and biofungicides. Work on barley disease control has indicated that elicitors and biofungicides work best in combination with reduced rate fungicides (Havis *et al*, unpublished). This approach may have to be adopted to overcome the seasonal variability observed with elicitors and biofungicides in the field trials reported here.

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INVESTIGATION OF MOLECULAR MECHANISMS ASSOCIATED WITH FUNGICIDE SENSITIVITY IN IRISH *PYRENOPEZIZA BRASSICAE* POPULATIONS

D E Bucur[1,2], Y J Huang[2], B D L Fitt[2], F Ritchie[3] and S Kildea[1]
[1]*Teagasc, Crops, Environment and Land Use Programme, Crop Science Dept. Oak Park, Carlow, Republic of Ireland*
[2]*School of Life and Medical Sciences, University of Hertfordshire, Hatfield, United Kingdom*
[3]*ADAS Boxworth, Cambridge, United Kingdom*
E-mail: stephen.kildea@teagasc.ie

Summary: Light leaf spot is amongst the most damaging diseases of oilseed rape and a significant threat to Irish crops. Unfortunately, the epidemiology of *Pyrenopeziza brassicae,* the agent causing this disease, remains poorly understood under Irish growing conditions and fungicides are relied upon to provide control. To investigate whether the cropping strategies currently used are the best for the control of disease, we screened three populations of *P. brassicae* isolates from different regions of Ireland for alterations in the genes targeted by azole and methyl benzamidazole carbamate fungicides. As molecular mechanisms associated with a decrease in fungicide sensitivity were observed in the populations, the results will be correlated with data from fungicide sensitivity tests using these classes of fungicides.

INTRODUCTION

Light leaf spot (LLS) disease of brassicas, caused by the hemibiotrophic fungal pathogen *Pyrenopeziza brassicae* (anamorph *Cylindrosporium concentricum*), is known as one of the most damaging diseases of winter oilseed rape (*Brassica napus* spp.) in northern Europe (Boys *et al.*, 2007). In Ireland, *P. brassicae* was observed for the first time during the 1964-1965 season, causing light leaf spot disease on broccoli, cabbage and Brussels sprouts (Staunton, 1967). As the area sown to oilseed rape in Ireland has increased from 2,300 ha in 2003 to 17,000 ha in 2012 (Collins & Phelan, 2018) and the Irish Tillage Sector Development Plan (2012) highlighted the potential to increase its production even more, LLS is regularly observed in Irish oilseed rape (OSR) fields.

Although believed to be a significant threat to Irish oilseed rape crops, the epidemiology of *P. brassicae* under Irish growing conditions is poorly understood. Currently, LLS control involves the use of resistant cultivars and fungicide applications, typically one in late autumn and a second in early spring. However, as disease symptoms are frequently not visible until late winter (Gilles, 2000), the correct timing for the first fungicide application is difficult to determine (Fitt *et al.*, 1998). Equally, in early spring the second fungicide spray is often applied to crops with symptoms, which may be too late for the fungicides to be effective. In addition, the potential for the development of fungicide insensitivity in *P. brassicae* populations (Carter *et al*., 2014) to further compounds will affect our ability to control the disease. As limted data are available on the fungicide sensitivity of the Irish *P. brassicae* population we aimed to investigate the molecular mechanisms correlated with decreases in sensitivity to azole and MBC, two of the classes of fungicides currently/previously used in controlling LLS. As a decrease in sensitivity to azoles has been correlated with alterations in the regulatory and coding region of the sterol 14α-demethylase gene (*CYP51*), the gene targeted by these fungicides, we investigated the presence of alterations identified by Carter *et al.* (2014) in Irish *P. brassicae* populations.

Similarly the presence of the mutations E198G and L240F in the β-tubulin, previously confirmed by Carter *et al.* (2013) as conferring MBC resistance, was determined.

MATERIALS AND METHODS

Collection establishment

Leaves presenting characteristic symptoms of light leaf spot, such as the white acervular conidiomata pustules with a circular distribution (Ashby, 1997) were randomly sampled from three oilseed rape crops (Cv. Phoenix) located in three different regions of Ireland: Co. Carlow (East), Co. Cork (South) and Co. Louth (North). At the time of sampling, early March 2019, each crop had received a fungicide treatment (Proline) in late autumn. At sampling, diseased leaves collected from each field were further incubated separately in polyethylene bags for 2-4 days at 4°C in order to promote *P. brassicae* asexual sporulation. Following this, single conidiomata were identified using a dissection microscope and isolated from each leaf using a sterile needle. The colonies obtained were subcultured several times on potato glucose agar amended with ampicillin and streptomycin sulphate to obtain single spore colonies. A subsample of the collections established was used for further molecular analysis.

DNA extraction

Single spore colonies grown from glycerol stocks at 18°C for 19 days were used to inoculate 50 ml Falcon tubes containing 30 ml potato dextrose broth. After 5 weeks of growth at 20°C and 240 RPM, *P. brassicae* mycelium was separated by centrifugation, freeze-dried for 24 h and homogenised using a benchtop mixer mill (Retsch Mixer Mill). The DNA was extracted using a GenElute™ miniprep kit according to the manufacturer's protocol (Sigma-Aldrich, Missouri, United States).

Amplification of *CYP51* regulatory region

A 662 bp sequence representing the predicted regulatory region of *CYP51* was amplified using the primer pair CYP51upstreamF / CYP51upstreamR (Carter *et al.*, 2014). PCR was done using *Taq* DNA polymerase with ThermoPol Buffer (New England BioLabs Inc., Massachusetts, United States). The PCR conditions were 95°C for 2 min, followed by 35 cycles at 95°C for 30 s, 57°C for 30 s and 72°C for 1 min, followed by a final extension of 5 min at 72°C. The PCR product obtained was migrated in electrophoresis on a 1% agarose gel and visualised under UV-light using an ENDURO™ GDS Gel Documentation System for Electrophoresis (Labnet International, Inc. New Jersey, United States).

Detection of codons 460 and 508 from *PbCYP51* coding region

The 1244 bp fraction of *PbCYP51* encompassing codons 460 and 508 was amplified using the primers pair CYP51expressionF1 / CYP51R (Carter *et al.*, 2014), using *Taq* DNA polymerase with ThermoPol Buffer (New England BioLabs Inc., Massachusetts, United States). The PCR reaction conditions used were as described above, with an annealing temperature of 56°C and an extension time of 1:30 min. Two units of *TspRI* (CASTG) (New England BioLabs Inc., Massachusetts, United States) were used to detect G460S, using approximately 60 ng of purified PCR product and 1 × NE buffer 4 in a total volume of 10 µl, and the mix was incubated for 2 h at 65°C. In order to detect the mutation S508T, approximately 60 ng of the purified PCR product were digested with 1 unit of *BssSI* (CACGAG) and 1 × NE buffer 3 in a total volume of 10 µl and the mix was incubated for 2 h at 37°C. The digested product obtained was separated on a 1% (w/v) agarose gel and exposed to UV-light to visualize DNA fragments.

PCR-RFLP detection of E198A and L240F substitutions in the *β-tubulin* gene

The primer pair PZtubF1 / PZtubR1 (Carter *et al.,* 2013) an 865 bp fragment from the *β-tubulin* gene, including codons 198 and 240 was amplified. The reaction mix used was similar to the one mentioned above, and the reaction conditions were 95°C for 5 min, followed by 30 cycles at 95°C for 1 min, 52°C for 1 min and 72°C for 1 min, with a final extension step of 5 min at 72°C. The PCR product obtained was purified and approximately 60 ng PCR product was digested with 1 unit of *BsmAI* enzyme (GTCTCN) (New England BioLabs Inc., Massachusetts, United States) and 1× NE buffer 4 in a total volume of 10 µl and incubated for 2 h at 55°C. The restriction fragments resulted were separated in a 3% (w/v) electrophoresis gel and visualised under UV-light.

RESULTS

A total of 47 *P. brassicae* isolates were used to detect the alterations in the genes targeted by azole and MBC fungicides (14 were sampled from Co. Carlow, 11 from Co. Louth and 22 from Co. Cork).

Detection of alterations in the regulatory and coding region of *PbCYP51* gene

The analysis of the regulatory region of *PbCYP51* showed the presence of the three inserts in all three populations screened. Wild type isolates without any type of insert were present in all three populations, representing 34% of the entire collection. Amongst the remaining isolates, the 151 bp insert was most frequent in the collection, followed by the 46 bp insert, however, differences existed between the collections in terms of the frequencies of the inserts in each (Figure 1).

Figure 1. Frequencies (%) of isolates presenting S508T and G460S alongside the different types of inserts observed for the regulatory region of *PbCYP51* in Irish populations of *P. brassicae* sampled from Carlow, Louth and Cork. Error bars represent SEM, df = 12.

The digestion of 1244 bp *PbCYP51* fragment with *BssSI* resulted in detection of S508T substitution in 4 out of the 47 isolates analysed, two of which came from the Louth population, one from Carlow and one from Cork (Figure 2).

Nine isolates presenting G460S were identified in the three populations screened following the digestion of the purified PCR product with *TspRI*, four of which came from the Louth population, two from Carlow and the remaining three from Cork (Figure 3).

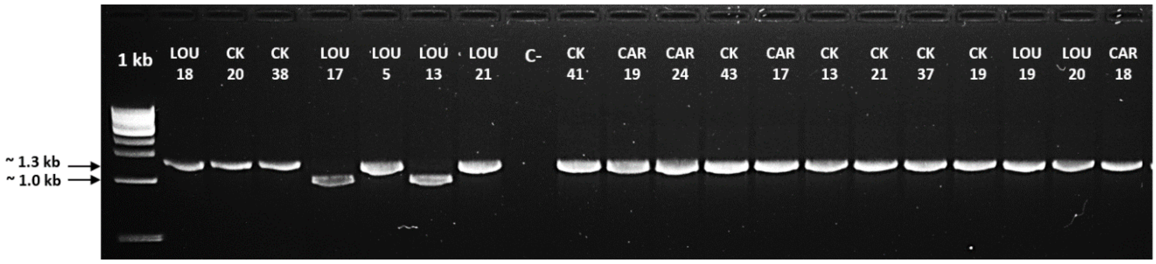

Figure 2. Restriction digest of 1244 bp fragment of *PbCYP51* using *BssSI to* detect the substitution S508T. Arrows indicate the size of bands obtained, 1 kb bands are specific for the isolates with the mutation. Sites from which *P. brassicae* isolates were obtained: LOU – Louth, CK – Cork, CAR – Carlow, C^- – Negative control.

Analysing the possible combinations of the two mutations in the coding region and the inserts from the regulatory region, six different combinations were observed in the *P. brassicae* populations: G460S with each of the different inserts, G460S + S508T, and G460S + S508T, with either the 151 bp or 232 bp insert.

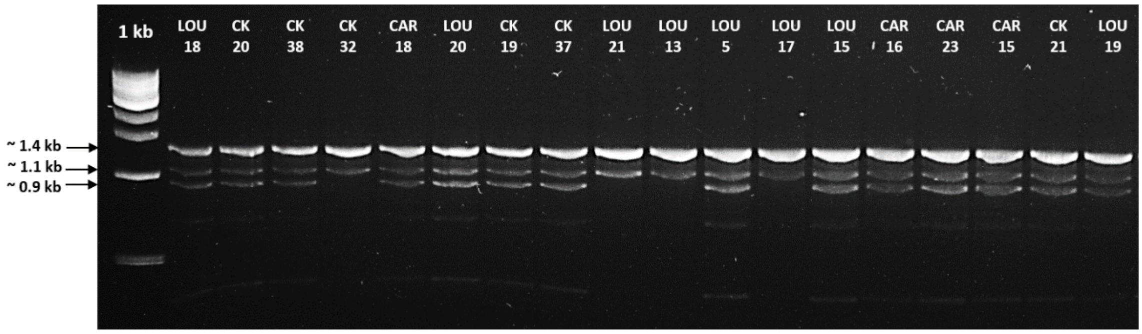

Figure 3. Restriction digest of 1244 bp fragment of *PbCYP51* using *TspRI to* detect the substitution G460S. Arrows indicate the size of bands obtained, the absence of 900 bp bands is characteristic for the isolates with the mutation. Sites from which *P. brassicae* isolates were obtained: LOU – Louth, CK – Cork, CAR – Carlow.

The average frequencies of all the possible alteration were not significantly different between the populations, and there were no significant differences observed between the three populations screened based on the frequencies of the two possible substitutions in the coding region (P = 0.125). This was also the case comparing the populations based on the different variants of the insert from the regulatory region, the P-value obtained being 0.18225.

Population screening for detection of resistance to MBC fungicides

After the digestion with *BsmAI* of the 865 bp purified PCR product, the isolates were divided into three groups: Sensitive (S), Moderately Insensitive (MI) and Insensitive (I), based on the size and number of bands obtained, as described by Carter *et al.* (2013): the isolates presenting five bands (461bp, 200 bp, 104 bp, 69 bp and 31bp) were grouped as sensitive, while the isolates with only 4 bands were classified as insensitive (565 bp, 200 bp, 69 bp and 31bp, the 69 bp) or moderately insensitive (461bp, 200 bp, 104bp and 100 bp) to methyl benzamidazole carbamates. According to Carter *et al.* (2013) the absence of 100/104 bp band is caused by the E198A substitution in the insensitive isolates, whereas the absence of 69 bp band is caused by L240F in the moderately insensitive isolates (Figure 4). The difference between the resistant isolates and the other two types was caused by the 565 bp band, while the difference between the sensitive and the moderately resistant isolates was caused by the presence of 69 bp and 31bp bands in the sensitive ones.

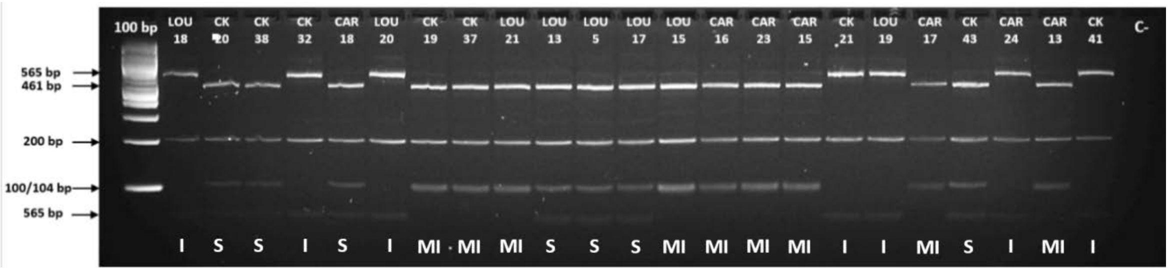

Figure 4. Restriction digest of 865 bp fragment *β-tubulin* gene detecting the substitutions E198A and L240F in order to differentiate between Insensitive (I), Moderately Insensitive (MI) and Sensitive (S) isolates. Arrows indicate the size of bands obtained. Sites from which *P. brassicae* isolates were obtained: LOU – Louth, CK – Cork, CAR – Carlow.

All types of isolates were present in all three populations screened. The isolates with sensitivity to MBC fungicides were predominant in the population sampled from Cork, while for Carlow the moderately insensitive isolates dominated in the population, whereas, in Louth, the same number of sensitive and insensitive isolates have been observed (Figure 5).

Figure 5. Frequencies (%) of isolates with different degree of insensitivity to MBC fungicides in Irish populations of *P. brassicae* sampled from Carlow, Louth and Cork. S – isolates without alterations in the β-tubulin gene, MI – isolates with L240F and I – isolates with E198A. Error bars as SEM, df = 4.

For all the locations screened, the frequency of isolates with E198A was the lowest (Figure 5). There were no significant differences observed between the three populations screened based on the frequencies of the three types of isolates monitored: presenting L240F, E198A or without any alterations, and the P-value obtained was of 0.4.

DISCUSSION

The results obtained from this preliminary molecular screening suggest that limited differences, if any exist between the main OSR producing regions in Ireland in terms of sensitivity of *P. brassicae* to either the azole or MBC fungicides. However, they do confirm the presence of alterations previously associated with decreased sensitivity to the azole and MBC fungicides in the Irish *P. brassicae* populations (Carter *et al*., 2014).

We observed that most of the population analysed had neither the substitution S508T or G460S, inserts in the regulatory region, potentially indicating a population largely sensitive to azoles. However, comparing the regulatory and coding regions of the gene confirmed the populations had a greater tendency to have inserts in the regulatory region than mutations in the coding region. Although Carter *et al*. (2014) detected the amino acid substitution G508T alone, in the

Irish collection of *P. brassicae* isolates it was detected only in combination with G460S alone, or with G460S and either the 151 bp or 232 bp insert. Interestingly neither G460S or S508T were detected in any of the isolates with the 46 bp insert in the regulatory region. Whilst most of isolates may not have G460S or S508T, complete sequencing of the *PbCYP51* is required to determine if additional mutations are present.

The frequencies of the two alterations in the *β-tubulin* gene, E198A and L240F, known to confer different levels of insensitivity, did not significantly vary between the three sites. However, as with the azoles, both mutations were detected in all three populations indicating the presence of insensitivity in the Irish populations. Again, isolates without either mutation dominated the populations. As the MBC fungicides have not been used in Irish OSR crops for a number of years, the low incidence of either mutation may reflect this.

Following the detection of the different inserts/mutations associated with azole and MBC insensitivity, the next steps will be to determine the sensitivity of the Irish population to these two classes of fungicides in order to confirm the degree of sensitivity established in the *P. brassicae* population.

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THE OPPORTUNITIES AND CHALLENGES OF REDUCING FUNGICIDE USE IN SPRING BARLEY

S Stetkiewicz[1], K Topp[2] and F J Burnett[2]
[1]*Lancaster Environment Centre, Pentland Centre for Sustainability in Business, Lancaster University, Bailrigg, Lancaster LA1 4YW*
[2]*Crop & Soil Systems Group, SRUC, West Mains Road, Edinburgh EH9 3JG*
E-mail: s.stetkiewicz@lancaster.ac.uk

Summary: This study assessed the potential of three integrated pest management techniques – crop rotation, varietal disease resistance, and forecasting disease pressure – to reduce the need for fungicide use in Scottish spring barley. To address this question, data from three sources was pooled and compared: a long-term experimental field trials database; a participative survey of stakeholders; and the Adopt-A-Crop database of commercial practice. Results suggest that IPM techniques such as varietal disease resistance rating have potential for reducing the need for fungicide use in this system, and that farmers are theoretically open to these management changes, but that they are not currently widely adopted.

INTRODUCTION

Integrated Pest Management (IPM) is an ecosystem approach to managing pests and diseases on farm while minimising the use of pesticides (FAO, 2017). IPM can encompass a range of techniques, including spraying pesticide, which vary based on the needs of the farming system. In this project, three IPM techniques with potential to reduce the need for fungicide use were focused on, within the context of Scottish spring barley production: crop rotation, disease resistance, and forecasting disease pressure. Barley is an important global crop, with over 53 million hectares harvested each year (FAOSTAT, 2013), and is of particular importance in Scotland, where spring barley is the main cereal crop (Scottish Government, 2016). Fungal pathogens are important pests of barley, and have been estimated to cause total yield losses in the range of 15% globally (Oerke & Dehne, 2004). Three fungal diseases of importance to Scottish spring barley were selected for this project's focus: powdery mildew (caused by *Blumeria graminis* formae specialis *hordei*), Rhynchosporium (caused by *Rhynchosporium commune*), and Ramularia (caused by *Ramularia collo-cygni*).

In order to more fully understand the potential for IPM to reduce the need for fungicide use in this system, data from three sources was compared: field trial experimental data, a stakeholder survey, and a commercial practice database. Using this interdisciplinary method, and bringing together these diverse sources of data provided a more holistic view of the production system than would otherwise have been possible.

MATERIALS AND METHODS

Experimental Field Trials Database

An SRUC database running from 1996 – 2014 collected field trial data from experimental plots which allowed the comparison of untreated and (best practice) treated plots at a range of locations across Scotland. Recent data (2011-2014) was used to assess impacts of fungicide on yield using analysis of variance. Due to a lack of detailed information available for previous years, stepwise regressions were used to assess data the full dataset (1996-2014). For more detail on the database and the methods used to analyse the Field Trials data, please see Stetkiewicz *et al.* (2019).

A simple economic analysis conducted compared mean difference between treated and untreated yields for each year to the cost saved by not purchasing fungicides in that year, based on data from the SAC/SRUC Management Handbooks from the relevant years (SAC Consulting, 2010), and barley price data from the AHDB (AHDB, 2016) (assuming comparable quality between untreated and treated barley). This estimate gives an indication of the financial impacts of reducing fungicide use, but does not take into account other related issues, such as changes to machinery, fuel, and labour costs.

Stakeholder Surveys

Stakeholder surveying of 43 Scottish spring barley farmers and 36 agronomists was carried out at SRUC/AHDB Cereal events in 2016, and focused on understanding current practice and attitudes towards taking up the selected IPM techniques in future. For more detail on the methods used in relation to the survey, and to see a copy of the survey used, please see Stetkiewicz *et al.* (2018).

Adopt-A-Crop (AAC) Database

The Adopt-a-Crop (AAC) database of annual surveys of commercial crops from over 500 farms across Scotland was used to assess the current levels of uptake of disease resistant varieties and crop rotation on-farm. For more detail on the AAC database and the methods used in relation to analysing the AAC, please see Stetkiewicz (2018).

RESULTS

Field Trials database

The majority of trials in the period 2011-2014 did not show a statistically significant impact of fungicide treatment on yield, and the average yield increase due to fungicide application during this period was 0.62 t/ha.

Table 1. Fungicide impact on yield in 2011 – 2014 Field Trials database.

	Mean yield (t/ha)	Standard error of mean (t/ha)	Median yield (t/ha)
Untreated	6.23	0.11	6.38
Treated	6.84	0.12	6.82
Difference	0.62		0.44

In the full 1996 – 2014 database, the difference between treated and untreated yields could be explained by disease resistance (varieties with a high level of resistance to one or more of the three diseases were deemed ‘resistant’ varieties), average season rainfall, and high combined disease severity. The final model R^2 was 21.2%, with season rainfall, disease resistance, and combined AUDPC for the three diseases each having a similar impact on R^2 when removed from the model (5.7, 5.5, 4.3, respectively).

The simple economic analysis estimated a resulting difference in profit between treated and untreated fields of less than 5% for both malting and feed varieties in the years assessed, with some years showing a net negative difference, highlighting that in certain years the costs of fungicides were not recouped by the increase seen in yields from treated plots.

Stakeholder Surveys

Stakeholders were open to taking up IPM measures, with disease resistant varieties being most frequently selected as the best technique in terms of both practicality and cost. However, farmers and agronomists overestimated the impacts of fungicide treatment on yield as compared with the yield impacts found in the analysis of the Field Trials database. Farmers also reported using IPM measures, such as varietal disease resistance, but when asked to provide lists of the varieties sown in the past five years, the mean disease resistance ratings for the varieties listed by farmers contradicted this (see Table 2). This is despite the fact that 66% of farmers indicated that varietal disease resistance was an important/very important source of information when choosing which variety to sow (see Table 3). Similarly, while the majority of farmers stated that they often/always used crop rotations, the majority also often/always sowed consecutive barley and/or cereals on their farms (see Figure 1).

Farmers also overestimated the impact of fungicide treatment as compared with the yield differences seen in the Field Trials database. While 71.8% of farmers believed that fungicide treatment increased spring barley yields by 1-2 t/ha, the average yield difference seen in the 2011-2014 Field Trials database was only 0.62 t/ha.

Table 2. Comparison of the percentage of surveyed farmers claiming to sow highly resistant varieties always/often with the percentage of varieties sown by these farmers which were highly resistant to each disease

	Percent of farmers who stated that they always/often sowed highly resistant varieties for this disease	Percent of varieties listed as sown in the past five years by the same farmers which were highly resistant to this disease
Ramularia*	66	27
Rhynchosporium	66	23
Mildew	76	85

*For all results relating to disease resistance for Ramularia presented in this paper, this has been calculated based on the data available for 2012 onwards only (the first year for which disease resistance ratings were published for this disease in the Cereals Recommended List (SAC & HGCA, 2012))

Table 3. Importance of sources of information to varietal selection

	Percent of surveyed farmers choosing this source as important/very important
Market demand for a particular variety	93%
Variety had malting/brewing certification	81%
Having prior experience with the variety on my farm	66%
Varietal disease resistance rating	66%
Agronomist selection	27%
Suggestion from/grown by another successful farmer in my area	23%

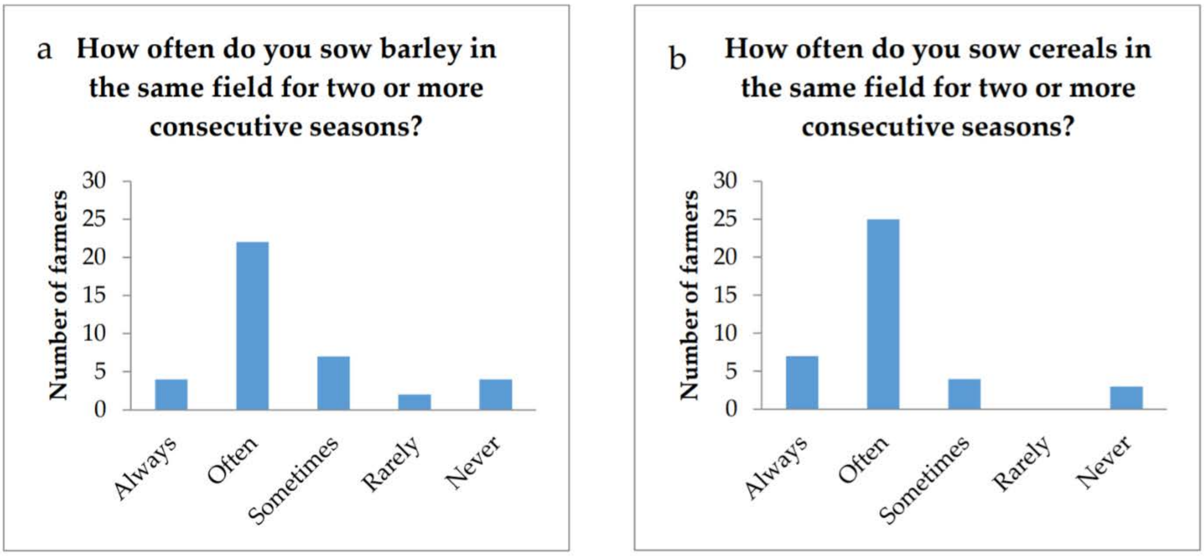

Figure 1a & b. Self-reported frequency of use of consecutive a. barley, or b. cereals

Adopt-A-Crop Database

The survey finding that farmers did not sow highly resistant varieties was further confirmed in the AAC database analysis – less than 15% of varieties sown were highly resistant to Rhynchosporium, with 26% being highly resistant to Ramularia, although more than half (58%) were highly resistant to mildew (see Table 3). Highly resistant is here defined as a score of 7 or above in that year's SRUC/AHDB Cereals Recommended List (SRUC & HGCA, 2019). In addition, nearly three-quarters of farms in the database had sown barley in two consecutive seasons, indicating great scope for improvement of crop rotation practices.

In addition, the majority of varieties sown in both the AAC and listed in the farmer survey were below the 'Best Choice' variety for that year (see Figure 2) – here defined as the highest rated variety which had full recommendation for distilling in the Cereals Recommended List for that year; this is intended as a measure of farmer uptake of resistance within the constraints of the pressures of the malting market.

Table 4 . Percent of varieties sown in AAC and survey which were highly resistant

	Ramularia	Rhynchosporium	Mildew	Any Resistance*
AAC	26%	14%	58%	75%
Farmer survey	18%	19%	84%	84%

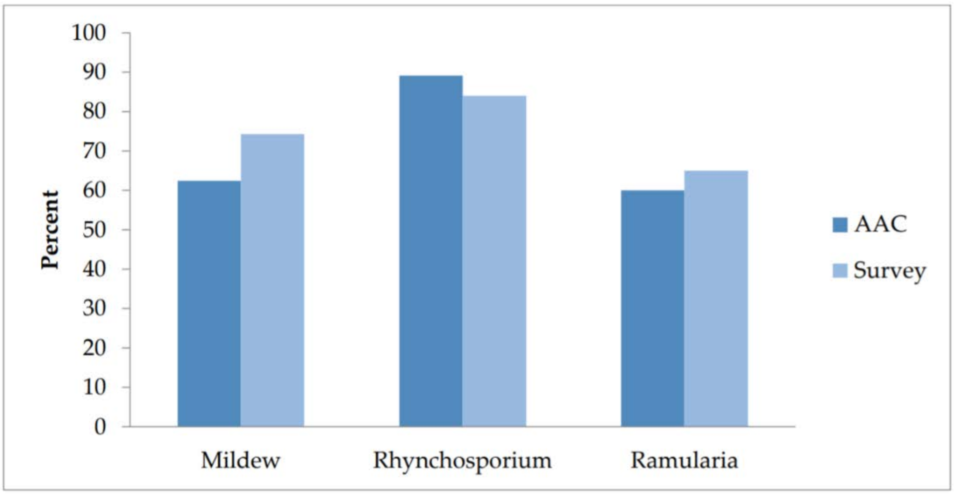

Figure 2. Percent of varieties sown in AAC and survey which were below the 'best choice' for that year

DISCUSSION

The main finding of this project was that there is scope for IPM uptake to be improved upon in Scottish spring barley production, while maintaining high yields. In particular, the use of more highly resistant crop varieties and crop rotation practices could help to reduce the need for fungicides, and could be taken up by a much larger segment of the farming population than is currently the case. In addition, given that yield differences between treated and untreated crops was explained in the regression models by a combination of disease resistance, average season rainfall, and high combined disease severity, there appears to be scope for weather-related decision support tool use in order to better target the seasons when fungicide application will provide the greatest benefit.

The interdisciplinary nature of the project, which brought together data from a range of sources, and allowed cross-comparison of, for example, yield impact figures from the field trials data with farmer perception of yield impacts from fungicide use allowed for a more detailed picture of the potential for IPM in this system. The gap between farmer perception and practice is of particular concern, as if farmers believe they are using IPM strategies such as disease resistance to their fullest, and yet seeing little benefit on farm, they may dismiss these strategies unfairly, if in reality the varieties planted are not highly resistant. This project also highlights the importance of long-term data sources, which offer a unique opportunity (and challenge) to assess trends and changes over a variety of climatic conditions, which may be particularly relevant for stakeholder directed messages, and for considering impacts in the future under variable climatic conditions.

Future research into highly resistant varieties which meet distilling requirements may be beneficial in this system, but attention must also be paid to the reasons behind lack of uptake of IPM measures, and the importance of market pressures in influencing farmer behaviour.

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This paper summarises the results reported in detail in the lead author's PhD thesis and related papers. For more detailed information regarding methods and outputs, please refer to these publications.

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ASSESSING THE STATUS OF PYRETHROID RESISTANCE IN CEREAL APHIDS

D J Leybourne[1] and S P Foster[2]
[1] *Pest Management & Entomology, ADAS, High Mowthorpe, North Yorkshire*
[2] *Biointeractions and Crop Protection, Rothamsted Research, Harpenden, Hereford*
E-mail: Daniel.Leybourne@adas.co.uk

Summary: Insecticide resistance is an ongoing problem for crop protection, and resistance is continually evolving against a decreasing number of available actives. It is therefore important to detect any reduced sensitivity to insecticides early on so that action can be taken to mitigate the development of full resistance. The two dominant cereal aphid species in the UK are the English grain aphid (*Sitobion avenae*) and the bird cherry-oat aphid (*Rhopalosiphum padi*). These are both significant vectors of cereal viruses, and with the loss of neonicotinoid seed treatments, through the recent EU ban, they are now primarily managed with pyrethroids. This limited availability of chemical control options will inevitably increase selection pressure for the evolution of pyrethroid resistance. Indeed, reduced sensitivity to pyrethroids has already been reported in *S. avenae*, however little information is available on pyrethroid sensitivity in *R. padi*. In this project, we will sample *R. padi* and *S. avenae* populations and subject these to insecticide resistance bio-assays. We will test aphid populations from the main cereal growing areas of the UK and provide updated information on the status of pyrethroid sensitivity while simultaneously highlighting populations at risk of developing full resistance which would lead to pyrethroid control failures.

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EVALUATING THE EFFICACY AND PERSISTENCE OF PRE EMERGENCE HERBICIDES ON BLACK-GRASS (*ALOPECURUS MYOSUROIDES*) UNDER TWO CROP ESTABLISHMENT METHODS IN WINTER WHEAT

C Simpson[1], W Smith[2], A J Hilton[3] G Anderson-Taylor[1] and W J Thomson[3]
[1] Bayer Crop Science, Cambridge Science Park, Cambridge, CB4 0WB
[2] NIAB, Huntingdon Road, Cambridge, CB3 0LE
[3] SRUC, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA
E-mail: craig.simpson@bayer.com

Summary: This study investigated the efficacy and persistence of diflufenican + flufenacet, diflufenican + flufenacet + aclonifen and diflufenican + flufenacet + metribuzin on black-grass *Alopecurus myosuroides* under two establishment methods. A crop trial in Cambridgeshire assessed the performance of the herbicides on black-grass in direct drilled and cultivated plots. The wheat plots were established and six weed assessments were made within the fixed quadrats from November to March. The results show that using an integrated approach of herbicides and the correct cultivation choice can help to reduce the overall black-grass burden. The study found the cultivated plots had improved blackgrass control and all three herbicides provided significant control over the untreated plots. Whilst differences between the products were not significant, the results indicate that the addition of metribuzin or aclonifen to flufenacet + diflufenican products could be valuable tools for controlling black-grass in future years as part of an integrated weed management strategy.

INTRODUCTION

Black-grass (*Alopecurus myosuroides)* is an annual plant that is often found in lowland arable areas. Black-grass is widespread in northern Europe including England, France, Germany and Belgium. In the United Kingdom, black-grass is mainly concentrated in the south and east of the country, although it is increasingly common in northern England and the Scottish Borders. Competition from black-grass plants has an adverse effect on crop yield and this consensus is supported by published literature (Storkey *et al* 2003). The impact of black-grass populations will vary, however in wheat crops where the black-grass density exceeds 100 plants/m^2 yield losses of over 2 t/ha are predicted (Moss, 2013). The majority of black-grass in the UK germinates in the autumn. The period of dormancy can vary due to environmental factors (Swain *et al*, 2006) resulting in a protracted germination. Therefore, the persistency of selected herbicides play an important role in controlling black-grass in these situations. It has been reported that the optimum germination depth for black-grass is within 0-2cm of the soil surface but some seeds are able to germinate whilst buried to a depth of 4cm (Froud-Williams *et al*, 1984). Deep cultivating and specifically ploughing can reduce the black-grass population (Lutman *et al*, 2013) by burying the seeds and reduce the risk of black-grass emerging. Despite this, different forms of reduced tillage remain popular in the UK where the effectiveness of black-grass control should be monitored, particularly in high-pressure situations. Herbicides remain an important control method despite black-grass developing resistance to a number of herbicides including: pendimethalin, iodosulfuron-methyl-sodium, mesosulfuron-methyl, propoxycarbazone-sodium, and pyroxsulam in the UK. The development of resistance to post emergence herbicides, has seen increased reliance on pre emergence herbicides with residual activity for black-grass control such as flufenacet (FFA) and diflufenican (DFF) (Bailly *et al*, 2012). A study published in 2019 has found some UK black-grass populations with reduced sensitivity to FFA and that additional active ingredients can help to control these difficult populations (Dückera *et al*, 2019). Bayer have recently gained approval for products containing FFA, DFF + metribuzin (MRB). Dückera *et al* (2019) showed that the addition of MRB to FFA would help to control difficult populations of black-grass, particularly as MRB has an alternative mode of action. MRB is in the HRAC group C1. Similarly, Bayer are seeking approval for products containing aclonifen (ACL). As with MRB, ACL has been found to increase control of difficult black-grass populations in co-formulation with FFA + DFF and additionally provides an alternative mode of action. ACL is in the HRAC group F3. The reported trial was conducted as part of the ongoing research into these new products under two different pre-drilling methods.

MATERIALS AND METHODS

Field Trial

The field trial took place at NIAB TAG's Hardwick trial site. The soil is classified as clay with a typical break down of 47% clay, 18% sand and 35% silt. The field has a history of black-grass and the crop of winter wheat followed a previous crop of winter beans. The variety KWS Siskin was drilled at 173kg/ha with a target population of 350 plants per m^2.

Trial Design

The trial consisted of two separate blocks, one direct drilled straight into the winter bean stubble with a John Deere 750A drill. The direct drill only cultivated the top 2cm of soil with the intention of creating a low level of soil disturbance and having a higher level of previous crop residue. The other block was cultivated with a tine and disk cultivator to below 12cm of the soil surface before being sown with the John Deere 750A drill. Within each cultivation block, there were four treatments replicated three times in a random complete block design. Each plot measured ten meters by two.

Treatments

The plots were treated with the following herbicide programme using a knap sack sprayer at a 200l/ha spray volume:

- 60g diflufenican(DFF) and 240g flufenacet(FFA) - (0.6L/ha Liberator)
- 60g diflufenican(DFF) and 240g flufenacet(FFA) + 70g metribuzin(MRB) - (1L/ha Alternator Met)
- 60g diflufenican(DFF) and 240g flufenacet(FFA) + aclonifen(ACL) - (Coded SP29995, product and active rates not provided due to commercial confidentiality)
- Untreated

Within each plot, five fixed 0.5m^2 quadrats were monitored throughout the season for untreated plots and herbicide treated plots within both the minimal cultivation and cultivated blocks of the trial.

Establishment methods and black-grass assessment method

The quadrats were inspected throughout the growing season, with six assessments taking place between November and March. Most black-grass within the UK germinates in the autumn therefore two inspections took place in November, with an inspection per month taking place after this (Table 1). The number of black-grass plants present within each quadrat was recorded at each stage before being compiled to generate a mean number of black-grass plants present at that timing. In the third (middle) quadrat within each plot the black-grass were marked individually with different coloured tags (at each inspection) to ensure weeds were clearly identifiable and also to provide a visual representation of the pattern of germination.

Table 1. Dates of establishment procedure and plant counts

Procedure	Date(s)
Stale seedbed	27/SEP/2018
Plots sown	23/OCT/2018
Pre emergence herbicides applied	25/OCT/2018
Plant Count 1	6/7/NOV/2018
Plant Count 2	22/23/NOV/2018
Plant Count 3	10/11/DEC/2018
Plant Count 4	4/7JAN/2019
Plant Count 5	1/4/FEB/2019
Plant Count 6	5/6/MAR/2019

The data was then recorded in Microsoft Excel© before being transferred to Genstat and a repeated measures ANOVA was conducted to identify statistical differences between the sampling time, herbicide treated and untreated black-grass as well as between the products.

RESULTS

The results from the untreated plots show the majority of black-grass emerged before December (Figure 1). The black-grass numbers peaked in February before declining in March in both cultivation plots. Throughout the trial, the direct drilled plots had a greater level of black-grass recorded compared to the cultivated plots.

Impact of cultivation method on black-grass emergence in Untreated Plots

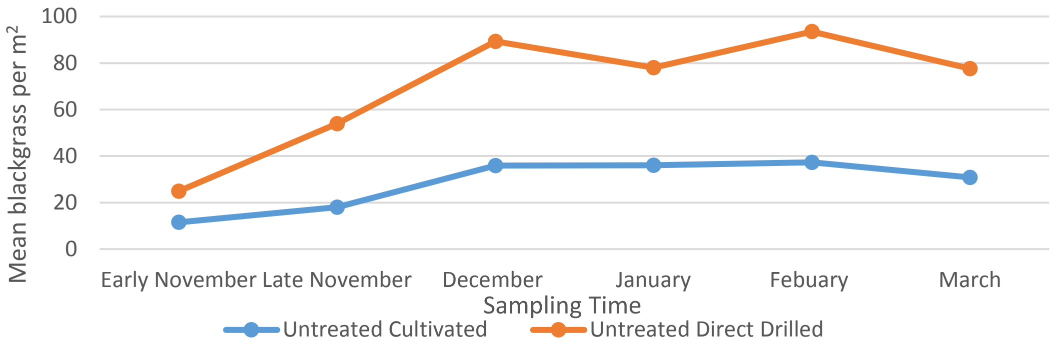

Figure 1 Mean black-grass/m^2 in untreated plots for both cultivation methods

Impact of herbicide treatments on black-grass emergence in Direct Drilled Plots

In the direct drilled trial, there was a significant time and treatment effect ($P<0.001$) which confirmed that untreated plots had consistently more black-grass than those treated with herbicide (Figure 2). In the untreated plots, the February plant count had the highest mean incidence of black-grass at 93.5 per m^2 whereas the mean black-grass in the herbicide treated plots was highest in December at only 22.3 per m^2 (mean of all three herbicides). Whilst a statistically significant difference between the individual herbicide products was not seen, the addition of MRB and ACL to FFA + DFF did result in these herbicides having a lower population of black-grass throughout the inspections compared to straight FFA + DFF (Figure 2).

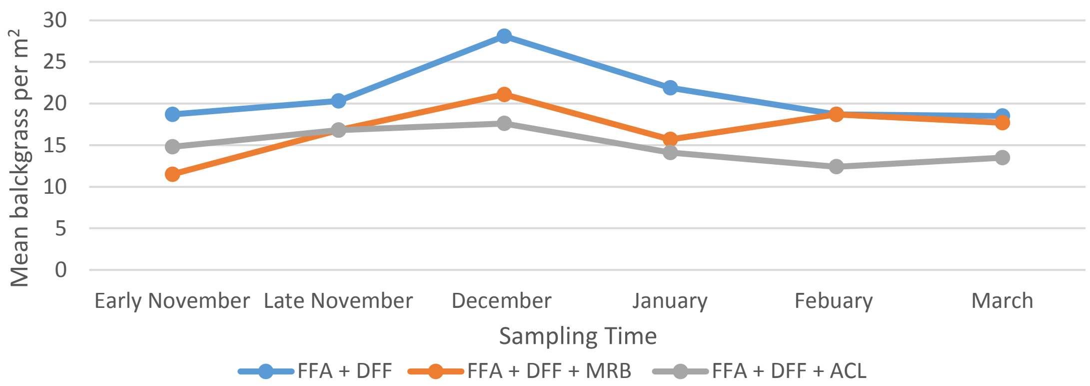

Figure 2 Mean black-grass/m^2 per sampling time and product in direct drilled trial

Impact of herbicide treatments on black-grass emergence in cultivated plots

Although the level of black-grass recorded in the cultivated plots was lower than recorded in direct drilled plots, there was still a significant difference between the black-grass recorded at the different sampling timings ($P<0.001$) and a significant difference between those which were treated and untreated ($P<0.001$). As found for the direct drilled plots, the untreated cultivated plots in February had the highest mean incidence of black-grass, whereas the herbicide treated cultivated plots had the highest incidence of black-grass earlier in the season. All treatments significantly reduced black-grass numbers compared with the control. Whilst no statistical differences were observed between the individual herbicide treatments, the application of FFA + DFF + MRB had the lowest weed levels throughout the season (Figure 3). Despite the FFA + DFF + ACL having the highest level of black-grass in late November, the counts from December until March were lower than the plots which had just received FFA + DFF. In comparison to the direct drilled plots all three herbicide treated cultivated plots had a lower level of black-grass present at the time of the final inspection.

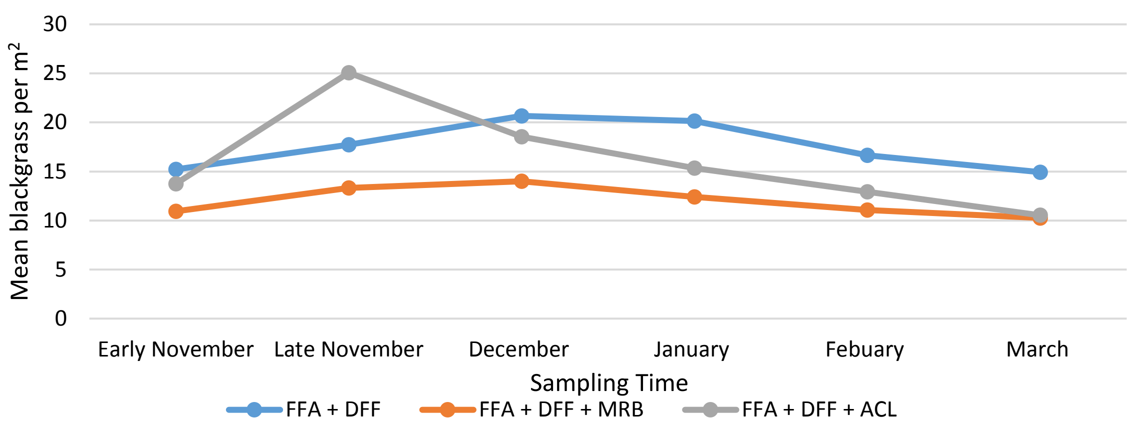

Figure 3 Mean black-grass/m^2 per sampling time and product in cultivated trial

DISCUSSION

The results from this trial have highlighted the value of herbicides to control black-grass under different cultivation methods. Across both trials, the statistical analysis found the treated and the untreated to be different with all three herbicides being effective at controlling black-grass. The addition of ACL or of MRB to DFF + FFA provided a lower mean black-grass count in both cultivation systems but this was not a significantly different to that provided by DFF + FFA alone. This lack of significance may in part be attributable to the methodology, which was restricted in terms of the number of marked plots feasible to assess in the time available, highlighting the difficulty in obtaining such basic data in a field situation. Despite this, the results generally agree with the conclusions of Dückera *et al* (2019) that ACL and MRB can help to control black-grass populations with reduced sensitivity to FFA, and that the new products incorporating ACL or MRB with the new modes of action have the potential to play a part in an anti-resistance management strategy to control black-grass. Across both cultivation methods, the statistical test showed that the plant count timing was a significant factor in the black-grass numbers recorded. All three herbicide treated plots recorded their highest level of black-grass in December in the direct drilled trial. Whilst in the cultivated trial, FFA + DFF and FFA + DFF + MRB had the highest level of black-grass in the December inspection before dropping off later on. The untreated plots however had the greatest levels of black-grass in February across cultivation systems. Winterkill is likely to have had an influence on these results with the black-grass numbers decreasing from December to January in the treated plots (Figure 2 and 3), especially as January 2019 had a lower mean daily air temperature in comparison to November and December. Maréchal *et al* (2012) suggested black-grass required significant growth to withstand the harshness of winter, while the trial reported by Meiners (2015) found black-grass in the 1-2 leaf stage was eliminated over winter due to a frost damage, whilst tillering plants survived. With this considered, it is possible that the herbicides had reduced the rate of growth and thereby increased the chance of winterkill occurring in these treated plots. The study also highlights the importance of cultivation method when considering black-grass control. When cultivated with a tine and disk cultivator the number of black-grass plants in the untreated plots was reduced by 59% compared with the untreated plots that were direct drilled. Similar work to this was highlighted by Lutman *et al* (2013). The authors reported that when compared to non-inversion tillage, direct drilling can increase the black-grass population by 16%. Although Lutman *et al* (2013) did comment that there was a large level of variation in these results and ploughing remains an important tool in controlling black-grass. The authors also emphasized that an integrated pest management plan remains an important strategy to control black-grass. The herbicide treated mean in the cultivated plots at the March inspection provided the lowest black-grass levels compared to the other counts in this study. This reinforces the case for using integrated black-grass control programmes by using the correct cultivation alongside suitable chemistry to reduce the overall burden of black-grass in a crop later in the season.

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THE EFFECTS OF GLYPHOSATE ON SPRING BARLEY GRAIN QUALITY IN THE NORTH EAST OF SCOTLAND

C A Harvie[1], C Baillie[2], K Drever[3] and A J Hilton[1]
[1]SRUC, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA
[2]Agrii, Rosehall, Aberdeenshire, AB53 4HD
[3]Aberdeen Grain, Whiterashes, Newmachar, Aberdeenshire, AB21 0QL
Email: alex.hilton@sruc.ac.uk

Summary: Environmental and health concerns about the use of glyphosate have meant that its use as a pre-harvest desiccant in cereals is under scrutiny. A field scale trial was established in 2017 to compare the effect of a pre-harvest application of glyphosate with an untreated control in two cultivars of spring barley, Laureate and Olympus. Glyphosate was applied at 0.75 kg/ha in 200 l/ha on 19th August. At harvest, application of glyphosate caused a reduction in moisture content, despite the dry summer. Yield was not affected in Laureate (5.47t/ha treated; 5.54 t/ha untreated) but glyphosate application did reduce yield from 6.26 t/ha in the untreated to 5.94t/ha in Olympus. After harvest samples were used for a range of quality measurements. Percentage skinning of grain was only 1 % Laureate and 2% in the Olympus from the combine. However, when a threshing machine was used post-harvest significant increases in skinning were seen with the use of glyphosate in Olympus but not in Laureate. Differences between the two cultivars in grain quality and yield were attributed to timing of glyphosate application. When grain samples were assessed for germination and subsequent growth in a glasshouse trial no numerical differences in emergence and tillering were observed. Some symptoms of negative geotropism were seen in germinated seed where glyphosate had been applied in both cultivars.

INTRODUCTION

Glyphosate was first introduced as a herbicide in 1974 by Monsanto and since that time it has become the most widely used pesticide in the world with 8.6 billion kilograms having been used globally up to 2014 (Benbrook, 2016). In Scotland, it is used as a non-specific herbicide prior to sowing a crop but also as a pre-harvest desiccant in cereals where it is used to encourage even ripening and to control problem grass weeds. In 2016 it was used on 35% of spring barley crops, predominantly as a desiccant to improve ripening (Monie *et al*.,2016). Using it in this manner potentially can cause issues with residues.

Use of glyphosate has been reviewed by Member States, the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA). On 12 December 2017, the Commission renewed the approval of glyphosate for a further 5 years until 2022 (European Commission, 2019).

Much of the spring barley grown in Scotland is used for malting for the distilling industry. A key quality criterion for this use market is to ensure that grain is viable and many maltsters stipulate a percentage germination of 98%. In some crops, such as potatoes, glyphosate is associated with a negative impact on initial growth. Where glyphosate has drifted into a seed potato crop from an adjacent field the subsequent daughter tubers do not grow normally and many will not emerge when planted the following year (AHDB, 2018). No literature exists on how a pre-harvest desiccation of barley crops affects the quality of grain for malting or for seed. As part of an Honours degree project an on-farm trial was established to answer these questions.

MATERIALS AND METHODS

Field Trial

Two separate farm-based field trials were performed in commercial crops of Olympus and Laureate spring barley, in Aberdeenshire, during 2018. Laureate was chosen due to its growing market share in the malting industry, whilst Olympus is an up and coming cultivar that is increasing in popularity at the time of the trial. The fields selected for the trial had been in an arable rotation since 2013, with spring barley being the main crop in the rotation. The fields were ploughed then sown using an 'Amazone' one pass harrow mounted pneumatic seed drill on 29th April for Laureate and 27th April for Olympus.

Glyphoaste was applied as Roundup Powermax (720 gai/kg) at a rate of 0.75kg/ha of product. This was applied in 200l/ha of water on the 19th August when grain was below 30% moisture content using the farms sprayer, with a boom width of 24m. This was compared with untreated plots and plots were laid out in a randomised block pattern with each of the two treatments being replicated 3 times. Field plots widths were set at 24m to accommodate the minimum distance between tramlines. Due to the different length of the two fields the plot size for Laureate was 110m and Olympus 140m. Crop management was consistent across fields with inputs being the same and following standard agronomic practice.

Field Measurements

Harvesting was done using a commercial combine on 4th September for the Laureate, with the Olympus being harvested on the following day. Grain yield data (t/ha) was gathered from the combine, with calibration taken place with grain gathered from the headland in the corresponding fields. The combine was cleaned between every plot to avoid contamination and samples were gathered for further analysis. Prior to harvest a 1m² ear samples was taken at random from each plot for skinning testing and further yield calculations.

Grain Quality

Moisture content was measured using a 'Wile 55 Grain Moisture Meter', which had been calibrated prior to the 2018 harvest season. Five moisture tests per plot were carried out at harvest and the average calculated and presented as % moisture.

Grain size and weight was assessed as Thousand Grain Weight, Specific Weight and as Screenings. Thousand Grain Weights were calculated using three samples of 1000 grains per plot. After harvest samples from each plot were dried down to 0% moisture in drying ovens over the course of 5 days. A thousand grain sample was counted out and then weighed. Specific weight was measured using a 'Farm-Tec chondrometer' with data calculated as kg/hectolitre. Screening tests were carried out using a 'Sortimat Grain Screener', which was composed of sieves of 2.5 and 2.25mm. A 1kg sample per plot was passed over the sieves and the weight of grain in each category was calculated.

A 1000 grain sample was taken from the combine for every plot. This was visually assessed and number of grains with symptoms of skinning were counted and presented as % skinning. A sample of ears from 1 x 1m area was cut prior to harvest and stored until February when it was threshed using a 'Walter & H. Wintersteiger' thresher. Percentage skinning was visually assessed after ears were put through the thresher.

Seed Quality

Germination was assessed using 100 grains per plot. The test was performed using petri dishes containing damp filter paper. 10 grains per sample were placed in each dish and this was placed in a warm room (17°C – 23°C). Dishes were checked daily for germination until no further increases were observed. Symptoms of negative geotropism were also observed and recorded.

To assess subsequent seed viability, a glasshouse experiment was performed to investigate whether growth was affected where the seed crop has been treated with glyphosate. For each field plot 5 grains were sown in each of five 0.5L pots containing 'Mother Earth' compost. Seed was sown at a depth of 2.5cm and placed in a randomised block design in a glasshouse set at 20°C. Plants were watered when required. At flag leaf emergence plants were assessed for numbers of tillers and height as measured from the base of the plant and extending to the ligule for the flag leaf.

Data Analysis

The two fields were treated as separate trials. Differences between glyphosate treatment and the untreated control were analysed as a two way T-test in Minitab 17.

RESULTS

Grain Quality and Yield

In 2018 the summer was relatively dry, with below average rainfall. The moisture content was low at harvest being 18.6 and 18.4% in the Laureate and Olympus respectively. In both crops the moisture content was significantly reduced, by 0.4% in the Laureate and 0.6% in the Olympus ($P<0.01$) (Table 1 and 3). The yield of the Laureate was on average 5.5 t/ha and there was no difference between with the use of glyphosate. In contrast, for Olympus there was a significant reduction in yield from 6.3 t/ha in the control to 5.9 t/ha where glyphosate was used($P<0.01$).

The effect of glyphosate on grain quality was cultivar specific. The specific weight was significantly reduced in Laureate where glyphosate was used from 60.9 kg/hl to 58.7 kg/hl where it had been applied ($P<0.05$). In contrast, no differences in specific weight were observed in the Olympus (Table 2 and 4). There was no effect on either screenings or thousand grain weight.

A low percentage of skinning (4%) was seen on samples taken from both cultivars at harvest and no differences were seen between treatments at this stage. However, when samples were put through a threshing machine skinning increased to 20% in Laureate and 40% in Olympus. Glyphosate significantly increased skinning to 47% in the Olympus ($P<0.001$) but there was no difference in the Laureate.

Table 1. Effect of glyphosate on Yield, Moisture content and Screenings in the cultivar Laureate

Glyphosate	Yield (t/ha)	Moisture (%)	Screenings <2.5mm (%)	Screenings <2.25mm (%)
Yes	5.47	18.2	4.61	1.06
No	5.54	18.6	4.63	1.01
P Value	0.48	0.04*	0.97	0.73

Table 2. Effect of glyphosate on Skinning, Specific Weight and Thousand Grain Weight (TGW) in the cultivar Laureate

Glyphosate	Combine skinning (%)	Threshing machine skinning (%)	Specific Weight (kg/hl)	TGW (g)
Yes	4.27	20.3	58.7	40.2
No	4.43	20.3	60.9	40.7
P Value	0.33	1	0.05*	0.24

Table 3. Effect of glyphosate on Yield, Moisture content and Screenings in the cultivar Olympus

Glyphosate	Yield (t/ha)	Moisture (%)	Screenings <2.5 (%)	Screenings <2.25 (%)
Yes	5.94	17.8	7.88	2.35
No	6.26	18.4	7.42	2.12
P Value	0.01**	0.01**	0.56	0.33

Table 4. Effect of glyphosate on Skinning, Specific Weight and Thousand Grain weight in the cultivar Olympus

Glyphosate	Combine Skinning (%)	Threshing machine skinning (%)	Specific Weight (kg/hl)	TGW (g)
Yes	4.43	47.3	60.8	37.8
No	4.27	39.7	61.3	37.7
P Value	0.286	0.01**	0.628	0.882

When grain from the field trial was sown in a glasshouse trial there was no obvious effect on plant growth (data not show). However, grains showing symptoms of negative geotropism were seen where glyphosate had been grown in both the Laureate and Olympus at 3% and 2.3% respectively.

DISCUSSION

Use of a pre-harvest treatment of glyphosate is a common practice in cereal production in the north east of Scotland. Despite being a dry year % moisture was consistently reduced in both barley cultivars in this trial and this highlights the value of glyphosate as a desiccant, where damp conditions exist or an early harvest is required. Similar studies have highlighted this effect in wheat in Brazil (Krenchinski *et al*., 2016) and Canada (Darwent *et al*.,1994). In a number of trials over a number of years and locations this was shown to be particularly effective when it is applied at grain moistures above 25% (Darwent *et al*., 1994).

A yield loss of 0.3 t/ha was recorded in Olympus but no negative impacts were seen in the cultivar Laureate. Using a Trimble Greenseeker to measure Normalised Difference Vegetation Index (NDVI) at the time of application (data not shown) it was shown that the Laureate was more mature than the Olympus when the glyphosate was applied. The greener canopy of the Olympus would have encouraged the glyphosate to be translocated around the plant and this could have reduced the proportion of assimilates moving to the ear from the leaves. Yield losses in wheat have been recorded where a range of desiccants were used in a trial at different growth stages from early milky stage to hard dough stage. Crops treated with paraquat at the milky stage showed reduced yield loss compared with plots treated between soft and hard dough, but no reduction was observed with glyphosate (Perboni *et al.*, 2018). In this trial treatments were applied at hard dough stage.

Many maltsters are concerned that a pre-harvest application may affect grain viability which would reduce the quality for both whisky and beer sectors. In the study, there was no evidence that % germination was effected. Wheat grain germination was not reduced when 1.0 and 2.0 kg/ha of glyphosate was applied pre-harvest but % abnormal grains increased using the higher rate (Jaskuliski & Jaskuliski, 2014). In the present study, lower rates of only 0.75 kg/ha were used and in this situation low levels of negative geotropism were seen. Although this does not affect % germination it is unclear if this could have an impact if this grain was subsequently used for seed. Higher rates of 1.5kg/ha-2kg/ha can be used where couch grass and other perennial weeds need to be controlled and it is unclear whether this would affect germination under Scottish conditions.

A small but significant reduction in specific weight was observed in Laureate where glyphosate was used, but not in Olympus. No differences in TGW were observed. In other studies, no difference in specific weight were seen in wheat except when there were very high moisture contents (>60%) at time of application (Darwent *et al*.,1994).

Low levels of skinning were observed at harvest time and no differences between treatments were seen. Using a thresher after a period of storage encouraged skinning to occur and this was more severe where glyphosate was used in Olympus. It is speculated that this is due to a weaker husk adhesion around the pericarp in this cultivar at the time when glyphosate was applied compared with the Laureate. However, further trials need to be performed to look at this in further detail.

This study highlights the value of using a pre-harvest, especially where grain moisture is high. Some negative impacts were observed in terms of yield and specific weight but these highlight the importance of using the product in accordance with the guidelines provided by AHDB Best Practice Guidelines (AHDB, 2018).

ACKNOWLEDGEMENTS

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PHYSIOLOGICAL INVESTIGATION OF NITROGEN USE EFFICIENCY IN DIFFERENT RICE GENOTYPES OF BAAP POPULATIONS

Y Hazzazi[1], A Y Tantray[1,2], G Norton[1] and A H Price[1]
[1]*School of Biological Sciences, University of Aberdeen, Aberdeen, United Kingdom*
[2]*Department of Botany, Aligarh Muslim University, Aligarh, India.*
E-mail: y.hazzazi.19@abdn.ac.uk

Summary: Rice is one of the most important agricultural crops around the world, because it is a main food for more than 50% of the population in many countries. In the future, some scientists expect that the global demand for food will have increased by 85% between 2013 and 2050. To achieve high productivity farmers increase large scale use of nitrogen applications in the rice field. But the rice crop only takes up 40% of the nitrogen applied while the rest causes environmental problems like increase of greenhouse gas and eutrophication in water bodies. To solve this problem there is need to increase the nitrogen use efficiency (NUE) in rice crop plants. Following this, we have conducted a green-house experiment to assess the NUE of rice under different nitrogen applications. During the experiment we completed weekly analyses of leaf nitrogen balance index (NBI) and plant height for 5 weeks. After the harvesting of the experiment, root length, shoot and root biomass, leaf nitrogen content were analysed. There was significant difference from the first week till harvesting in NBI and plant height. The leaf nitrogen content was significantly correlated with NBI in different rice genotypes. There was significant interaction between treatments and genotypes. The finding may be helpful in feature to assist low nitrogen in crop field by increasing the NUE in plants which contribute in crop production and help in reducing the N inputs.

Key words: Nitrogen, nitrogen use efficiency, rice, nitrogen balance index.

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LEAF (ORGAN-SPECIFIC) PROTEOME ANALYSIS OF RICE VARIETIES UNDER OPTIMUM AND LOW PHOSPHORUS LEVEL

A Y Tantray[1, 2], A Ahmad[1] and A H Price[2]
[1]Department of Botany, Aligarh Muslim University
Aligarh, 202002, Uttar Pradesh, India
[2] Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, AB243UU, UK
E-mail: Tantray.adil@abdn.ac.uk

Summary: Phosphorus (P) deficiency is one of the major limiting factors for crop productivity. The yield of rice (*Oryza sativa* L.) is severely limited by phosphorus deficiency. Therefore, constant high fertilizer application is required for getting high yield. High application of P fertilizers causes serious environmental pollution and economic loss as the P use efficiency is only 10-20%. Rice varieties that can grow and yield well at low level of P are urgently required. Functional proteome analysis of rice under low-P conditions can help deciphering the proteins involved in low-P tolerance in rice. In the present study, leaf proteome profile of two contrasting P-responsive rice varieties (cv. *Panvel* and cv. *Nagina 22*) was analyzed using two-dimensional gel electrophoresis and mass spectroscopy. These varieties were grown hydroponically in the nutrient medium under control environmental conditions at low-P level (2.0 µM). Differential expression pattern of proteins of the leaves of both the varieties was analyzed in 30-day-old plant. Identification of these proteins through mass spectrometry and MASCOT software revealed that these differentially expressed proteins were homologous to known functional proteins involved in energy metabolism, biosynthesis, photosynthesis, signaling, protein synthesis, protein folding, phospholipid metabolism, oxidative stress, transcription factors, and phosphorus metabolism. It is suggested that the rice varieties modified protein expression differentially in response to low-P supply to maintain the growth by arising as adaptations. The differential expression of proteins differed in both the varieties. Higher potential of protein stability, stress tolerance, osmoprotection and regulation of phosphorus uptake was observed in cv. *Nagina 22* than cv. *Panvel*. This study of low-P responsive proteins will emphasize the proteome responses to low phosphorus conditions, which can help in enhancing the PUE and understanding of its mechanism.

Keywords: Phosphorus use efficiency, phosphorus, proteomics, rice

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GENOME WIDE ASSOCIATION MAPPING OF SEEDLING SALINITY TOLERANCE IN RICE BENGAL AND ASSAM AUS PANEL (BAAP)

C Chen, G J Norton and A H Price
School of Biological Science, University of Aberdeen, United Kingdom
c.caijin.17@abdn.ac.uk

Abstract: Salinity is one of the major abiotic constraints on rice production in coastal, arid and semi-aid areas around the world. In the present study, salt tolerance at rice seedling stage was evaluated using 204 rice accessions from Bengal and Assam Aus Panel (BAAP) in salt stress treatments (3g L-1 NaCl) in the hydroponic system. Four traits Na, K concentration in shoots, Na/K ratio and salt injury score were investigated. One-way ANOVA results showed that there were significant cultivars differences in those traits. Genome wide association mapping was performed and a total of 25 QTLs were identified associated with the sodium phenotypes, seven of them associated with multiple traits. Twelve genes in identified QTLs regions were co-localised with known genes to be involved in salinity tolerance in rice, including a well-known major gene OsHKT1;5, which has been identified to be involved in retrieval of Na+ from the xylem. In additionally, three promising QTLs were investigated by constructing the Neigbor-Joining (NJ) tree that allowed cultivars which shared similar haplotypes to be assigned into clusters, and the phenotypic values across the clusters were further checked. A total of 31 cultivars were identified as the tolerance genotypes according to the Na/K ratio and cluster analysis of these three promising QTLs. The salt tolerance cultivars and QTLs identified in this study would provide useful information for breeding rice with increased salt tolerance.

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THE POTATO CYST NEMATODE EPIDEMIC – WHERE ARE WE NOW AND WHERE ARE WE HEADING?

J Pickup[1], K Davie[1], V Blok[2], A Kleczkowski[3], B McDougall[3], H Kettle[4], A Roberts[4], D Ewing[4] and L Kuhfuss[2]
[1]*SASA, Edinburgh, EH12 9FJ, UK,*
[2]*The James Hutton Institute, Dundee DD2 5DA, UK*
[3]*The University of Strathclyde, Glasgow, G1 1XQ, UK*
[4]*Biomathematics & Statistics Scotland, Edinburgh, EH9 3FD, UK*
E-mail: jon.pickup@sasa.gov.scot

Summary: SASA estimates that the total area infested with PCN in Scotland is now over 13% of the area regularly planted with potatoes. Seed potatoes cannot be grown on land recorded as infested and ware potatoes can only be produced under an officially approved control programme. In recent years the incidence of *Globodera pallida* has increased markedly, with Angus the most affected county. Statutory testing data collected by SASA shows that the area of land recorded as infested with *G. pallida*, currently 6,200 ha, is currently doubling every 7–8 years, whilst the area of land infested with *G. rostochiensis* is relatively static at c. 14,500 ha. In the 1970s, findings of *G. pallida* represented 2–3% of the all PCN findings, whereas now they account for nearly 70%. At the current rate of increase, the widespread presence of *G. pallida* may prohibit the production of seed potatoes on PCN-free land in as little as 30 years' time

CHANGES IN THE INCIDENCE OF *GLOBODERA PALLIDA* IN ANGUS

To determine how the incidence of *Globodera pallida* has changed in the past 10 years in the most infested county in Scotland, Strathclyde University used data provided by SASA to estimate the infestation by *G. pallida* throughout Angus parishes.

a. **b.** **c.**

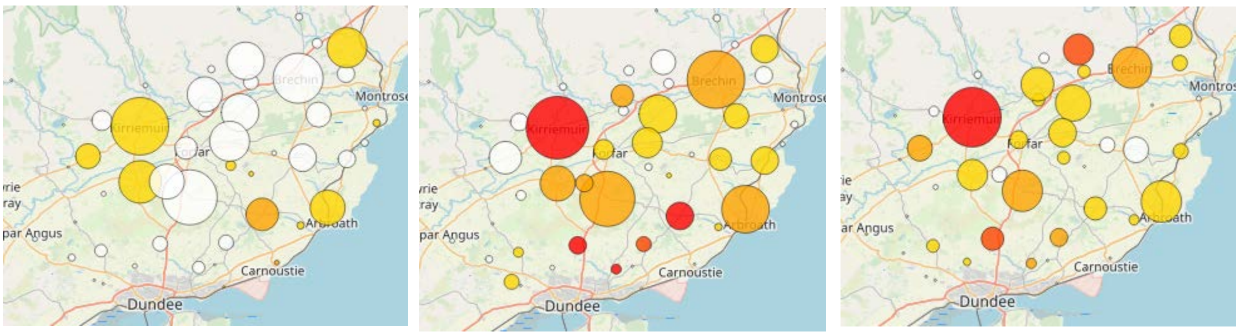

Figure 1. A map of Angus with visualisation of *G. pallida* positive testing. Size of circles corresponds to the area of land tested in a given year whereas colour represents the proportion of area that tested positive (white: no infestation, yellow = low infestation, red = high infestation. a.2010, b.2014, c.2018

To visualise the spread of PCN from 2010 to 2018, an interactive map was created in R package Shiny (Figure 1). The size of the plotted circles represents the area in each parish (according to the Agricultural Parish Map of Scotland) which was tested for PCN whereas the colour of the circles represents the number of samples testing positive for *G. pallida* within each parish, with darker circles representing parishes with a greater number of positive samples and white circles representing those with no *G. pallida* found in tested samples in a given year.

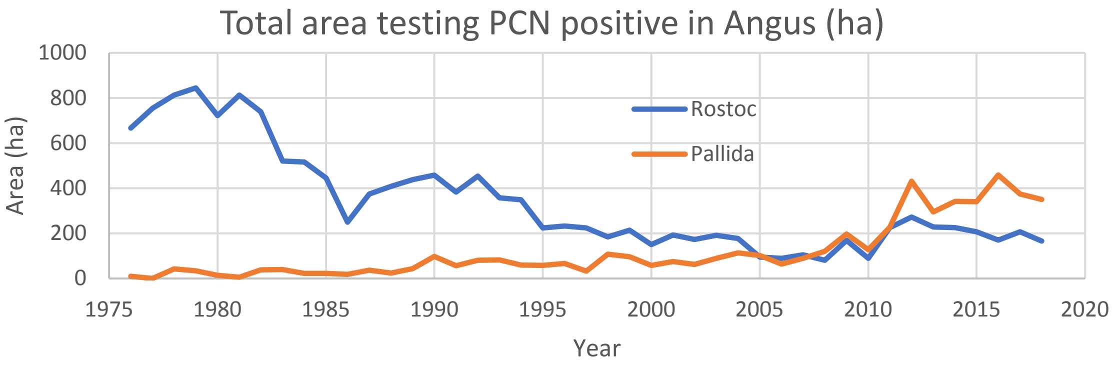

Figure 2. Total area testing positive for *G. rostochiensis* (Rostoc) and *G. pallida* (Pallida) between 1976 and 2018 in Angus

In 2010, most parishes in Angus had little or no land infected with *G. pallida* with the average area of infected land per parish being 1.43 hectares. By 2014, the area infested with *G. pallida* rose rapidly and the average area over the parishes reached 6.45 hectares. The pest has also spread substantially; the number of infested parishes in Angus almost doubling over a four-year period. *G. pallida* and *G. rostochiensis* infestations in Angus were compared for the period of 1975-2018 using positive and negative testing results.

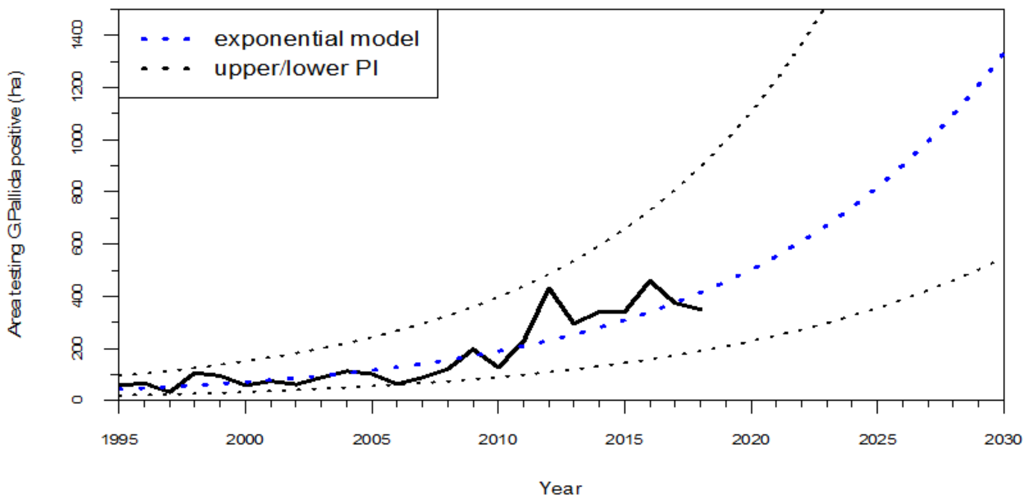

Figure 3. Increase in the area testing positive for *G. pallida* (thick line) compared with the model prediction (dotted line) and the upper and lower predictive 95% confidence intervals.

The area with *G. pallida* testing positive in each year was low from 1976 onwards and began to slowly increase between 2005 and 2010. This area then rose rapidly from 128 hectares in 2010 to 432 hectares in 2012. In contrast, *G. rostochiensis* was recorded from approximately 800 hectares p.a. in 1970s, but this incidence has gradually declined to match the area infested similar with *G. pallida* between 2005 and 2010 (Figure 2).

SASA data for the period 1995 to 2018 were used to estimate the parameters for a model to describe the rate at which infestation with *G. pallida* has been expanding in recent years. The exponential model captured the data well and predicted that the area testing positive each year is doubling approximately every 7 years. Thus, this result was used to describe the actual cumulative acreage testing positive with *G. pallida* by treating the data shown in Figure 2 and in Figure 3 as yearly increments and calculating the sum up to a given year. This assumption ignores the process of de-recording by which land is returned to the infestation-free status. Thus, the results will provide a 'worst-case' scenario.

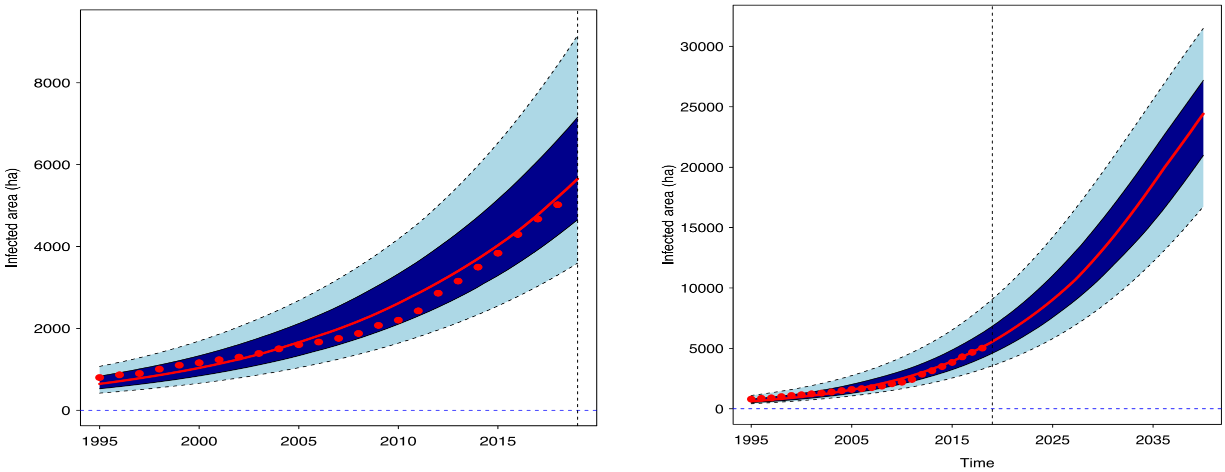

Figure 4. (a) The cumulative area testing positive with *G. pallida* over time. Data (running sum of data in Figure 3) are represented by points and the model outcome by red line (median), 50% (dark blue) and 95% (light blue) confidence intervals.
(b) extends the prediction time to 2040. Vertical dashed line marks 2019.

There is a good agreement between the model output and the data (Figure 4a) so the model can be used to predict the levels of *G. pallida* infestation under the assumption that the current approach continues. By 2040, over 20,000 ha of the land in Angus is predicted to be unsuitable for growing seed potatoes (Figure 4b) and will limit production of ware potatoes (out of the 50,000 ha of 228,000 ha in Angus estimated to be used for potato production). This prediction currently does not consider the decline in infestation levels if potatoes are not grown in a land with a positive test and ignores the effects of planting resistant varieties. Using the model and assuming 80% yield loss and costs and prices from J. Nix Farm Management Handbook 2017, an estimate loss of £5,093 per ha is predicted. Multiplying the loss by the area infested in 2019 suggests a current loss of £25m p.a. (5,000 ha multiplied by £5,093 ha^{-1}), rising to £125m per year in 2040. This figure represents the opportunity loss not the actual loss, i.e. the value of potatoes that could have been grown had the land not been infested by *G. pallida.*

PCN MANAGEMENT IN SCOTLAND

Seed potato production is prohibited on infested land. Directive 2007/33/EC permits ware potato production on infested land, providing that this is done as part of a control programme aimed at reducing the population of PCN. In Scotland, control programmes are evaluated using the AHDB PCN calculator for *G. pallida* https://potatoes.ahdb.org.uk/online-toolbox/pcn-calculator. The calculator estimates outcomes dependant on nematicide use, varietal choice and rotation length (Table 1). Where *G. rostochiensis is* found, there is a wide range of commercial varieties with resistance to this species. In the Netherlands, there are strong incentives to produce resistant ware (resistance score of 7-9) on infested land, including reduction of the period for which seed production is prohibited from 6 to 3 years.

Table 1. Rotation periods required to prevent population increase (AHDB PCN Calculator)

Light Silt	Intolerant		20% Decline Rate					
Resistance Score	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
No Treatment	13	10	7	5	2	1	1	1
Nematicide - 70% control	11	7	4	2	1	1	1	1
Nematicide - 50% control	12	8	5	4	1	1	1	1

The first UK cultivar with resistance to PCN, Maris Piper, was released in 1966 (Dale & Bradshaw, 2006). Maris Piper was been remarkably successful and is the most widely grown cultivar in the UK. Resistance to *G. rostochiensis* has not been observed to have been broken in the UK despite the reliance on the *H1* resistance gene for several decades. However, Maris Piper is fully susceptible to *G. pallida* and thus its widespread cultivation, and the use of other varieties with *H1* resistance, has inadvertently led to the spread of *G. pallida*, which is now the predominant species in the UK (Minnis *et al*., 2002).

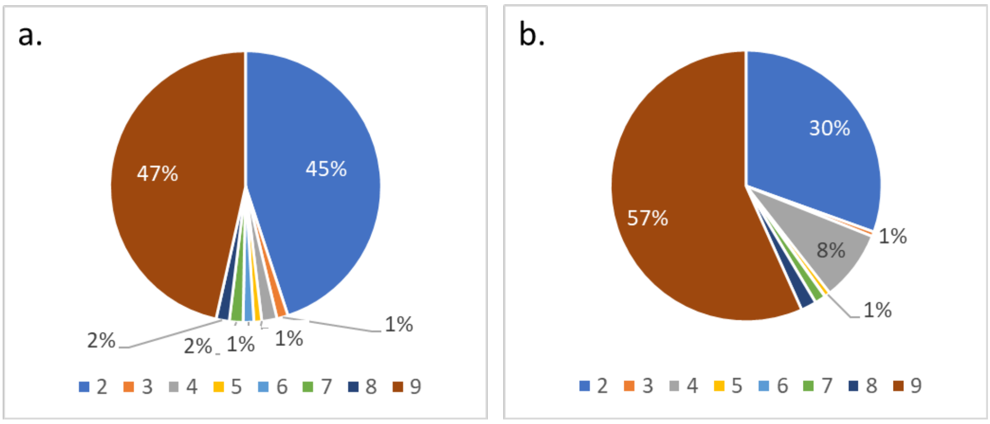

Figure 5. Resistance scores of varieties grown to *G. rostochiensis* for a. seed and b. ware in 2019

In Scotland in 2019, varieties highly resistant to *G. rostochiensis* (resistance scores of 7-9) were grown on 50% of the area cultivated for seed potatoes and on 57% of the area used to grow ware (Figure 5). Susceptible varieties (resistance score = 2) were grown on 45% of seed land and 30% of ware land. The overall Scottish seed crop has an average resistance score of 5.6 to *G. rostochiensis,* whereas the ware crop has a slightly higher average of 6.3.

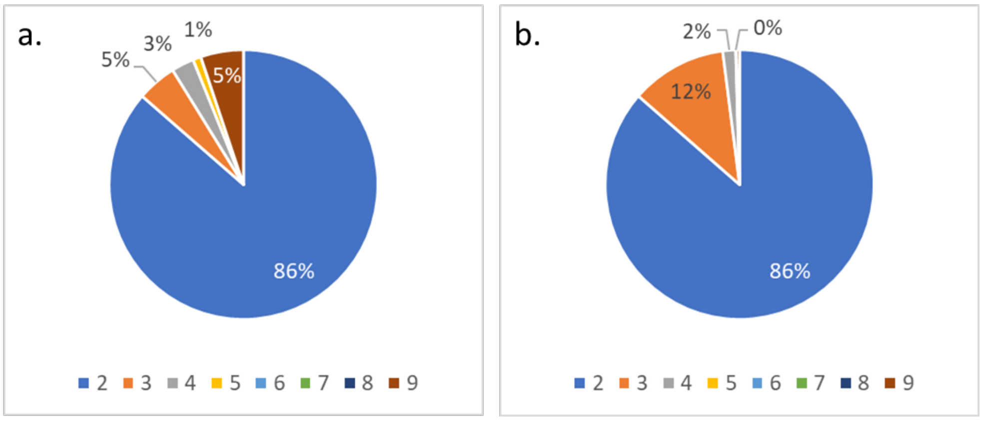

Figure 6. Resistance scores of varieties grown to *G. pallida* for a. seed and b. ware in 2019

Varieties highly resistant to *G. pallida* (resistance scores of 7-9) were cultivated on 5% of the area used for seed potatoes but only on 0.4% of the area used to grow ware (Figure 6). Varieties susceptible to *G. pallida* (resistance score = 2) were grown on 86% of both seed and ware land. The overall Scottish seed crop has an average resistance score of 2.5 to *G. pallida*, whereas the ware crop has a lower average of 2.2.

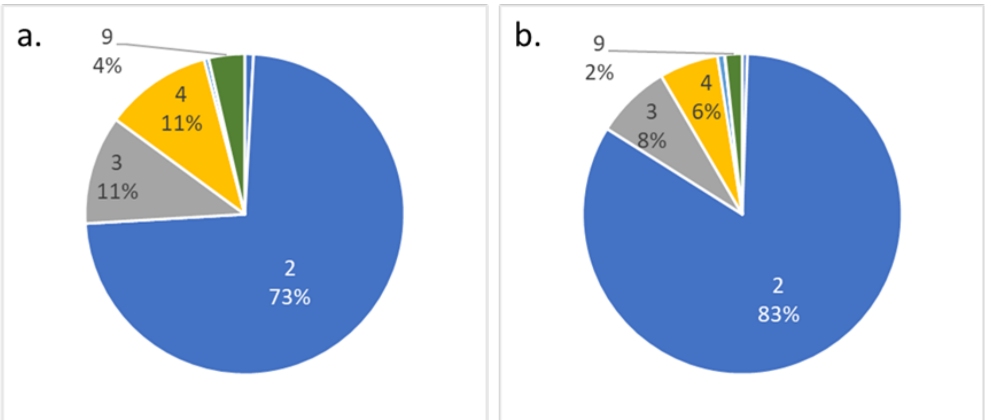

Figure 7. a. *G. pallida* resistance scores of crops grown in *G. pallida* infested land 2010-19 (1485 crops)
b. *G. pallida* resistance scores of all crops grown in Scotland 2010-19 (70,385 crops)

SASA recorded *G. pallida* in statutory soil tests drawn from 5352 ha between 2010-19. Whilst the cultivation of potatoes for seed is prohibited in infested land, parcels within the same field that have tested clear of PCN can be used for seed and ware can be grown under a control programme on land known to be infested. Since these findings, 1485 crops were planted in fields with *G. pallida*. 74% of these crops were of varieties with no resistance to *G. pallida*, in comparison to an 'across Scotland' average of 84% of susceptible potato production (Figure 7). This suggests that only in an estimated 10% of cases, are growers being influenced in their choice of variety resistance by the results of SASA's PCN tests.

A similar situation was observed for *G. rostochiensis,* even though commercially successful varieties are widely available for most market uses. SASA recorded *G. rostochiensis* in statutory soil tests drawn from 4172 ha between 2010-19. Since these findings, 1364 crops were planted in fields with *G. rostochiensis*. 33% of these crops were of varieties with no resistance to

G. rostochiensis, in comparison to an 'across Scotland' average of 43% of susceptible potato production (Figure 8). This again suggests that only in an estimated 10% of cases, are growers being influenced in their choice of variety resistance by the results of SASA's PCN tests.

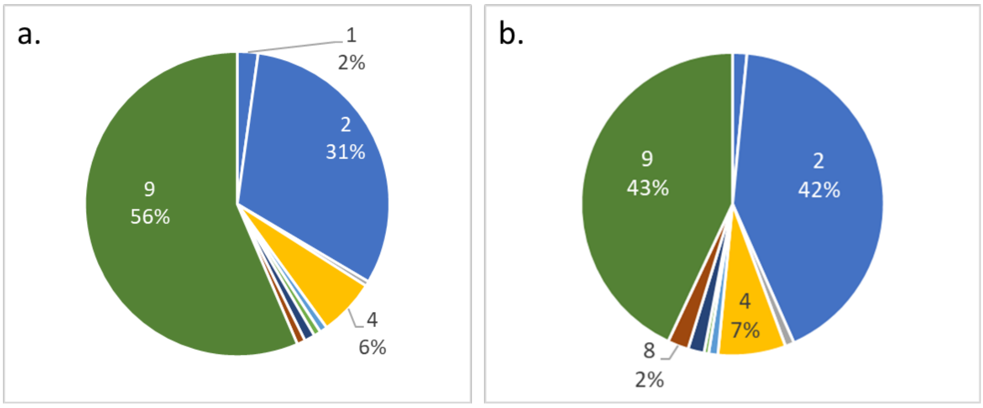

Figure 8. a. *G. rostochiensis* resistance scores of crops grown in *G. rostochiensis* infested land 2010-19 (1364 crops)
b. *G. rostochiensis* resistance scores of all crops grown in Scotland 2010-19 (70,520 crops).

Success with breeding for resistance to *G. pallida* has lagged behind that for *G. rostochiensis*. This is partly because the 2 main sources of resistance used for *G. pallida*, have been less amenable for breeding. Unfavourable agronomic qualities and the polygenic nature of the resistance (Gebhardt, 2013) has complicated the breeding process. However, recently cultivars with resistance from both sources have been produced and these now provide parental material for the breeders and the prospect of increasing the production area of varieties with high resistance to *G. pallida*.

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POTATO CYST NEMATODES IN SCOTLAND – OBSTACLES WE FACE AND OPTIONS TO OVERCOME THESE

K Davie[1], J Pickup[1], V Blok[2], L Kuhfuss[2], H Kettle[3], D Ewing[3] and A Roberts[3] and A Kleczkowski[4]
[1]*SASA, Edinburgh, EH12 9FJ, UK,*
[2]*The James Hutton Institute, Dundee DD2 5DA, UK*
[3]*Biomathematics & Statistics Scotland, Edinburgh EH9 3FD, UK*
[4]*The University of Strathclyde, Glasgow G1 1XQ, UK*
Email: kim.davie@sasa.gov.scot

Summary: Historically Scotland has had a relatively low incidence of potato cyst nematode (PCN). However, land recorded with *G. pallida* is increasing in accordance with an exponential model, and if left unchecked could result in a loss of land available for seed potatoes within a few decades. Several control options for PCN have been proposed and are utilised with varying degrees of effectiveness worldwide. Trap crops and biofumigants are not typically suitable for Scottish conditions, nematicides are expensive, not used extensively, and their future availability is at risk. To manage PCN populations successfully, resistant varieties must be used as a key component of any integrated control programme. Many commercially successful varieties with resistance to *G. rostochiensis* are available to Scottish growers and can be used to effectively control this species. However, only recently have cultivars with resistance to *G. pallida* started to become available. At present less than 3% of potatoes grown in Scotland are resistant to *G. pallida*. To better understand the practicalities of PCN management and barriers to the use of resistant varieties growers were interviewed about PCN. This work has highlighted some of the practical hurdles we have to overcome if long term management is to be achieved.

INTRODUCTION

Globodera pallida is an increasing problem in Scotland with the area of land being officially recorded as infested doubling approximately every six or seven years (Pickup *et al.*, 2018). On the current trajectory there could potentially be no land available for seed production in as little as thirty years. Scotland currently produces around 80% of all UK seed (https://potatoes.ahdb.org.uk/sites/default/files/publication_upload/B9624%20World%20Service_Eng_FINAL.pdf). This seed meets high health standards due to the low levels of virus and other diseases common in warmer climates. *G. pallida* poses a threat to the seed potato industry in Scotland and in the longer term could also adversely impact upon the yields from ware crops. The aims of the EU PCN Directive (2007/33/EC) are to stem the spread of PCN and to keep populations at low levels. As seed potatoes are produced over several generations and may be moved between farms, and often from country to country, ensuring freedom from PCN at all stages of the seed chain is key to preventing the introduction of the pest. Therefore, any land in which seed potatoes are intended to be planted must be tested and found free of PCN. Ware potatoes which are destined directly for consumption or processing may be planted provided the infested land is subject to an official control programme, which has an aim of reducing the population of PCN. Tools available to manage PCN infestations include the use of resistant varieties, trap crops, biofumigants, chemical nematicides and increasing rotation length.

Rotation length

Scotland has traditionally used long rotations for of one in six or longer for seed potato land, and this is sometimes increased to as much as one in ten. Studies suggest decline rates for PCN are ca. 20-30% annually in the absence of a host crop (Trudgill *et al*., 2014). This decline can vary with differences in soil structures and environmental factors and is therefore difficult to predict (Devine *et al*., 1999). *G. pallida* is generally thought to decline at a slower rate than *G. rostochiensis*. Taking advantage of decline rates enables rotation length to be used as a tool in the management of PCN. This is however dependent on removing or killing any groundkeepers as these will act as reservoirs for the nematodes, with populations increasing on susceptible cultivars and selecting for resistance breaking populations on resistant ones.

Nematicides

Nematicides are usually applied at planting to control the juveniles emerging from cysts in response to exudates from the roots of the growing potato crop. Treatments can prevent early damage to root systems of the plants and help to achieve a healthy yield. Nematicides reduce the initial population invading the plant roots, but any successful nematodes will still undergo a rapid multiplication on susceptible hosts. Minnis *et al.* (2004) showed significant ware yield increases from 22 to 35 t/ha with granular nematicide. Sterilant and fumigant nematicides are not used in Scotland.

Biofumigants

'Biofumigation' is a term used to describe the suppression of soil borne pests, using natural biocidal compounds (principally isothiocyanates). These are released in soils when glucosinolates in plant residues (predominantly brassicas) are hydrolysed. To provide the greatest benefit crops used for biofumigation these generally must be planted throughout the summer growing season for a period of approximately 12 weeks then macerated and incorporated immediately into the soil (Ngala *et al*., 2014). The performance of biofumigation depends on which type of crop is used and a range of environmental and agronomic factors.

Trap Crops

Trap crops come in two forms, firstly a potato crop, which must be killed within 40 days of planting, this is used in the Netherlands where high infestations occur. The nematodes are captured and killed within the plant prior to maturation. The second method of using a trap crop is to grow a crop related to *Solanum tuberosum* which can induce hatching but is an unsuitable host for PCN replication. There have been several candidate crops studies for this and *Solanum sisymbriifolium* (sticky nightshade) shows the most potential to date (Dandurand *et al*., 2014).

Biosecurity and knowledge

Where land is free from PCN, keeping it that way is critically important. Cysts are sedentary and cannot move by themselves. Other than introduction with the planting of infested seed, the predominant method of spread is through the movement of infested soil, primarily by machinery or animals. Thorough biosecurity measures are required to ensure soil is not moved between fields by any means. A key factor in the management of PCN populations is understanding how PCN work: how they multiply, how they are spread. Testing potato land regularly and understanding what the results from laboratories mean and what to do to prevent the spread of PCN is vital to effective management.

Resistant varieties

Resistance is probably the most effective management tool available to control PCN. Highly resistant varieties can limit multiplication to just 1% of multiplication on susceptible varieties. Resistance to PCN is scored on a 1-9 scale: a variety scoring one unit higher than another will limit PCN reproduction to half of that on the other variety. Around 50% of potatoes planted in Scotland are resistant to *G. rostochiensis* (score 7-9), while for *G. pallida* only 3% of the potato crop has comparable resistance. As potato growers are responsible for producing crops, their behavioural practices strongly influence whether management of PCN is successful. The aim of our research was to gain an understanding of growers' perceptions of the PCN threat and what if any measures they were taking to reduce this. Understanding why resistant varieties are not being commonly used in Scotland could help in determining measures to encourage greater uptake.

MATERIAL AND METHODS

In total 35 potato growers were surveyed from across the all the main potato growing regions in Scotland. In total there are approximately 450 potato growers in Scotland, so this represents ca. 8% of the industry. 27 growers were contacted by telephone and face-to-face interviews were arranged. Discussions lasted for 1-2 hours. Initially 18 growers were interviewed in 2017, with 9 additional face-to-face interviews in regions that had not previously been targeted in 2019. These face-to-face interviews were complemented by a shorter paper questionnaire composed of closed-ended questions based on important factors identified during qualitative interviews. This questionnaire was used for data collection at the Potatoes in Practice event (8 August 2019 at the James Hutton Institute's Balruddery Farm), and 8 additional questionnaires were collected from Scottish growers.

RESULTS

Farm sizes of surveyed growers ranged from less than 100 ha to several thousand hectares. Seed and ware potato growers were represented, rotation lengths varied from 6 to 10 years and the number of cultivars each grower produced ranged from 1 to 27.

Table 1. Summary statistics of farmers interviewed

	n	%
Tenure		
Own most or all land	12	35.3
Rent most or all land	15	44.1
Half owned	7	20.6
Type of production		
Seed only	22	62.9
Ware only	6	17.1
Both seed and ware	7	20
Presence of PCN		
G. rostochiensis only	2	6.1
G. pallida only	3	9.1
Both PCN species	13	39.4
Yes, but PCN species unknown	7	21.2
No presence of PCN detected	8	24.2

Most of the farmers interviewed did have PCN on their land, with many having both PCN species present, although this may be historic or limited to only one field. In total 8 farms claimed to never have had any findings of PCN. Growers that owned the land they produce on and growers that rent the majority of their land were represented, with land owners seeming to be favouring seed over ware production.

Most damaging pest or pathogen

Growers were asked what they believe to be the most damaging pest or pathogen. The results are shown in figure 2 below, some growers gave two pests/pathogens as being equally important.

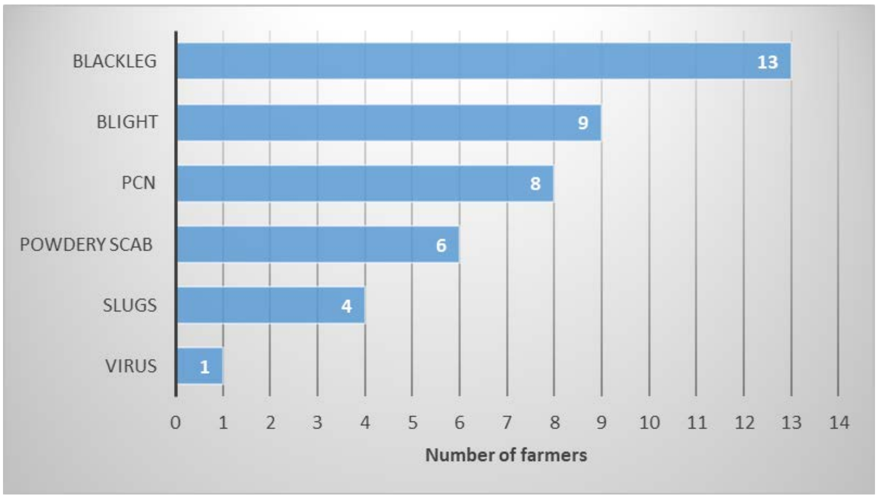

Figure 1. Growers' opinions of the most damaging pest or pathogen

In recent years, most down-gradings of seed potato crops in Scotland have been due to blackleg, so many growers see this as having the greatest impact. While blight is currently well managed, there was concern over new resistant strains emerging. Growers that cited PCN as the greatest concern all had PCN on their land and were taking steps to manage it or had lost land to seed production as a result. The growers who had not had land test positive for PCN were less concerned. Most of the growers interviewed believed that PCN was an important issue and were worried about the consequent imposition of statutory restrictions on their land. Some growers said they were not sure if the distribution and infestation levels of PCN were increasing or if the changes reported were solely due to the increased sampling rate introduced in 2010 under the revised EU Directive. The biggest concern regarding PCN was loss of land availability for seed as opposed than the effect that PCN may have on yields. Some growers told us that the restriction of seed land due to PCN either meant a shift to ware production themselves or a loss of land to ware growers. Land owners who do not grow potatoes may preferentially to rent land to ware growers as there is no clearance from PCN required.

Variety choice

Most growers stated they had little choice with varieties they planted. Many seed growers are tied into contracts as these provide a guaranteed buyer for the produce. Those that did not grow on contract tended to grow Maris Piper or other multi-purpose varieties free from breeders' rights. Where possible, growers tended to stick with varieties they know well. In the north of Scotland variety choice was also restricted by ability to produce a yield in the soils. Potato marketers/producers are aware of end markets, the varieties and quantities required. The demand for varieties is therefore driven by the end processor or seller e.g. the supermarkets, frozen food manufacturers or fast food restaurants. One producer informed us that for ware, the furthest North that it is possible grow potatoes for processing is Yorkshire. Almost all varieties available with high levels of resistance to *G. pallida* are processing varieties, and for this reason very few resistant cultivars can be grown for ware in Scotland.

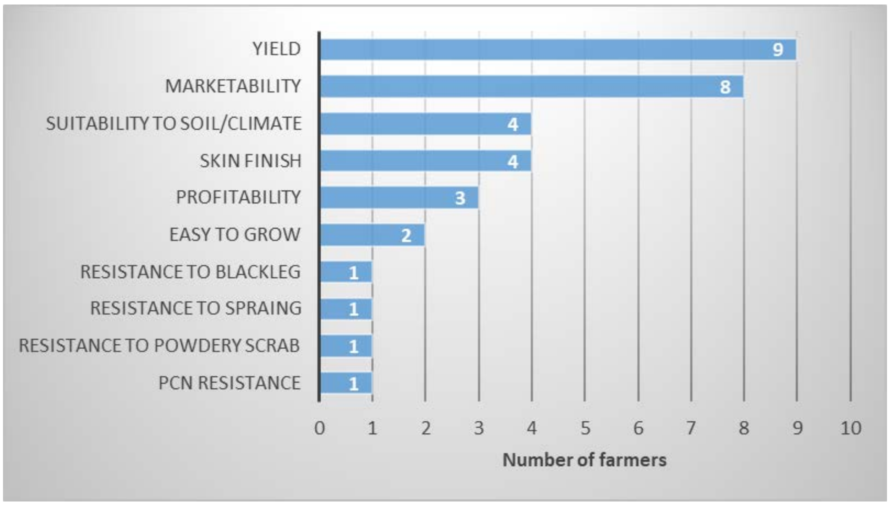

Figure 2. Grower's opinions of the most important variety characteristics

Marketability and yield were considered the most important factors in a potato variety. Two thirds of the farmers we spoke to knew whether the varieties they were growing were resistant to PCN. However, only a couple of the growers actively sought out and used resistant cultivars in rotation. A large majority of growers (26 out of 33) would be willing to use resistant varieties if available and marketable.

Grower opinions on PCN management

We asked growers what they believed was the best way to tackle problems with PCN. Most believed that increasing the length of rotation or using resistant varieties were the most effective tools available to manage PCN. Other suggestions included leaving infested land free from potatoes, controlling groundkeepers and trying biofumigant crops. In the north of the country there was a strong feeling that ware growers should also be testing for and acting on PCN findings. Nematicides are not widely used in Scotland (ca. 4% of seed and 19% of ware crops treated, SASA pesticide usage data). None of the farmers we interviewed used them as they didn't currently have a problem producing yields. When asked about biofumigants and cover crops, no farmer had tried biofumigants and a few had used cover crops, but this was usually to help improve soil structure. To reduce the spread of PCN, most growers believed good hygiene and biosecurity would be effective, although practices such as cleaning machinery between fields wasn't practical. Most of the growers we spoke to use their own machinery, but the ones who used contractors were concerned about the levels of cleanliness.

DISCUSSION

Growers were generally only concerned about PCN if it meant a restriction in the land they had available for seed production, few were aware of potential yield loses or the long-term consequences of a PCN introduction. Ware growers and land owners who rent out land to potato growers, currently see little economic impact from PCN so in general don't worry about it. Education is required to increase awareness of the future risks for the whole industry. Increasing understanding of detection rates, likelihood of low-level infestations, PCN reproduction and spread and management options are all required. Current attitudes in Scotland could be counterproductive in the management of PCN. Some growers, particularly where there is a history of PCN, have moved towards ware production to avoid the risk of land being recorded and the imposition of restrictions. This action will allow PCN populations to grow unchecked and reduces the availability of land for seed production. Measures should be taken to improve control programmes for ware crops and to restrict less seed land where possible. While rotations can be an effective tool in managing PCN, control of groundkeepers is imperative. We discussed the use of cover crops, biofumigants and trap crops, however, crop rotations and Scottish conditions don't favour these control measures. *Solanum sisymbriifolium* does not establish sufficiently well in Scotland's cooler conditions to work as a trap crop and few farmers would be willing to grow it in place of a summer cash crop. A similar situation exists for biofumigant crops, unless subsidies can be provided. Trap crops suitable for Scottish conditions should be investigated. Chemical nematicides are not widely used in Scotland and the availability of these controls may decrease in the future. The most effective tools available to manage PCN infestations are resistant cultivars. These are highly effective at supressing *G. rostochiensis* and are an integral part of any control programme. Virtually no suitable *G. pallida* resistant varieties are available for ware production. Priority should be given to the breeding of *G. pallida* resistant varieties suitable for Scottish conditions. The industry needs to work with end users (processors and supermarkets) to increase uptake of the few resistant varieties that are available, to drive market demand, enabling growers to grow them profitably. Overall the range of options to control *G. pallida* are currently limited in Scotland, but in order to preserve the seed potato industry for future generations workable solutions must be found urgently.

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INTEGRATED POTATO LATE BLIGHT MANAGEMENT IN RESPONSE TO AN EVOLVING PATHOGEN POPULATION

D E L Cooke, J Lynott, L Sullivan, P Skelsey, A Torro-Galiana and A K Lees
The James Hutton Institute, Invergowrie, Dundee DD2 5DA
E-mail: david.cooke@hutton.ac.uk

Summary: Potato late blight (*Phytophthora infestans*) remains one of the most destructive and economically important crop diseases. Control options include the use of host resistance, disease risk forecasting and optimal fungicide use, alone and in combination. However, in practice, management typically relies on a prophylactic fungicide programme at 5-7 day intervals to minimise the potentially severe impact of a field outbreak. The efficacy of the fungicide programme depends on an understanding of the pathogen. Three key questions are; a) is the pathogen present? b) what is the current risk of infection? c) what lineage of the pathogen is present and how will this affect its management? An AHDB Potatoes-funded survey of late blight outbreaks in British crops from 2003 to 2019, potato cropping and meteorological data and a detailed record of pathogen genetic diversity on a national scale is being exploited to examine these questions and this paper presents current progress.

INTRODUCTION

Effective management of potato late blight relies on the integration of several different approaches that exploit knowledge of the pathogen, its interactions with both its host and the environment and the effectiveness of fungicide chemistry. Research at The James Hutton Institute on several avenues is working to improve integrated pest management (IPM) of potato late blight.

The potato late blight pathogen is capable of very rapid multiplication from initially small local sources of primary inoculum. Understanding and managing such sources is a key method of supressing early disease pressure. Relatively few pathogen clones dominate outbreaks in UK crops (Cooke, 2019) which demonstrates that asexual mycelium surviving overwinter in potato tubers causes most outbreaks. These tubers may be in storage as seed, within discard piles or those that emerge as volunteer plants in fields adjacent to the subsequent year's potato crop. The pathogen can also reproduce via sexual oospores that form when A1 and A2 mating type strains meet in infected plants. Such spores are more long-lived than the asexual mycelium form and the associated meiotic recombination and reassortment generates novel genetically diverse forms of the pathogen. Crop management practices should aim to suppress both forms of primary inoculum but understanding what is most relevant locally can inform management. Previous studies have indicated an elevated genetic diversity of *P. infestans* in northeast Scotland compared to other potato-growing regions (Cooke *et al*., 2016) and factors responsible for this are under investigation (Torro-Galiana *et al*., 2019).

Fungicide sprays remain the core of effective blight management and routine weekly application schedules are commonplace. This risk-averse approach is understandable given the destructive power of this aggressive polycyclic pathogen. However, there are increasing economic and environmental pressures on the industry to reduce agrochemical inputs. Within a season, decisions are required by growers and their advisors on when to start the spray programme, which product to use and when to apply successive applications to prevent blight infection and/or spread. Decision Support Systems (DSS) are important to inform growers of the timing of the first infection risk periods and subsequent threats throughout the growing season and are designed to optimise spray timings. High risk conditions for potato blight based on temperature and humidity criteria were first defined in Britain by Beaumont and then Smith in the 1950s (Smith, 1956) and have recently been updated to The Hutton Criteria (Dancey, 2018; Skelsey & Cooke, 2014). Since 2017 Hutton Criteria reporting has been provided at GB postcode district resolution by the Agricultural and Horticulture Development Board (AHDB) BlightWatch system (www.blightwatch.ac.uk). There are however, few objective comparisons of field-scale fungicide programmes based on such DSS systems. The Centre for Sustainable Cropping (CSC) is a research platform based at Balruddery Farm near Dundee provides an opportunity for such studies. Potato crops within the rotation are used to compare 'conventional' agronomic practices to those considered 'sustainable' (Hawes *et al*., 2016) allowing a comparison of calendar-based spray schedules to those informed by the Hutton Criteria.

A changing pathogen population is an important consideration in the planning of late blight IPM. It is known that *P. infestans* is an evolutionarily high-risk pathogen (McDonald & Linde, 2002) which has a dynamic population able to defeat host resistance (Fry, 2008; Lees *et al*., 2012) and develop insensitivity to key fungicide active ingredients such as metalaxyl (Davidse *et al*., 1981) and fluazinam (Schepers *et al*., 2018). The AHDB Fight Against Blight (FAB) campaign has been sampling and genotyping the pathogen from GB potato crops since 2003 (Cooke *et al*., 2012). Almost 9000 GB samples have been genotyped using Simple Sequence Repeat (SSR) markers and this should be viewed in the context of wider European monitoring in the Euroblight consortium which has typed a further 7000 isolates (Cooke *et al*., 2019). This work has revealed the emergence and spread of dominant clones such as 13_A2 across large areas of British and European crops and the presence of sexually recombining populations in some regions. The traits of each clonal lineage are being characterised to understand their impact on blight management and the significance of this work in shaping best practice guidelines is communicated to the industry through conferences, field days, press releases to the agricultural media and scientific publications.

In this study we demonstrate three areas of research in which late blight IPM in Northern Britain is being impacted by Hutton late blight research. We are investigating the role of primary inoculum and the underlying factors that shape it in Scottish crops, quantifying the effectiveness of DSSs in sustainable cropping practices and, through pathogen genotyping and phenotyping, examining how a changing population is impacting the effectiveness of host resistance and the efficacy of fungicides.

MATERIALS AND METHODS

The database of more than 2500 potato late blight outbreaks sampled by FAB since 2003 has yielded almost 9000 samples of *P. infestans* genotyped using SSR markers (Li *et al*., 2013). A dataset of these samples, geo-located to postcode districts combined with AHDB potato cropping data and hourly meteorological data has been generated. Using ArcGIS, a clustering analysis as detailed in Torro-Galiana *et al*., (2019) has been applied to the data to examine the factors associated with the clustering of non-clonal outbreaks.

A potato crop at the CSC was used to compare the late blight fungicide spray programme according to standard practice (conventional) to a more sustainable schedule shaped by Hutton Criteria data generated by BlightWatch (www.blightwatch.ac.uk). Disease progress in the crop, spore trapping and detection technology (Lees *et al*., 2019) to monitor inoculum presence and outbreaks in the local (DD2) FAB postcode district were used evaluate this IPM approach.

Genotyping data from the Hutton FAB blight monitoring work was examined to determine whether the clonal genotype EU_37_A2, with known insensitivity to fluazinam (Schepers *et al*., 2018) was present in Scotland. Additional testing of isolates of contemporary clonal lineages to five fungicide active ingredients assessed using *in vitro* leaflet assays was conducted (Lees, 2018).

RESULTS

Analysis of the genetic diversity of *P. infestans* from disease outbreaks in Scotland shows a consistently higher proportion of non-clonal samples in the northeast of the country (Northern Aberdeenshire, Moray and Highland) than in the production areas to the south (Angus, Fife, Perthshire and the Lothians). Of the 95 Scottish non-clonal outbreaks recorded between 2006 and 2017, 90 were from the northeast with only five recorded from other regions. Spatial cluster analysis over space and time confirmed the localisation in this region. Additional structured sampling of multiple samples per crop from eleven outbreaks in these regions in 2019 crops was conducted to better understand the nature and source of the pathogen diversity. Genetic diversity levels were high with 94 multi-locus genotypes (MLGs) detected amongst the 186 collected samples and more than seven unique MLGs in each of seven of the eleven sampled outbreaks.

Despite the relatively warm and dry 2018 growing season there were 26 Hutton Criteria alerts for the DD2 postcode district. The spray programmes on both the 'conventional' and 'sustainable' treatments commenced on 27th June but with eleven and eight applications, respectively, the schedule based on the Hutton Criteria resulted in a saving of three fungicide applications. Disease pressure was low and no blight was detected in either treatment.

The EU_37_A2 clone of *P. infestans* was first detected in the Netherlands in 2013 but was not sampled in a British crop until June 2016 in Shropshire (Cooke, 2019). Data uploaded to the EuroBlight database allows mapping of the FAB data and indicates a wider GB geographic distribution in 2017 but no findings in Scotland (www.euroblight.net). Late blight disease pressure was low in 2018 but the EU_37_A2 clone was sampled from a trial in Ayrshire in September. In 2019 it was found twice in the main potato growing regions of Angus and Fife.

DISCUSSION

The successful application of IPM relies on a broad understanding of the disease triangle of host, pathogen and environment seen in the context of management practices that limit the negative impact of pests and diseases. In this paper a summary of findings on primary inoculum, DSSs and pathogen population change and their impact on potato late blight IPM is presented.

There is strong indirect evidence for the presence of soil-borne sexual oospores of *P. infestans* that are initiating local late blight epidemics in northeast Scotland. Such sexual recombination generates novel pathogen diversity which poses a threat to existing management practices via the emergence and spread of clones able to break down host resistance or with insensitivity to fungicide active ingredients (Andrivon, 1995; Fry, 2008; Yuen & Andersson, 2012). However, the novel MLGs reported in this region have, to date, proved ephemeral with none as aggressive or invasive as clones that have emerged in other parts of Europe (Cooke, 2019; Cooke *et al*., 2012). This is consistent with observations in the north and east of Europe (Yuen & Andersson, 2012). The viability of oospores will decline over time in the soil and long rotations are therefore considered the best form of management. Potato rotations are longer in land used for seed production so the increased evidence of oospore-borne infection in northeast Scotland compared to other regions is counterintuitive. The proximity of seed and ware crops and presence of volunteer potatoes are, however, other factors influencing the turnover of both sexual and asexual forms of primary inoculum. The underlying cause of the hotspot, its impacts and ways it can be managed are the subject of ongoing Hutton research using modelling (Torro-Galiana *et al*., 2019) and phenotyping (Cooke *et al*., 2016).

The deployment of effective and durable host resistance in combination with reduced levels of fungicide has proven an effective means of managing potato late blight (Kessel *et al*., 2018) with the two mechanisms suppressing selection in the pathogen population (Ritchie *et al*., 2019). However, crop yield and agronomic and quality traits often take priority in potato breeding programmes and, to date, there are no cultivars available that combine these traits with high blight resistance. The industry therefore remains reliant on blight-susceptible or moderately resistant cultivars protected by multiple fungicide applications. The decisions that growers and crop consultants make regarding when and what product to apply are critical to establishing and maintaining blight protection throughout the season. In this study, the modification of the spray schedule according to the Hutton Criteria alerts provided field-scale experimental evidence that a reduction in the number of fungicide applications was possible without compromising late blight control. Although blight pressure was low in 2018 another trial in the higher blight pressure 2017 season also saved one fungicide application. Such demonstrations are an effective way of increasing grower's confidence in DSS methods and further improvements such as a system that considers the impact of UV light on inoculum survival (Skelsey *et al*., 2018) are under development.

Blight fungicide choice is shaped by factors such as the specific characteristics of the product, the stage of crop development, blight pressure and product price. Fungicide insensitivity has been reported in the pathogen population and may result in ineffective applications that also increase selection pressure and add to the economic and environmental costs of potato production. Reports of insensitivity to Metalaxyl-M (mefenoxam) in the EU_13_A2 lineage (Cooke *et al*., 2012; Dey *et al*., 2018) has, for example, dramatically reduced the use of this fungicide in the UK (https://secure.fera.defra.gov.uk/pusstats/index.cfm). More recently, insensitivity to fluazinam and control failures have been reported due to the two clonal lineages EU_33_A2 and EU_37_A2 (Schepers *et al*., 2018). Prior to the reporting of fluazinam insensitivity in clone EU_37_A2 it spread rapidly from initial outbreaks in Shropshire in 2016 to crops in many other regions by the end of the 2017 season. The emergence of EU_37_A2 in Scotland in two outbreaks in 2019 is a cause for concern. However, widespread dissemination of the insensitivity issue in Britain via the farming press, AHDB Potatoes activities and the Fungicide Resistance Action Group, UK accounts for a significant reduction in fluazinam use (Garthwaite *et al*., 2019). Such a reduction will reduce the selection pressure on the pathogen population and may account for the presence of EU_37_A2 in only two outbreaks in Scotland in 2019 compared to the 13 outbreaks caused by another aggressive clone, EU_36_A2, that was also sampled in Scotland for the first time this season.

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AN UPDATE ON A CURATIVE LATE BLIGHT FUNGICIDE DECISION AID

K Maloney[1], N Havis[1], D E L Cooke[2], G Loake[3] and R A Bain[4]
[1]Crop and Soil Systems Group, SRUC, West Mains Road, Edinburgh EH9 3JG, UK
[2]The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK
[3]Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh EH9 3BF, UK
[4]Crop and Soil Systems Group, SRUC, Auchincruive Estate, Ayr KA6 5HW, UK
E-mail: kyran.maloney@sruc.ac.uk

Summary: This paper summarises the development of a simple decision aid, which aims to assist growers and agronomists when decisions about the use of curatively acting fungicides in the management of potato late blight (*Phytophthora infestans*) are taken. The decision aid is parameterised from glasshouse bioassays and field trials which explored temperature-dependent pathogen development and curative control decline with disease development. Pathogen growth was explored using a qPCR assay, which generated data within the incubation period, before visible symptoms have developed. In its current form, the decision aid provides a categorical prediction on the likely level of curative control based on temperature and time inputs. It has the potential to incorporate modifying factors such as cultivar resistance. The decision aid now requires validation using a large dataset to assess the utility of its predictions in different geographic areas and local conditions.

INTRODUCTION

Late blight of potato, caused by the oomycete pathogen *Phytophthora infestans* is one of the most important biotic stresses facing potato production in temperate regions (Fry, 2016). Frequent applications of fungicides are required for adequate management, generally on a weekly basis during the growing season (Schepers *et al*., 2017). All blight spray programmes should be protectant in nature, ensuring that fungicide is present on the foliage prior to the arrival of *P. infestans* spores. However, there are situations in which very recently established infections can be arrested by fungicide active ingredients which are taken-up by plant tissue; the so-called curative effect (Schepers, 1998). Only a small sub-set of active ingredients used commercially for late blight control have curative properties, but their importance within spray programmes has been increasing in recent years. In some situations a grower or agronomist may suspect that infection is likely; for example, if a high-risk weather period has just occurred, and that the use of an active ingredient with curative properties is warranted.

End users currently have somewhat limited information on which to justify the inclusion of an active ingredient for its curative properties within a given blight spray. It is known that the duration of the curative effect is very short; it is by definition confined to the pathogen's incubation period, which may be as short as 3 days, given optimum conditions and aggressive strains. A ranking of some formulations is available, but this is at least partially subjective. It has been demonstrated that the temperature at which the pathogen develops can have a modifying effect on curative activity (Genet *et al*., 2001), but this has not been explored in detail.

Previously (Maloney *et al*., 2018), we introduced a project, the goal of which was to produce a simple data-driven decision aid, and outlined some of the methodology used in its construction. Here, an updated overview of the decision aid is given, explaining how we envisage it being used, and suggesting an approach to its validation.

METHODS AND MATERIALS

Temperature-Dependent Development

The prototype decision aid consists of two linked deterministic models, the first of which describes temperature-dependent pathogen development and the second the likely curative control given an expected level of pathogen development. In both cases, the models consist of very simple equations which were parameterised using data from laboratory-based assays.

For the temperature dependent development model, information on pathogen growth within the incubation period of the pathogen's life cycle was the most relevant driver in relation to curative control. We hypothesised that the rate at which the pathogen colonizes host tissue strongly influences the level of curative control possible; i.e. the level of curative control declines as pathogen biomass increases. With this in mind, a qPCR assay was used to determine the quantity of *P. infestans* DNA present during the pathogen's incubation period.

A detached leaf assay (cv. King Edward) was used under controlled climatic conditions to obtain estimates of pathogen growth within the incubation period over a range of physiologically relevant temperatures. The same assay was used to obtain data on rates of symptom development, which has been described previously (Maloney *et al*., 2018). Specific details of the assay can be found in Maloney *et al*. (2020), but briefly: detached leaves were artificially inoculated with single isolate *P. infestans* spore suspensions. Leaflets were then incubated in sealed transparent boxes at fixed temperatures between 6 – 30°C with a photo-period of 16 hours. An initial 12 hour period at 18°C was included at each temperature so that infection rates were standardized.

At 12-hour intervals, up to and including 72 hours the boxes were opened and six randomly selected leaflets were removed, and frozen individually using liquid nitrogen. Genomic DNA was then extracted from individual leaflets, using the same method as Fraaije *et al*. (2001. Total *P. infestans* genomic DNA within each sample was then quantified using a qPCR assay, developed and described by Lees *et al.* (2012). Methods used were very similar to Cullen *et al*. (Cullen *et al*., 2001) and Lees *et al.* (2012), and can be found in full within Maloney *et al.* (2020). The assay was performed in 96-well plates using an AriaMx Real-Time PCR System (Agilent Technologies, USA), all wells were prepared in duplicate. Primers and probes, as well as other reaction components (TakyonTM MasterMix) were obtained from Eurogentec (UK). Within each plate, a serial dilution of *P. infestans* DNA from concentrations of 20 ng μL^{-1} to 2 x 10^{-5} ng μl^{-1} (seven dilutions in total) were included in order to generate a standard curve.

Curative Control

A number of methods were used to explore the relationship between pathogen development and the level of curative control, ranging from a laboratory-based leaf-disc bioassay through to field trials using staggered inoculations and curative fungicide treatments. Details of the leaf-disc assay have been given previously (Maloney *et al*., 2018), and data arising from the most aggressive isolate tested using this bioassay were used to obtain parameters for the curative control component of the decision aid. Experiments used a representative curative fungicide (propamocarb-HCl + fluopicolide, as Infinito, Bayer Crop Science) which is rated in the EuroBlight table as providing a 'good' curative effect.

It was also important that a similar pattern of declining curative control with disease development could be detected in the field, and so a small-scale field trial was conducted at SRUC's trial site at Auchincruive, South Ayrshire in 2016. The trial consisted of 28 plots (each 4.95 m long x 1.7 m wide) encompassing two ridges. Seed tubers of the late blight-susceptible variety King Edward (foliar resistance rating = 3) were hand planted, with 0.33 m spacing within rows. The experiment consisted of six blocks, each block had four plots, to which were allocated to different curative fungicide treatments times, or to an untreated control. Select sites within each plot were assigned for artificial inoculation, but four plots (not within blocks) were left uninoculated and untreated to assess the level of natural background late blight infection. Two artificial inoculation timings: (i) early morning and (ii) mid-afternoon, were applied to each plot, i.e. plants of the first ridge within a plot were inoculated at (i) and those in the second ridge at (ii). Thus, there were 36 possible disease development time intervals, the product of two inoculation times, three curative treatment times and six plots per treatment time.

Plants for inoculation were selected one day before the start of the experiment. Each inoculation site consisted of two stems which could be bunched together easily and these were tagged with loop-lock labels, of separate colours for the different inoculation timings. A total of four inoculation sites (= eight stems) per plot per timing were selected. Inoculum was prepared approximately an hour before the start of inoculations, with two separate batches, from samples of the same infected plant material, prepared for the different inoculation times. Details of inoculum preparation and inoculation method can be found in Maloney *et al.* (2020). Assigned plots were treated curatively at 1 day, 2 days, and 3 days from the start of inoculations, with precise spray times recorded. The treatment of plots took ~ 20 minutes for each of the timings. Plots received the curative treatment propamocarb-HCl + fluopiclode at 1.6 L ha^{-1} in a 200 litre water volume. Plots were sprayed using either an AZO backpack sprayer (350 kPa spray pressure and Lurmark F03-110 nozzles), or a tractor-mounted sprayer (350 kPa spray pressure and Lurmark F03-110 nozzles) depending on availability of equipment. Symptoms were assessed 7 days post inoculation. Inoculated stems were identified via the loop-lock tags and a record was made of the number of late blight lesions per leaf. Leaves between the first fully unfurled leaf below the stem apex (leaf 1) to leaf eight were assessed as these were the most likely to have intercepted both inoculum and fungicide spray.

RESULTS

Temperature-Dependent Development

An example of the results obtained from the qPCR assay are presented in Figure 1, which shows the increase in *P. infestans* specific genomic DNA at 22°C for the isolate 2012_10702B (genotype 8_A1). For most temperatures there was an exponential increase in the quantity of *P. infestans* DNA with time (data not shown). It was possible to assign growth rates within the incubation period at the different tested temperatures. These data were then described with a growth function (the Kontodimas equation (Kontodimas *et al*., 2004)) which met several pre-specified criteria (goodness-of-fit, simplicity, and biologically interpretable parameters). This function was used as the basis for the temperature dependent development component of the decision aid.

Curative Control

In the laboratory bioassays, curative control declined rapidly with time (data not shown). This was also the case it the field trial conducted at SRUC's Auchincruive site there was a statistically significant linear relationship between mean lesion count per compound leaf (natural log + 1 transformed) and increasing disease development time ($p < 0.01$).

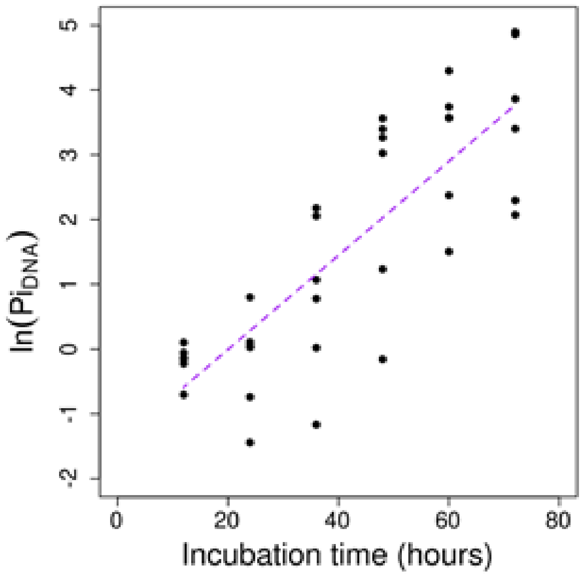

Figure 1. DNA quantity in a sample (pg) and time elapsed (hr) between inoculation and sampling. Data shown are from one isolate and temperature combination (2012_10702B, 22°C). Each point (●) represents an extraction and quantification from a single inoculated leaflet (n = 6, per time point). The data are described by a simple linear relationship ($p < 0.01$, $R^2 = 0.67$).

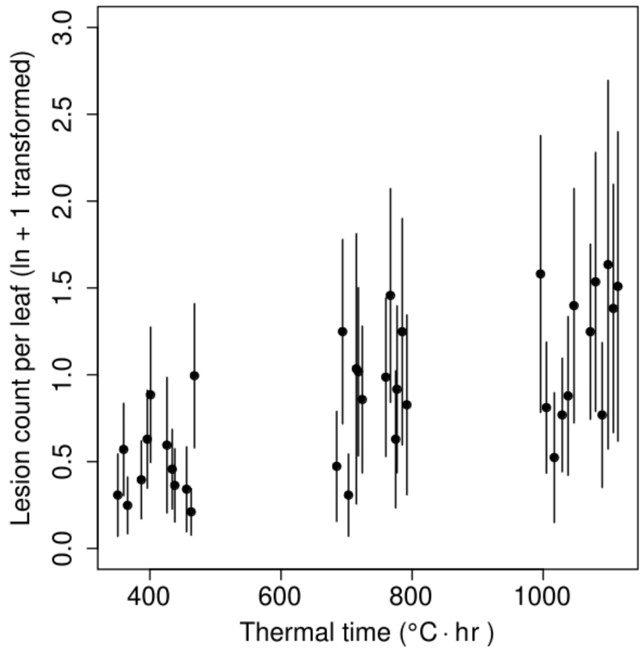

Figure 2. Transformed lesion count per compound leaf with increasing thermal time between inoculation and curative treatment (propamocarb-HCl + fluopiclode). Each point represents the mean from four inoculation sites (= 64 leaves), lines represent the 95% confidence intervals. Data are from a field experiment. Thermal time was calculated for the interval from inoculation to time of treatment and has units °C · hr.

Prototype Decision Aid

An overview of the decision aid is given in Figure 3, (Maloney, 2020). The aid uses the Hutton criteria (Dancey *et al*, 2017) as a starting point, and must be supplied with a set of air temperature readings (or predictions) and time intervals; from these the expected pathogen development is calculated. This expected development, is in turn used as input for the curative activity section of the aid. In its current form this provides a curative effectiveness category based on the expected level of curative control ('very likely', 'somewhat likely', 'unlikely' and 'very unlikely' to be effectual). The aid is designed in such a way that modifying factors such as the level of host resistance can also be incorporated.

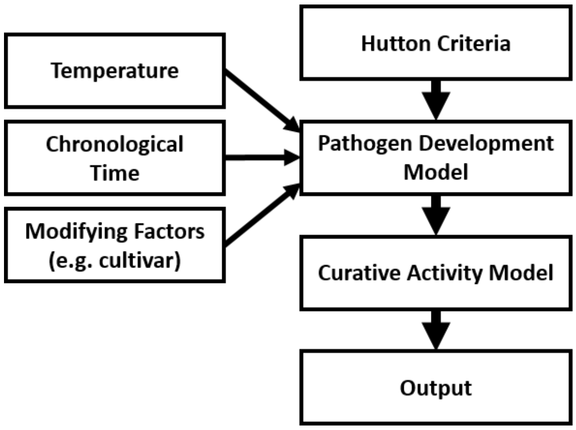

Figure 3. A schematic overview of the decision aid in its current form.

DISCUSSION

The *P. infestans-S. tuberosum* pathosystem is one of the most challenging systems for the implementation of Integrated Pest Management (Ritchie *et al*., 2018). The costs of control failure are high and growers therefore adopt a risk-adverse approach of regularly scheduled fungicide sprays. The decision aid developed within this project provides those tasked with managing potato crops with a tool to help guide the choice of fungicide products in different situations. The time window for curative control is too short for spray timings to be determined by the decision aid's predictions, but the aid can suggest whether the use of a product with curative activity is appropriate in a given situation.

The results from the field trial suggest that the relationship between decline in curative control with increasing disease development follows a similar pattern in field and the lab, increasing confidence that the decision aid will be applicable to the field situation. However, the decision aid must be properly validated before it is made available to the potato industry, as its utility is not yet proven. Very limited validation has been carried out in this project, and although this suggests the decision aid does provide sound guidance on the level of curative control expected, but it is not yet clear if it is always informative, and/or if it performs well in different geographic locations and for different potato crops. Useful data for pre-symptomatic growth of a number of *P. infestans* isolates have been obtained during this project, which may be of use to other researchers and modellers who require estimates of tissue colonization in the early stages of infection. Additionally, a simple novel methodology for assessing curative control has been developed. This may prove useful as the basis for assessing a wider range of curative fungicides with a standardized protocol.

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IS THERE A ROLE FOR MODERATE LEVELS OF CULTIVAR RESISTANCE IN LATE BLIGHT IPM?

R A Bain
SRUC, Niven Building, Auchincruive Estate, Ayr KA6 5HW
E-mail: ruairidh.bain@sruc.ac.uk

Summary: There is very considerable trials evidence that moderate levels of foliar resistance can make a substantial contribution to the integrated control of late blight but this evidence has generally not persuaded the potato industry to reduce fungicide inputs on cultivars with higher resistance ratings. The aim of this work was to investigate the suitability of cultivars as integrated control components based on their 1 to 9 resistance ratings for not only foliar blight but also tuber blight. In spite of severe foliar blight, weather conditions favourable for tuber infection and no input of zoospore-active fungicides, the incidence of tuber blight on four cultivars (Carolus, Setanta, Harmony and Orla) was either 0% or not significantly greater. For Gatsby, tuber blight incidence was within the Basic seed grade tolerance of the SPCS scheme. Similar results had been obtained in the previous year. The hypothesis, that cultivars with a combination of a moderate rating for both foliar and tuber blight offered sufficiently effective control of tuber blight through a reduction in foliar inoculum density to a value below the threshold for infection of tubers with a moderate level of resistance, was correct but the trial results were more complex than anticipated. One possible explanation is some non-contemporary published resistance ratings. Results are preliminary at this stage but six top-50 cultivars (by GB planted area) have a combination of resistance ratings the same, or superior, to those that gave very good control of tuber blight experimentally.

INTRODUCTION

Cultivar resistance should be a key component of integrated pest management (IPM) of potato late blight caused by *Phytophthora infestans*. Foliar resistance ratings of several cultivars were downgraded from resistant to moderately resistant when exposed to more aggressive and virulent genotypes such as 13_A2 (Lees *et al.*, 2012). However, there is strong evidence that such moderate levels of foliar resistance can make a substantial contribution to integrated control of late blight (Bain *et al.*, 2014). This evidence supports the conclusion reached in many earlier studies worldwide (Fry, 1978; Kirk *et al.*, 2001; Kirk *et al.*, 2005; Nærstad *et al.*, 2007). However, this considerable body of scientific evidence has not convinced growers and agronomists to reduce fungicide inputs on cultivars with moderate to high foliar blight resistance ratings. The most likely explanations for this failure to convert science into practice are as follows: 1) the evidence produced is strong for foliar blight control but not tuber blight and yet the potato industry is very aware that the negative economic impact of blight is considerably greater for the tuber phase of the disease than for foliar blight alone, 2) grower experience and applied research have shown that, under some circumstances that are not rare, substantial tuber blight can result from a very low severity of foliar blight. The potato crop is very expensive to grow and therefore growers are cautious to reduce fungicide inputs because of the perception that such an action could increase the risk of tuber blight.

A prevalent opinion is that cultivar resistance ratings are not sufficiently high for resistance to be included as an active component of integrated control. However, it is unlikely that waiting until they are available is a sound approach because it is questionable whether cultivars with resistance ratings of 7 to 9, combined with the attributes acceptable to the market, will be available in the short term.

Data in the AHDB Potato Variety Database showed that in 2017 the level of cultivar resistance for the 50 most widely grown cultivars tended to be low to moderate, not high. This is illustrated by 0, 77 and 19% of cultivars having foliar resistance ratings of 7 to 9, 4 to 6 and 1 to 3 respectively. The corresponding values for tuber blight resistance were 11, 65 and 20%. Although the resistance ratings of the most widely grown cultivars are lower than ideal, they may be more useful than their values suggest. Currently, cultivar resistance ratings for foliar blight and tuber blight are generated in separate tests. In each test the inoculum density is standardised. Whilst this generates robust resistance ratings for foliar blight and tuber blight individually, any link between them, i.e. the effect of foliar resistance on the quantity of inoculum challenging the progeny tubers (which can be large), is overlooked. This is an artificial situation because the source of the *P. infestans* zoospores challenging the tubers in a grower's crop is almost invariably the leaf lesions in the crop. In some scenarios moderate foliar resistance will result in the threshold number of sporangia for tuber infection not being reached.

The aim of this work was to investigate the suitability of some cultivars for integrated disease control based on their 1 to 9 resistance ratings. Field trials were used to determine which combinations of foliar resistance and tuber resistance ratings have the potential to offer acceptable control of tuber blight in a system of reduced fungicide inputs. All cultivars received the same blight fungicide programme, designed to allow controlled foliar blight development towards the end of the growing season (to simulate the IPM situation that growers are concerned about) coupled with poor direct protection of the tubers through fungicide product choice.

MATERIALS AND METHODS

Test cultivars were selected to give a range of combinations of foliar and tuber blight resistance ratings (Table 1). The ratings were presumed to be appropriate for genotype 13_A2 having last been updated between July 2014 and July 2018 on the AHDB Potato Variety Database. It should be noted that cultivar Setanta had two ratings for tuber blight resistance. Two cultivars, Shepody and King Edward, were included as susceptible references. Classified seed tubers (Seed Potato Classification Scheme) were sourced in Scotland and machine planted to achieve a uniform planting depth. In the randomised complete block design there were five replicate plots per cultivar; each plot consisted of 4 rows (3.4 m) x 5.1 m. The seed tuber spacing within drills was 23 cm.

The foliar blight fungicide programme deliberately used fungicides with zero, or very little, efficacy against tuber blight, i.e. Revus (150 g mandipropamid/ha, Syngenta) or mancozeb, without (Dithane NT, 1500 g mancozeb/ha, Sumitomo) or with cymoxanil (either Option, 90 g cymoxanil/ha, Corteva; or Curzate M, 90 g cymoxanil + 1360 g mancozeb/ha, Corteva). Fungicide products were applied at full UK label rates at a target 7-day interval. The severity of foliar blight in each plot was recorded from its first occurrence until desiccation (Anon., 1976). All tubers from the centre two rows of the four-row plots were harvested, washed and assessed twice, after weeks and then months of ambient storage. An average of 184 tubers per replicate plot was assessed.

Statistical analysis

At this stage the statistical analyses used were preliminary; all used Genstat 16th Edition. The foliar blight severity data and the tuber blight incidence data were not suitable for analysis of variance and this was not rectified by transformation (Shapiro-Wilk and Bartlett's tests). Kruskal-Wallis analyses of variance were carried out to test for a significant effect of cultivar resistance on both variates. Mann-Whitney U tests were used to compare Carolus (0% tuber blight) with the other seven cultivars individually to identify which cultivars resulted in a tuber blight incidence not significantly greater than zero.

Table 1. Resistance ratings of the cultivars used.

	Foliar resistance rating	Tuber resistance rating	Source of rating[1]	Last updated
Reference cultivars				
Shepody	2	3	NL & IVT	July 2015
King Edward	3	4	NL & IVT	February 2017
Test cultivars				
Orla	4	8	NL & IVT	February 2017
Harmony	5	3	NL & IVT	May 2018
Setanta	5	9 or 3[a]	NL & IVT	July 2014
Gatsby	7	3	NL & IVT	October 2014
Red Cara	7	7	NL & IVT	August 2017
Carolus	9	9	Breeder	November 2016

[a] two ratings given in AHDB Potato Variety Database
[1] NL = National List; IVT = Independent Variety Trials

RESULTS

In 2018, conditions were very favourable for tuber infection, as indicated by 13.2 % and 8.9 % tuber blight in the reference cultivars Shepody and K. Edward (Table 2). For five cultivars (Carolus, Setanta, Harmony, Orla and Gatsby) the incidence of tuber blight was very low and within the SPCS scheme Basic seed grade tolerance. Similar results had been obtained in 2017.

DISCUSSION

These preliminary results suggest that the risk of tuber blight was acceptably low in a simulated IPM system for five of the six test cultivars. The very good control of tuber disease by cultivars rated 9 for tuber blight (Setanta) (intriguingly also listed as a 3) or 9 for both phases of the disease (Carolus) was expected, but there are few cultivars with such high ratings that are agronomically acceptable.

Table 2. Tuber blight incidence in relation to final foliar blight severity for six test cultivars (2018 trial) (foliar blight resistance rating, tuber blight resistance rating).

Cultivar	Foliar severity (%) 26 Sept.	Tuber blight incidence (%)	Significance of difference from 0%	SPCS grade, prior to grading out of blight
Shepody (2,3)	36.0	13.2	*P*<0.001	No grade
King Edward (3,4)	40.0	8.9	*P*<0.001	No grade
Red Cara (7,7)	4.0	1.9	*P*<0.001	No grade
Gatsby (7,3)	7.7	0.5	*P*=0.026	Basic
Orla (4,8)	3.8	0.5	*P*=0.056	Basic
Harmony (5,3)	6.1	0.3	*P*=0.119	Basic
Setanta (5,9 or 3)	3.5	0.2	*P*=0.246	Pre-Basic
Carolus (9,9)	0.0	0.0		Pre-Basic
Significance of Kruskal-Wallace anova	*P*<0.001	*P*<0.001		

Table 3. Current areas in GB of cultivars with the same, or a superior, combination of resistance ratings to those used to achieve very good control of tuber blight experimentally

Resistance ratings (foliar, tuber)	Cultivar(s)	GB area 2019 (ha)	Rank (out of 50, by area in GB)
7, 7	Cara	630	46
6, 7	Eurostar	670	43
5, 7	Cara[1]	630	46
5, 7	Markies	6090	2
5, 7	Nectar	3510	5
5, 5	Mozart	690	42
5, 5	Nectar[1]	3510	5
5, 3	Harmony	590	47

[1] Indicates cultivar with more than one rating in AHDB Potato Variety Database

The hypothesis being tested in this work was that cultivars with a combination of moderate ratings for both foliar and tuber blight offer sufficiently effective control of tuber blight through a reduction in inoculum density originating from the foliage to a value below the threshold for infection of tubers with a moderate level of resistance. The hypothesis was correct, but the trial results were more complex than anticipated. Suitably effective control of tuber blight was achieved for cultivars with the following combinations of cultivar resistance: Setanta (5, 9 or 3), Harmony (5, 3), Gatsby (7, 3) and Orla (4, 8). Control of tuber blight was poorer with the combination 7, 7 (Red Cara). There do appear to be discrepancies between official resistance ratings and some of the results obtained.

Although the field experiment was inoculated with a single isolate of 13_A2 only, by the end of the growing season genotype frequencies had changed substantially: the genotype percentage incidences in September were 8% 13_A2, 79% 6_A1 and 13% 37_A2. Clearly cultivar resistance can only be reliably used in IPM if the ratings information is contemporary for the virulence genes in the *P. infestans* population challenging the crop.

As stated earlier, the inoculum for tuber infection in a crop almost invariably originates on the haulm. The quantity of haulm-derived inoculum is related to the 1 to 9 resistance rating of the cultivar for foliar blight. Separate tests for foliar and tuber resistance can't take account of any interactions between foliar and tuber resistance ratings. The interaction is clearly illustrated by the incidences of tuber blight in the trial for Shepody, Harmony and Gatsby. These three cultivars are rated 3 for tuber blight resistance. However, in the field trial tuber blight incidence was 13.2% for Shepody (foliar resistance rating of 2) but only 0.5% and 0.3% for Gatsby and Harmony respectively. The latter two cultivars are rated 7 and 5 respectively on the 1 to 9 scale for foliar resistance.

The information in Table 3 challenges the validity of the widely-held view that cultivar resistance-based IPM is not possible with current popular cultivars. That said, although the tabulated information is the most up-to-date available from the AHDB Potato Variety Database, firm conclusions would require the resistance testing of the experimental cultivars against appropriate genotypes of *P. infestans* individually. What is clear is that, with some further work, there is scope for cultivar resistance to contribute substantially to IPM.

The results presented here provide unintended evidence that some cultivars, e.g. Harmony and Gatsby, could perhaps be grown under high-risk (of tuber blight) conditions without the use of zoospore-active fungicides. Therefore, there is scope for these cultivars to contribute to reducing the risk of resistance developing in *P. infestans* to the two remaining highly effective modes of action against the tuber phase of the disease, i.e. QiI fungicides (amisulbrom & cyazofamid) and benzamides (fluopicolide), now that there is resistance to fluazinam in some genotypes of *P. infestans* (Schepers *et al.*, 2018). Fungicides with alternative target sites to the two listed above could be used for tuber blight control on less tuber-susceptible cultivars as an additional contribution to resistance management strategies.

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POTENTIAL IMPACTS OF '*CANDIDATUS* LIBERIBACTER SOLANACEARUM' (LSO) AND ITS PSYLLID VECTORS IN THE UK

J C Sumner-Kalkun[1], S Madden[1], F Highet[1], A Karley[2], Y M Arnsdorf[1], and D Kenyon[1]
[1]*SASA, Roddinglaw Road, Edinburgh, EH12 9FJ*
[2]*The James Hutton Institute, Invergowrie, Dundee, DD2 5DA*
E-mail: jason.sumner-kalkun@sasa.gov.scot

Summary: '*Candidatus* Liberibacter solanacearum' (Lso) is a psyllid transmitted bacterium which causes disease in Solanaceous and Apiaceous plants. It has recently been found to be present in several species of psyllid within the UK. Concerns were raised over *Trioza anthrisci* as it was found to be carrying Lso haplotype C (which is responsible for carrot crop loss in Scandinavia) and its preferred hosts are Apiaceous plants. This study examines the distribution and potential agricultural impact of this species. Field surveys across Scotland indicate that this species has a localised distribution but is found within crops. Laboratory experiments show that *T. anthrisci* can reproduce and transmit Lso on carrot plants, however symptoms were not observed during the 12-week study period. Initial studies suggest that *T. anthrisci* could pose a risk to agriculture due to potential transmission of Lso into crops and highlight the threat posed by the introduction of new vector species into the UK agricultural system.

INTRODUCTION

The bacterium "*Candidatus* Liberibacter solanacearum" is associated with disease in Solanaceous (Munyaneza 2012) and Apiaceous crops (Nelson *et al*., 2013), including Zebra Chip of potato. Symptoms in apiaceous crops include yellowing/ reddening of leaves, stunted growth and shoot and root proliferation. This bacterium has been found in the UK in plants and psyllid vectors but there have been no reports of Lso associated disease in the UK. There are currently 9 described Lso haplotypes with A, B and F associated with Solanaceous crops and C, D, and E associated with disease in Apiaceous crops such as carrot and celery. These haplotypes show close association with certain psyllid vectors. *Bactericera cockerelli*, the tomato-potato psyllid (TPP), is responsible for the transmission of haplotypes A and B in North and Central America and has recently been found in South America as far south as Ecuador (Castillo Carrillo *et al*., 2019). TPP has also invaded New Zealand via human-mediated distribution where it has caused millions of dollars-worth of damage to the agricultural industry (Teulon *et al*., 2009). TPP is present in Australia but without its associated Lso haplotypes (Australian Government Department of Agriculture and Water Resources 2017). In parts of Europe and Africa Lso is found in several different Apiaceous crops, particularly carrot. In Scandinavia the most damaging psyllid is *Trioza apicalis* (carrot psyllid) which is the main vector of haplotype C (Munyaneza *et al*., 2010). Feeding of this psyllid induces a gall-forming response from the carrot plant, causing damage which is further exacerbated by Lso infection sometimes leading to 100% crop losses. In the Mediterranean region (Alfaro-Fernández *et al*., 2012), Northern Africa (Othmen *et al*., 2018) and the Middle East the main vectors of Lso are *Bactericera nigricornis* and *B. trigonica* which harbour haplotypes D and E. The impact of the remaining haplotypes (G, H, and U) on crops of importance are unknown. Haplotype U is associated with *Trioza urticae* and is found in stinging nettle (Haapalainen *et al*., 2018). Haplotype G was found in herbarium specimens of *Solanum umbelliferum* (Mauck *et al*., 2019) but its presence or impact on the wider environment is unknown. Haplotype H was found within symptomatic carrots and Polygonaceae weed plants in Finland (Haapalainen *et al*., 2019) but its psyllid vector and overall impact is yet to be determined. In the UK *T. apicalis* is present but population sizes are not known. In a survey of suction trap catches as part of the Rothamsted Insect Survey *T. apicalis* was found in one location near York but made up <1% of total psyllid catches across the UK (Bell *et al*., 2017). In 2017 the psyllid *Trioza anthrisci* was found to harbour Lso haplotype C and was collected in suction trap samples from Moray, Scotland and Sweden (Sjölund *et al.,* 2017). The known host plant of *T. anthrisci* is *Anthriscus sylvestris* (cow parsley), which is significant as this weed plant is in the Apiaceae and closely related to cultivated carrot (*Daucus carota* subsp*. sativus*)*.* This raises concern over the potential for *T. anthrisci* to transmit Lso to important crops such as carrot and potato. We surveyed 19 sites across Scotland for the presence of known Lso vectors, with a focus on determining the distribution and population size of *Trioza apicalis* and *Trioza anthrisci*. We also aimed to establish colonies of *T. anthrisci* for further transmission studies to examine their potential to transmit Lso to carrot plants. This information will help us understand the risk posed to agricultural systems by Lso infected psyllids which are present in the UK.

MATERIALS AND METHODS

Psyllid Collection and Sampling

Psyllids were collected from 19 sites across Scotland were surveyed between 2018 and 2019 using sweep-netting across a transect of the field margins. Each site was visited at least twice during May-September and covered major carrot growing regions in the East and North-East of Scotland from Edinburgh in the south to Elgin in the north. Sweep-netting was performed at intervals of 30m or at points in the field margin where large amounts of Apiaceous crops were present. A transect through the carrot crop was also performed every 10th row of carrots, with continuous sweeping along the row. Psyllids were immediately separated from other taxa collected at each sample point using a pooter and stored in 25ml universal tubes. Specimens were stored on ice in transit and put into a -20 °C freezer upon arrival to the lab.

Psyllid Identification and DNA extraction

Psyllids were identified using a combination of classical taxonomy and molecular DNA barcoding. DNA was extracted using a non-destructive method which allows the retention of a voucher specimen from which the identification can be confirmed using classical taxonomy. Psyllids were DNA barcoded using one or two gene regions. The internal transcribed spacer 2 (ITS2) and cytochrome c oxidase subunit 1 (CO1) were amplified and sequenced for identification of different psyllid species. For amplification of ITS2 primers CA55p8sFcm-F and CA28sB1d-R (Peccoud *et al*., 2013) were used; and for amplification of CO1 gene regions arthropod barcoding Primers LCO1490 and HCO2198 (EPPO 2016) were used. All reactions were performed in 20 µl consisting of: 10 µl 2x Type-It Microsatellite PCR Kit Master Mix (Qiagen); 0.4 µl (10 µM stock) each forward and reverse primer; 7.2 µl molecular grade water (Sigma-Aldrich) and 2 µl of psyllid template DNA. Reactions were run on a Veriti 96-well thermal cycler (Applied Biosystems) using the following programs. ITS2: 95°C for 5 mins; 25 x cycles of (95°C for 30 s, 56°C for 90 s, 72°C for 30 s); and a final extension at 72°C for 10 mins. CO1: 94°C for 5 mins; 5 x cycles of (94°C for 30s, 45°C for 30s, 72°C for 1 min); 25 x cycles of (94°C for 30s, 51°C for 1 min, 72°C for 1 min); and a final extension of 72°C for 10 mins. PCR clean-up was performed using EXO-SAP and Ethanol precipitation, then sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Forward and reverse complementary DNA strands were sequenced separately for each sample and analysed in-house using a 3500xL Genetic Analyser (Applied Biosystems). Sequences were aligned, trimmed and identified in Geneious R11 v11.1.5 (Biomatters Ltd.). Contigs were assembled using Clustal-W multiple sequence alignment method and compared using BLAST against an internal psyllid barcoding database held at SASA and against GenBank and BOLD databases.

Psyllid Rearing

Thirty live individuals of *T. anthrisci* were collected from four carrot fields in the Elgin, Moray area using sweep-netting and direct collection with a pooter. These individuals were transported from the field in sealed 25 ml universal tubes provided with fresh cow parsley leaves to prevent desiccation and as a temporary food source in transit. Psyllids were introduced to cow parsley (*Anthriscus sylvestris*) plants and reared in a controlled environment at 18 °C on a 16:8 light:dark cycle. A subset (n =13) of these insects was tested for Lso using real-time PCR (Li *et al*., 2009).

Transmission studies

Lso infected *T. anthrisci* were introduced to one-month-old carrot plants. Ten plants were each provisioned with 5 immatures and 5 adults. Counts of immatures and adults observed feeding on the plants were taken at weekly intervals for 12 weeks. Each week a sample of leaf and stem was tested for Lso from each plant. Control plants were also grown on which no psyllids were added to rule out transmission of Lso from seed. DNA from plant material was extracted using the "BioSprint 15 Plant DNA Kit" (Qiagen) on a "Kingfisher mL Purification System" (Thermo Scientific). DNA extracted from plant material was screened for Lso using real-time PCR (Li *et al*., 2009).

RESULTS

Distribution of *Trioza anthrisci* and *Trioza apicalis*

Nineteen sites across Scotland were surveyed for the presence of *T. anthrisci*, focussing on areas of the East coast where carrot production is high. *Trioza anthrisci* were present only in Elgin, Moray and were absent from all other sites. *Trioza anthrisci* was found on carrot and cow parsley in four out of four carrot fields in Moray locations. The first adults were collected at the end of July and were present on carrot until the end of August. In these locations *T. anthrisci* was the most abundant psyllid on carrot. Immatures were not observed on carrot plants, but immatures and eggs were found on *Anthriscus sylvestris*. All *T. anthrisci* tested were positive for Lso haplotype C (n=16); remaining adults and immatures were used to establish live colonies of *T. anthrisci* at SASA. *T. apicalis* was not found in any of the field sites surveyed.

Reproduction of *T. anthrisci* on carrot

We set up 10 one-month-old carrot plants and introduced 10 immature and five adult *T. anthrisci* to each plant. Observations of immatures, eggs and adults on each plant were made weekly to assess host suitability and insect survival/reproduction. The number of adults present on carrot plants slightly increase over the first 3 weeks (Fig. 1). As this coincided with a reduction in immature stages found on plants this is likely to indicate the development of those initial immatures into adults. In week 4 the first eggs were observed, laid on the edges of leaves. In week 5 the number of adults had decreased due to mortality of the initial adults placed on the plants. The number of immatures began to increase in week 6 representing the hatching of the first batches of eggs laid (~week 3). From weeks 7-12 the number of immatures increased beyond the number of immatures initially introduced to the plants. Although number of observed adults did decrease from week 4 to week 8 the number of adults increased past its initial point after 11 weeks.

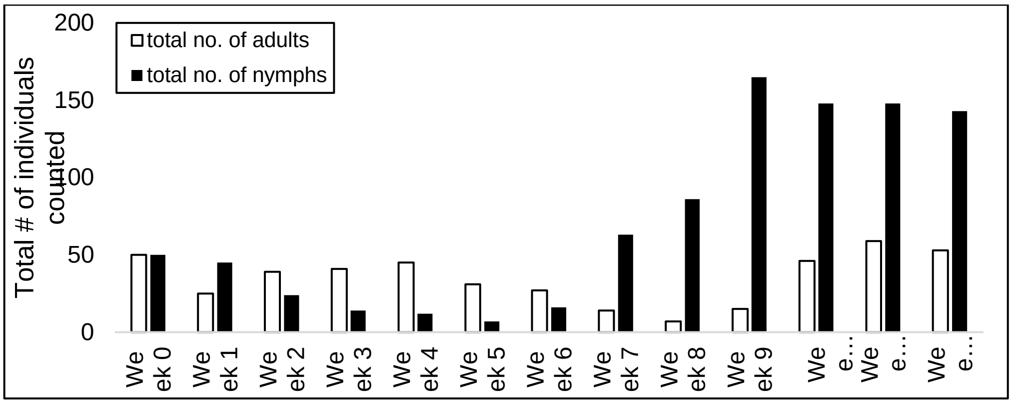

Figure 1. Numbers of immature stages and adults of *T. anthrisci* on carrot plants over a 12-week period. Fluctuations in the numbers of immatures and adults with an overall increase beyond the starting numbers show *T. anthrisci* was able to breed and reproduce and develop adequately on carrot plants.

Transmission of Lso to carrot by *Trioza anthrisci*

T. anthrisci infected with Lso haplotype C were introduced to carrot plants and reproduction, feeding and Lso transmission to the plant was monitored for 12 weeks. After 1 week no Lso positive plants were found (Fig. 2). Lso positives did occur after 2 weeks in a small number of samples but these did not come up in all technical replicates (triplicates) suggesting either false positives or a very low level of infection just below levels for reliable detection. Definitive true positives did not appear until week 7 when reactions showed positives across the triplicates (Fig. 2). Number of Lso positives from plants increased by week 10 but decreased during week 11 and 12. By week 12, positives had been confirmed in 70% of the plants tested (n= 10) but no symptoms of Lso were observed. All control plants were negative for Lso throughout the experiment.

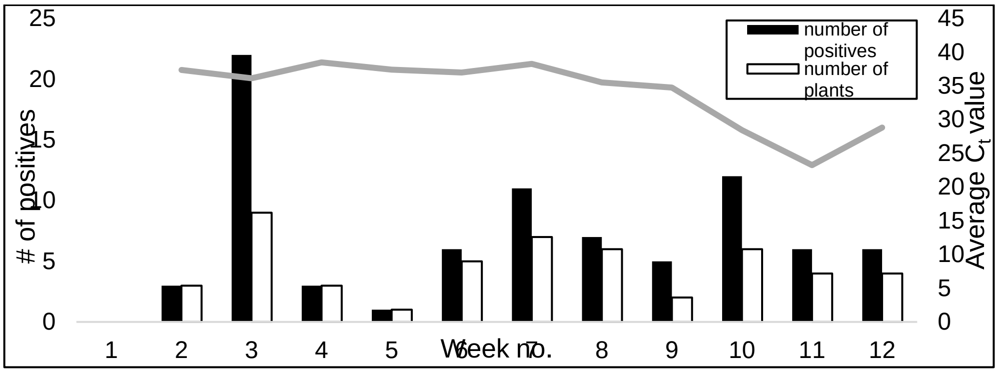

Figure 2. Number of Lso positive plants and plant samples over 12-weeks. Average C_t across all positives is also overlaid. Positives increased over time, with the first true positives found after 7 weeks. Average C_t decreased over time suggesting an increase in the titre of Lso infection.

DISCUSSION

A survey of crops and field boundaries over 19 sites across Scotland indicated that psyllids were rarely found within the crop and were mainly associated with known non-crop hosts. The carrot psyllid *T. apicalis,* prevalent in Scandinavia, was not found at any of the sites surveyed. The most abundant psyllid found on carrot crops was *T. anthrisci* only found from four sites located in the Elgin, Moray area in North-Eastern Scotland. The reasons for this localised distribution pattern and the lack of *T. apicalis* are not known but may be due to the availability of suitable overwintering sites in the UK as some Triozidae require coniferous plantations to overwinter successfully. Preliminary results in controlled environment conditions with no-choice bioassays show that *T. anthrisci* can complete its life cycle on carrot plants and is able to transmit Lso to carrot. However, whilst adults of this species were collected on carrot plants, no evidence of reproduction was observed within the crop. This may be due to sampling methods overlooking cryptic life stages (eggs and immatures) or due to the timing of sampling activities, i.e. sampling was performed when eggs and immatures are not present or are at low levels. However, all life stages of *T. anthrisci* were found on cow parsley (*A. sylvestris*) during the same sampling efforts. We have confirmed the original finding of haplotype C within *T. anthrisci* and found this bacterium within wild weed hosts (*A. sylvestris*). The bacterium was found in 100 % of adults tested suggesting it has a high infection rate in *T. anthrisci* populations and is persistent. We have confirmed that *T. anthrisci* is able to reproduce on carrot plants in controlled conditions and this is the first observation of this in UK populations. However, the tendency of this psyllid to colonise carrot in the field depends on its feeding preferences and whether it will colonise carrot when other available Apiaceous hosts are available. In addition to reproducing on carrot, *T. anthrisci* was also able to transmit Lso to carrot plants. However, no symptoms of Lso infection were observed during the 12-week period. Whilst it is still unclear whether *T. anthrisci* is a potential threat to potato the closely related species *T. apicalis* was not able to transmit Lso to potato (Nissinen *et al*., 2012) although Lso C has occasionally been found in potato plants in Finland (Haapalainen *et al*., 2018). Similarly, in the Mediterranean region, *Bactericera trigonica,* was shown to be a low risk to potato crops due to high preference for Apiaceous hosts and less efficiency in feeding on potato plants, nonetheless low-level transmission was observed in potato from a psyllid usually associated with carrot (Antolínez *et al*., 2017). The ability of *T. anthrisci* to transmit Lso to potato still needs to be assessed. In Scotland the areas of carrot production heavily overlap the areas of potato production and volunteer potato plants are regularly found within carrot crops. Further study should include assessment of *T. anthrisci* host plant preference and feeding/transmission studies to advise the risk of this psyllid causing damage to carrot and potato, although field observations suggest that it prefers to complete its life cycle on cow parsley.

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A NEW WAY TO SPOT DISEASE AND ROGUES? REAL-TIME DRONE BASED SEED POTATO INSPECTIONS

S Gibson-Poole[1], T Davey[2], J Ellicott[2], M McCreath[2] and A Hamilton[1]
[1]*Scotland's Rural College, Peter Wilson Building, Kings Buildings, Edinburgh, EH9 3JG, UK*
[2]*SASA, Roddinglaw Road, Edinburgh, EH12 9FJ*
E-mail: Simon.Gibson-Poole@sruc.ac.uk

Summary: Unmanned aerial vehicles (UAV) continue to evolve, with new functions and sensors being developed every year. Due to their aerial viewpoint, they could prove to be useful tools for detecting the presence of disease within agricultural crops. This paper investigates the use of an "off the shelf" UAV with real-time video goggles, to determine if such technology can give detailed enough feedback to allow the identification of varieties, variations, rogues and disease within a crop of seed potatoes (*Solanum tuberosum L.*). Trial plots used for examining prospective seed potato inspectors were assessed using the UAV and whilst the results indicate that the current technology is not yet effective, once certain enhancements are made then UAVs could potentially form part of the potato inspection process in the future.

INTRODUCTION

Unmanned aerial vehicles (herein termed drones) are expected to provide significant agronomic benefits in the future, with research previously conducted at Scotland's Rural College (SRUC) highlighting their potential in detecting the onset of disease within potato crops (Gibson-Poole *et al*., 2017). Other researchers have also shown their uses to assess the presence of diseases and pests such as late blight (*Phytophthora infestans*; Suigiura *et al.* 2016; Franceschini *et al.* 2019), Colorado potato beetle (*Leptinotarsa decemlineata*) infestation (Hunt & Rondon, 2017) and environmental canopy damage (Zhou *et al.* 2016).

Almost a quarter of the total UK potato production comes from the Scottish potato industry and approximately half of the potato area planted in Scotland is dedicated to producing seed potatoes. As a result, almost three quarters of UK potato production is derived from Scottish seed (SASA, 2019a). The Seed Potatoes (Scotland) Regulations 2015 set out the minimum requirements that Scottish seed potatoes must meet for certification, grading and marketing. It is SASA's responsibility to enforce the certification regulations within the Seed Potato Classification Scheme (SPCS) as governed by the 2015 Regulations. Certification is based on visual inspections of the crop by Scottish Government Plant Health Officials (herein termed inspectors), who are trained and assessed annually by SASA for competence using a purposely designed set of trial plots (~3000 plots, ~1300 varieties), that contain the top 30 commonly grown varieties and a large range of common diseases and varietal genetic variations.

Potato inspections typically do not cover the entire crop area but employ a calculated number of zigzag passes through the crop to ensure an effective inspection (typically 0.1-0.2 ha of the crop is directly inspected depending on the size of the planted area; SASA, 2019a). Inspection is carried out on foot, typically with two or more inspectors at a time with each inspection taking 30-60 minutes depending on the size of planted area. Drone technology is already being used for direct inspection purposes (i.e. a human operator is directly viewing and analysing the video footage from the drone), primarily for infrastructure inspection, to reduce exposure of the inspectors to potentially hazardous environments and to speed up the inspection process

(Agnisarman *et al*., 2019). Therefore, the use of this technology could also allow seed potato inspectors to remotely identify varieties, genetic variations, rogues and the presence and type of disease, potentially increasing the area of crop covered within each inspection whilst negating the need to move though the crop, which itself could cause damage to the plants and provide a vector for disease.

As an alternative to typical remote sensing experiments using drones (e.g. regular monitoring and analysis of imagery via computer aided classification), the aim of this experiment was to identify if current "off the shelf" drones can be utilised as effective real-time crop inspection tools by trained potato inspectors.

MATERIALS AND METHODS

The trial plots used for this experiment were located at SASA's Gogarbank Farm near Edinburgh and were part of their annual seed potato trials that are used for training new and experienced potato inspectors. A small number of plots are set aside for use as part of the seed potato inspectors' exam. Sixteen plots are used for the exam (comprising 2 rows of 25 tubers), with each plot being of a specific variety and containing a number of faults (cases of disease, variation and rogues). These sixteen plots are replicated three times to ensure that at least one set of sixteen good trial plots is available to use for the exam.

A ground-based visual inspection of the plots was carried out by SASA staff prior to the flights taking place. The variety in each plot was identified, the number and variety of each rogue per plot and the number of plants showing signs of mosaic virus infection, leaf roll, blackleg or an unwanted variation were recorded. All plots were assessed and eight were chosen to be candidates for the aerial assessment. Points were awarded per plot as follows; each correct identification of a variety scored two points; each correct fault identification scored two points; one point was awarded for each rogue identified and a further point if the variety was also correct; for each incorrect variety name or fault, one point was subtracted.

Aerial methodology

The aerial assessment was carried out in the afternoon of the 28th June 2019, after the plots had been used for the main inspector examinations. A DJI Mavic 2 Pro drone (DJI, Shenzhen, China) was used as the aerial platform, and connected to a video headset (DJI Goggles Racing Edition; DJI, Shenzhen, China; Figure 1). These video goggles were set to move the drones camera within its gimbal in conjunction with the head movements of the aerial inspector, so the inspector could look around from the perspective of the drone, with 90° lateral movement to the left and right, 45° to nadir and 30° upward. The camera was set to show video using the 4K HQ setting (at 30 frames per second), which gives the appearance of zooming the video footage, when in fact it is simply cropping the footage (to 55° field of view) and providing the highest image quality. The camera was also set to auto white balance with the default video encoding of H.264.

Seven potato experts (current inspectors or people directly involved in the industry) volunteered to be aerial inspectors. Three of them inspected all eight plots, and the other four inspected only four plots each (due to time constraints). Therefore, each plot was viewed by five different inspectors. The drone was manoeuvred around the plots at ~2 m above the ground to replicate a similar viewing angle as that used by the inspectors for the traditional ground-based exam (Figure 2). The drone would always start looking towards the centre of each plot, and the inspector would instruct the drone operator to move the drone as desired to view the plot from different angles. As with the traditional ground-based exam, each plot could only be viewed for

1 ½ minutes, and a scribe recorded what the aerial inspector could identify, along with any other comments relating to the experience of viewing the plots using this equipment.

Figure 1. Example of the trial plots on 28/05/2019. The drone is shown in flight (red circle) at ~3 m above ground level. An inspector (red arrow) uses video goggles to view the footage from the drone and drone operator (orange arrow) controls the drone in flight.

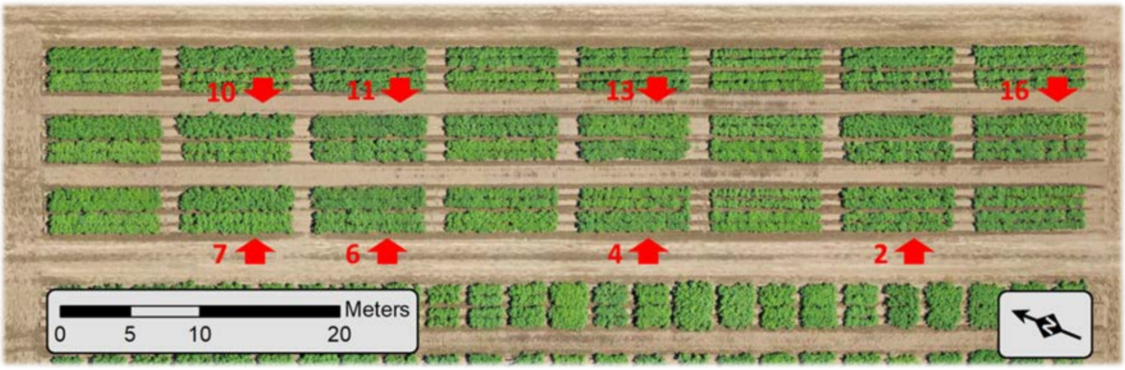

Figure 2. Trial plots on the 27/06/2019. Each of the plots viewed is indicated (red numbers) with an arrow showing the direction of view.

RESULTS

The identification of varieties gave mixed results (Table 1) with some being strongly identified by all inspectors (e.g. King Edward, Hermes and Maris Peer) and others being identified with varying degrees of accuracy or not at all (e.g. Cabaret). Similarly, when rogues were identified (average detection rate of 21%), the variety was only correctly given 20% of the time, as most inspectors could not name the variety even when confident a rogue was within the plot. None of the inspectors identified the presence of all the rogues in each plot and only one inspector identified the single variation that was present within one of the plots (giving an average detection rate of 20%). The identification of disease within the plots also gave mixed results (Table 2), with blackleg not being detected at all and leaf roll only sporadically (average detection rate of 13%). Mosaic virus was detected to varying degrees in every plot (average detection rate of 20%), with one inspector correctly identifying all three virus infected plants within plot 7 (variety Hermes), but no one identified the four virus infected plants within plot 11 (variety Rooster). One inspector also identified more virus infected plants than were present (plot 10, variety Estima).

Table 1. Varieties, variations and rogues present and identified within each plot. “Total” indicates the total number of that type of fault present per plot. “Avg” indicates the average identification rate for the fault based on all the inspectors. “Max” indicates the maximum number of that type of fault identified by a single inspector.

Plot	Variety	Varieties Avg (%)	Variations Total	Variations Avg (%)	Variations Max	Rogues Total	Rogues Avg (%)	Rogues Max
2	King Edward	100				2	30	1
4	Cabaret	0				2	10	1
6	Slaney	50				2	25	1
7	Hermes	100	1	20	1			
10	Estima	80				1	0	0
11	Rooster	60						
13	Burren	20				1	20	1
16	Maris Peer	100				2	30	1

Table 2. Number of disease faults present and identified within each plot. “Total” indicates the total number of that type of fault present per plot. “Avg” indicates the average identification rate for the fault based on all the inspectors. “Max” indicates the maximum number of that type of fault identified by a single inspector.

Plot	Mosaic Total	Mosaic Avg (%)	Mosaic Max ID	Leaf Roll Total	Leaf Roll Avg (%)	Leaf Roll Max ID	Blackleg Total	Blackleg Avg (%)	Blackleg Max ID
2	15	21	5						
4	3	13	2						
6	3	17	2				1	0	
7	3	20	3						
10	1	80	3						
11	4	0	0	1	40	1			
13	1	20	1	2	0	0			
16	4	25	2						

When looking at the points score for the exam, a perfect score for all the plots inspected would total 114. The best individual inspectors’ points score was 33 (29%) and the average points score for all the inspectors was 26.2 (23%). Four of the inspectors had completed the ground based exam earlier in the day so the possibility of bias within the results exists. However, the plots viewed were a mixture of two of the exam replicate plots, and the best individual score was from someone who had not previously seen the plots. A score of 69 (60%) was required to pass the exam, so on this basis all the inspectors would have failed to pass the potato inspection exam.

DISCUSSION

Although this experiment was both small and conducted over a short period of time, it is clear from the results of the aerial examination that the drone equipment used did not fulfil the role intended. This is to be expected as the equipment was designed for drone racing and not for visual inspection roles, but it received very positive reactions from all those who tried it, with most seeing potential in such a system once certain areas could be improved.

One of the areas that caused difficulty for identification of varieties was that of colour, in that some inspectors could not see distinct changes in colour between varieties, but rather a washed out “green” or the wrong shade of green. The video encoding was set to the default of H.264 which should have given a bit depth (colour depth) of at least 24 bit (~16 million colours), so the range of colours should have been sufficient. The option for high dynamic range (HDR) video could have improved this further but was not tested at the time. The white balance (or colour temperature) may also need to be altered by the inspector to improve the visual representation. The varieties that were strongly identified had distinct attributes such as the numerous red-violet flowers of Maris Peer (SASA, 2019b), making identification easier. However, the video feed itself was flat (2 dimensional) and if it were 3 dimensional (3D) then identification of structural features such as Cabaret’s deeply veined leaflets or Burren’s corrugated leaflets might have become more apparent (SASA, 2019b). Coincidently, live 3D video transmission technology from drones is possible (Smolyanskiy & Gonzalez-Franco, 2017), with some systems having been developed for use with custom built drones to assist with depth perception during drone racing.

An extra element that would potentially assist with spotting disease would be the use of wavelengths of light beyond the human visual system. For instance, being able to view a plant based on its reflectance of near infra-red wavelengths could more effectively show signs of stress (Mahlein *et al*., 2012). DJI have very recently produced a drone with a multispectral camera (The P4 Multispectral) that can transmit both a visual video feed and normalised difference vegetation index (NDVI) video feed. NDVI can be used to identify plant stress at the canopy level and, along with other sensors such as thermal imagers, could show clear signs of disease induced cellular damage at the leaf level (Mahlein *et al*., 2012).

Some inspectors also indicated issues with viewing angle, focussing and remembering where they were (or had already looked) when viewing the plots. This would be partly resolved by the inspectors having control of the aircraft movements and settings themselves as later testing showed that this made the act of inspection using the drone more instinctive. However, a simpler and more intuitive mechanism for changing settings such as white balance and focussing whilst wearing the video goggles would be more desirable than those currently available. Finally, for drone technology to be an effective real-time inspection tool, weather proofing is also required. Current “ready to fly” drones are typically not waterproof, and the drone used in this experiment should not be flown in wind speeds above 10 m/s (DJI, 2018). Some designs of waterproof drones are however available, although they were created more specifically for filming within marine environments (Hughes *et al*., 2019).

To conclude, as drone regulation changes due in 2020 look set to enable easier adoption of commercial drone operations within rural areas (CAA, 2019), the use of this technology is becoming more appealing. Although the technology as it stands is not yet ready to be used as part of the SPCS, enhancements could lead to even more innovative designs and sensors that could improve the speed and accuracy of seed potato inspections in the future.

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ALTERNATIVE GROWING MEDIA STRATEGIES FOR MINI-TUBER PRODUCTION

R C McHugh and G R D McGrann
SASA, Roddinglaw Road, Edinburgh, EH12 9FJ
E-mail: ros.mchugh@sasa.gov.scot

Summary: In recent years, there have been renewed efforts to reduce peat used for cultivating plants. UK government white papers published in 2011 and 2018 call for the elimination of commercial peat use by 2030. Early stage seed potato production in Scotland relies on peat to raise mini-tubers, the first tubers in the potato production chain, whose high health status is paramount to the health of future generations of potatoes. Growing media were assessed for their potential to reduce or eliminate the use of peat without compromising the quality, productivity or health of minitubers. Controlled environment and glasshouse experiments assessed the potential of using reduced peat, coir, wood products and wool compost blends to support production of healthy minitubers. All growing media tested supported the production of healthy minitubers. However, significant variation in overall tuber yields, individual tuber numbers and tuber size was observed between different growing media. The potential of these alternative growing media to reduce the use of peat in Scottish seed potato production will be discussed.

INTRODUCTION

Pre-basic tissue culture (PBTC) produces the first tubers in the potato production chain and preservation of their high health status is of paramount importance to the industry. Mini-tuber growers rely on peat as a major constituent of their growing media as peat is widely considered to be pathogen-free (Schmilewski, 2008). However, they are under increasing pressure to reduce their use of peat. In 2011, the UK Government released the White Paper “The Natural Choice: Securing the value of nature” (DEFRA, 2011). This set out targets for reduction in the use of peat. The aim was to end peat use in the public sector by 2015, by amateur gardeners by 2020 and by professional fruit, vegetable and plant growers by 2030, albeit on a voluntary basis. Although these targets have not yet been met the overall aim was reinforced by “Our 25 Year Plan to Improve the Environment”, published in 2018 (DEFRA, 2018). Growers need to be prepared for the likely eventuality that peat use will be eliminated or much reduced.

The suitability of alternative growing media for potato minituber production must be determined to allow PBTC minituber growers to make informed choices about the growing media they use to safeguard future production of seed potatoes. This has also been recognised in other sectors, including a large-scale peat alternatives evaluation in the horticulture sector (Mulholland, 2016). The four most popular growing media used in horticulture as alternatives to peat are: wood-based media such as wood fibre (33% of alternatives per volume supplied in 2014), green compost (22%), coir (20%) and bark (17%) (Denny and Waller, 2016). More than a third of growing media used by the horticulture industry were peat alternatives during the period 2012-2014 whilst alternatives constituted around half of all amateur use (Denny and Waller, 2016). This contrasts to the relative amounts used in 1999 (Alexander, 2014) where peat alternatives were used much less frequently with ~6% of total growing media used in horticulture being peat-alternatives. Despite the promise of alternative growing media, little is known regarding the production of disease-free plants from these products. Research in the horticultural industry has shown that alternative growing media show promise (Mulholland et al., 2016) although results have been mixed depending on the crop tested. Here we evaluate a range of peat-alternative growing media to produce disease-free minitubers suitable for the UK seed potato industry.

MATERIALS AND METHODS

Growing media

Peat-based minituber mix (Sinclair Pro, Cheshire, UK) was used as an industry standard control and compared to coir, pine bark 0-8mm, wood fibre (ICL Speciality Fertilisers, Suffolk, UK) and wool compost (Dalefoot Composts, Cumbria, UK) growing media. A blend of peat and coir (60:40 v/v) was also included in the experiments as professional growers routinely use blends with up to 30% non-peat constituents (Barrett, 2018) and, based on discussions with PBTC growers, 40% coir was considered the limit for a peat blend for mini-tuber production.

Microplant propagation

Microplants of the varieties cv. Hermes and cv. Maris Piper were obtained from the Nuclear Stock Unit at SASA, Edinburgh, UK. Murashige and Skoog medium (1 x Murashige and Skoog medium [MP Biomedical, California, USA], 3% sucrose w/v, 0.7% w/v Technical agar No. 3 [Oxoid, Thermo Fisher Scientific]) was used to subculture stock microplants which were grown for 21 days at 18°C with a 16:8 hour light:dark cycle in a Fisons Fitotron 600H (Weiss Technik, Loughborough, UK).

Microcosm experiments

Plant growing medium was added to a sterile 570 cm^3 polypropylene cup (Nupik-flo Ltd., West Sussex, UK) to a depth of 35 mm and the medium wetted with sterile distilled water (SDW). A single microplant was transplanted in to the growing medium in each plastic cup and a second sterile 570 cm^3 polypropylene cup sealed on top of the first using micropore tape to complete the microcosm. Each variety and growing medium combination was tested in three microcosms in at least two independent experiments. Plants were grown in a Fitotron Standard Growth chamber SGC120 (Weiss Technik) at 20°C for 12 weeks with a 16:8 light:dark cycle and watered with SDW as required. Plants were harvested after 12 weeks and minitubers were washed in SDW and measured lengthways from rose end to stolon end using a tape measure to give a measurement for tuber size. Each minituber was visually assessed for surface blemish diseases prior to storage in an extraction bag (BioReba AG, Reinch, Switzerland) at -20°C.

Glasshouse trials

Each growing medium was placed into 5 litre plastic pots. Four replicate pots were prepared for each variety and media combination and a single four-week-old microplant transplanted into each pot. The experiment was designed with randomised complete blocks with two treatment factors (variety and media). Microplants were grown to maturity in a temperature controlled glasshouse under a 16:8 hour day:night photoperiod set at 18°C during the day phase and 15°C during the night phase. Pots were watered as required with tap water. Tubers were harvested from each replicate pot after 16 weeks. Individual minitubers were measured and weighed. The weight of all tubers from a single replicate pot unit was determined as the overall yield of each plant. Disease assessments were made on each minituber, scoring the proportion of the tuber surface area covered with surface blemishes or the incidence of internal rots. Peel samples were taken from heel to rose end of tubers using a standard nylon handled potato peeler (Victorinox AG, Schmiedgasse, Switzerland), placed in an extraction bag (BioReba AG, Reinch, Switzerland) and stored at -20°C until required. Two independent experiments were performed.

Molecular diagnostics

DNA was extracted from minituber tissue, using the BioSprint 15 DNA Plant kit (Qiagen) with a KingFisher™ mL Purification system (Thermo Fisher Scientific, Paisley, UK) following the manufacturer's instructions. Quantitative PCR (qPCR) assays were performed to detect *P. pustulans* (Lees *et al*., 2009), *Colletotrichum coccodes* (Cullen *et al*., 2002), *Streptomyces scabies*, *Spongospora subterranea* (Qu et al., 2011), *Helminthosporium solani* (Cullen *et al*., 2001) and *Pectobacterium atrosepticum* (Humphris *et al.*, 2015) in tubers using the Takyon™ Rox Probe Mastermix dTTP Blue system (EuroGentec, Liege, Belgium) following the manufacturer's protocol. All qPCR reactions were run on a CFX96 Real-time PCR detection system (BIO-RAD, Hertfordshire, UK).

Statistical analyses

All data were analysed using GenStat v.14 (VSN International, 2011). Variation in minituber size produced in the microcosm experiments by different growing media was assessed using a general linear model (GLM) with variety, growing medium and the interaction between these two terms as factors within the model. For the glasshouse experiment GLM analysis was used to assess variation in tuber number per plant, tuber yield per plant, individual tuber size and individual tuber weight attributed to the effects of experiment, block, variety, growing medium and the interactions between variety and growing media.

RESULTS

Potato minitubers were produced in microcosms for each growing medium assessed. There was no significant difference in the size of minitubers produced by the two varieties ($P = 0.930$) but there were significant differences in tuber size when grown in the different growing media (Fig. 1; $P < 0.001$). Minitubers produced in pine bark ($P = 0.023$) or wood fibre ($P = 0.016$) were significantly smaller than those produced in peat, whereas wool grown plants produced larger minitubers than those from peat ($P = 0.004$). No disease symptoms were observed on any of the minitubers produced in any of the growing media. qPCR analysis confirmed the absence of any pathogens in minitubers produced in any of the growing media (data not shown).

Glasshouse trials further assessed the potential of the different growing media for producing disease-free minitubers. There was no significant effect of experiment ($P=0.106$) or variety ($P=0.338$) on the number of tubers produced per pot but growing medium did have a significant effect ($P<0.001$) with all media yielding fewer tubers compared to peat except coir which yielded a higher number (Fig. 2A).

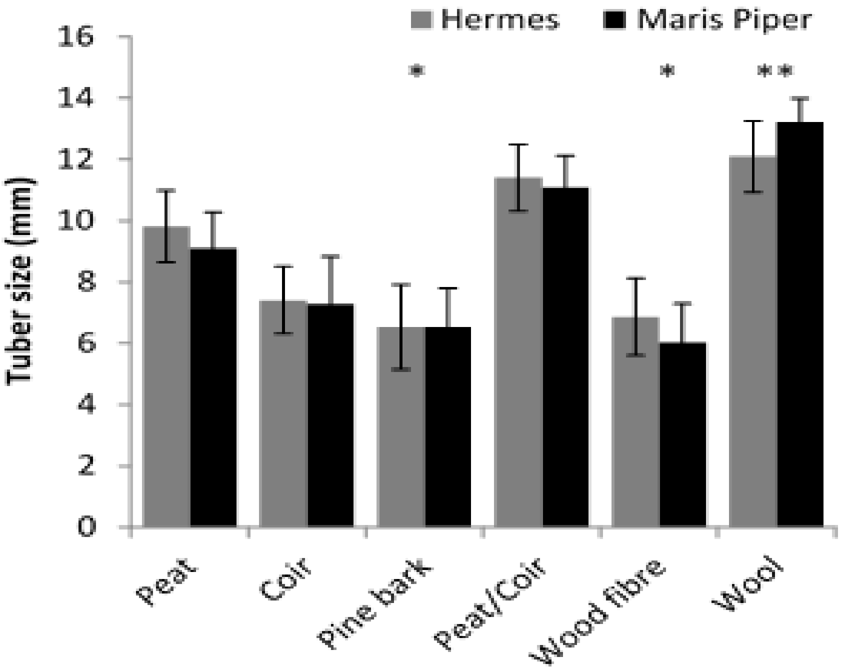

Figure 1. Size of minitubers produced in peat and peat-free growing media under controlled environment cabinet microcosm conditions. Error bars indicate ±1 SE. ** = P < 0.01; * = P < 0.05 differences comparing peat-alternative growing media to peat.

Growing media also had a significant effect on total tuber yield weight per pot (P<0.001) with all growing media except coir yielding significantly lower than peat (Fig. 2B). Variety had a significant effect on tuber size (P=0.017) with cv. Maris Piper tubers being larger than cv. Hermes tubers. Significant effects on tuber size were also observed for growing medium (P<0.001). Tubers produced in coir, wood fibre or wool were all significantly smaller than tubers produced in peat compost (Fig. 2C). There was no effect of variety on tuber weight (P=0.936) but growing medium did have a significant effect (P<0.001). Tubers produced in bark, coir, wood fibre and wool compost were significantly lighter than tubers produced in peat (Fig. 2D). Tubers produced in any of the growing media showed no signs of surface blemish diseases or internal rots. No pathogen DNA could be amplified from any of the tubers grown in any of the growing media except a single tuber produced in peat/coir (60:40 v/v) which indicated the presence of *S. subterranea*. None of the other tubers in this replicate were positive for this pathogen.

DISCUSSION

The drive to limit environmental impact by reducing or eliminating peat used in growing media poses a serious threat to the seed potato industry. As the first commercially produced tubers in the potato production chain, the propagation of pathogen-free PBTC minitubers is critical to safeguard the industry. Therefore, identifying peat-free growing media that can be used to produce commercially viable pathogen-free minitubers is of great importance to the seed potato industry.

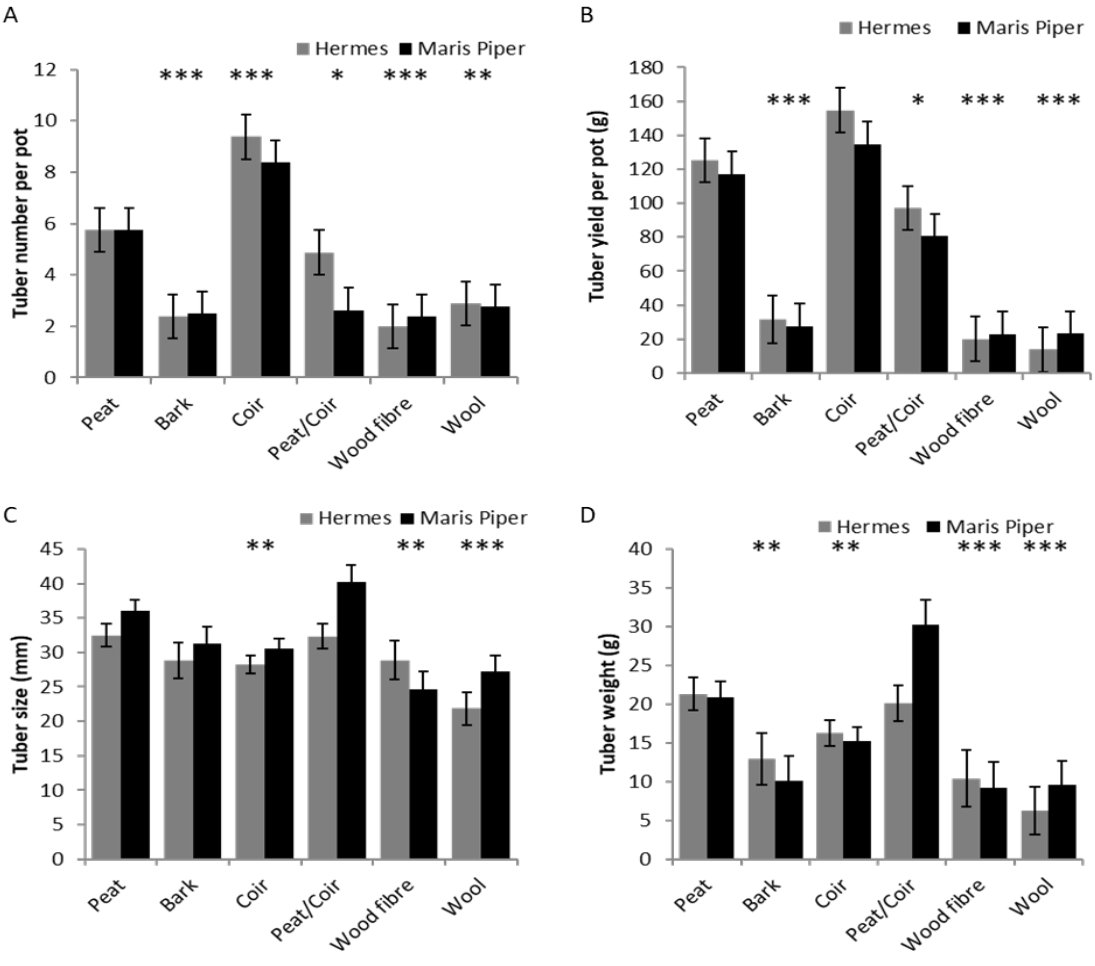

Figure 2. Effect of different growing media on tuber production under glasshouse conditions. (A) total number of tubers produced per pot; (B) total tuber yield produced per pot; (C) individual tuber size; (D) individual tuber weight. Error bars indicate ±1 SE. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$ differences comparing peat-alternative growing media to peat.

Controlled environment experiments demonstrated that potato plants could develop in peat-free and peat-reduced media and produce minitubers free from symptoms of surface blemish and rot diseases. However, yields assessed as total tuber weight, individual tuber weight, number and size varied between the various growing media. Visual analysis and molecular diagnostics for surface blemish and blackleg suggests that none of the growing media used poses a considerable risk of pathogen transfer. Wool compost showed strong potential as a peat-free growing medium for minituber production based on the microcosm experiments, but the tuber yields under glasshouse conditions were significantly lower than plants grown in peat. Wool-based products have shown some potential as amendments for producing various horticultural crops including tomato, pepper and aubergine (Gorecki and Gorecki, 2010). Assessing how these peat-free and peat-reduced growing media performed under PBTC minituber commercial production conditions will provide further insights in to the possible reduction of peat use for minituber production.

None of the products tested appear comparable to peat for the production of PBTC minitubers when used alone. An alternative method to reduce the use of peat in this industry could be the use of growing medium blends in order to achieve a medium with the characteristics to support healthy production and good yields. Peat blended with coir at a 60:40 (v/v) ratio produced fewer tubers than peat alone in the glasshouse experiment but the size and weight of the tubers are similar between the two growing media. As government policy aims to limit peat use by professional growers by 2030 (DEFRA, 2011, 2018) the use of blends that reduce peat use in potato minituber production may offer a pathway to encourage uptake of these alternatives to peat in the short term.

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PARAMETERS AFFECTING THE EPIDEMIOLOGY AND DETECTION OF VIRUSES INFECTING POTATO

C Lacomme, Y Nova, L Mullholland and J Pickup
SASA, Roddinglaw Road, Edinburgh EH12 9FJ
E-mail: christophe.lacomme@sasa.gov.scot

Summary: While virus incidence remains relatively low in Scottish seed crops, aphid-transmitted viruses represent about 90% of virus cases in symptomatic plants, accounting for 4.7% of seed crop area downgraded this year. Amongst the aphid-transmitted viruses, *Potato virus Y* (PVY) has become the most prevalent species. PVY isolates representing the main phylogenetic groups and strains suggest that they can differentially overcome *Ny*-mediated hypersensitive response mechanisms. However, *Ry*-mediated (extreme) resistance still provides efficient resistance to all PVY isolates tested. The most prevalent $PVY^{EU\text{-}NTN}$ species was able to infect older plants in comparison to PVY^{O}. Re-growth foliage after flailing resulted in a 15-fold lower tuber infection in comparison to unflailed plants. 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. The nature of PVY species and physiological state of the plant has a significant impact on PVY infectability and detection.

INTRODUCTION

Viruses of the genus Potyvirus such as 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 transmit them 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 plant.

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 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 define subspecies such as $PVY^{EU\text{-}NTN}$ (European), $PVY^{NA\text{-}NTN}$ (North-American) and $PVY^{N\text{-}Wilga}$. We have characterised PVY field isolates by assessing their serology, genome structure and pathogenicity. Yearly surveys revealed a shift from PVY^{O} towards PVY^{N} serotypes in seed potato crops during the past decade. 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 different host-mediated resistance mechanisms including mature plant resistance, a broad-spectrum resistance mechanism where potato plants inoculated late in the growing season display increased resistance to PVY infection (Gibson, 1991).

MATERIALS AND METHODS

Pathogenicity of PVY species identified in seed crops.

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 classification scheme. Data reports the number of virus cases identified during the growing season, where a virus case is defined as a crop in which either PVY^{N} or $PVY^{O/C}$ was detected by ELISA as for other potato-infecting virus species. 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 cvs. carrying different resistance genes was undertaken by monitoring virus titres in upper non-inoculated leaves of the plant by real-time PCR (Lacomme *et al.*, 2015). Five potato cvs. or breeding 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 cv. Tacna and G8866 breeding line (obtained from The James Hutton Institute, Dundee, UK). For each cultivar and isolate, two plants were inoculated at the 6–8 leaf stage using infectious sap of each PVY isolate. Plants were grown for a further 7 weeks. For each plant, two upper uninoculated leaves from the top and middle of the stem were sampled and five leaf discs (1 cm in diameter) were taken from each leaf. RNA was extracted from the pooled leaf discs and PVY titre was assessed by real-time RT-PCR.

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Potato cultivars with a comparable level of susceptibility to PVY (cvs. Estima and Maris Piper) were assessed for their ability to develop mature plant resistance in field trials over a 3-year period (unless indicated). Plants emerged 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). Tubers were collected from each plant and tested by growing-on DAS-ELISA to assess PVY incidence as previously described (Davie *et al.*, 2017, Lacomme *et al.*, 2015). Non-inoculated control potato plants (n=24 plants for each of the cultivar tested) were exposed for the duration of the trial to assess potential background primary infection from incoming viruliferous aphids. All non-inoculated plants were tested negative for PVY. Tubers were harvested 3 weeks after inoculation and PVY infection monitored by growing-on ELISA as previously described (Davie *et al.*, 2017).

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RESULTS

Population dynamics of virus species infecting potatoes

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). PVY^{N} represents more than 90% of PVY cases (Figure 1). Data from a previous survey on the molecular nature of isolates of the PVY^{N} serotype revealed that the PVY^{N} group was composed of distinct molecular groups with about 88% belonging to the European EU-NTN molecular group, 8% to the North American NA-NTN and 4% to the EU-N groups (Davie *et al*., 2017).

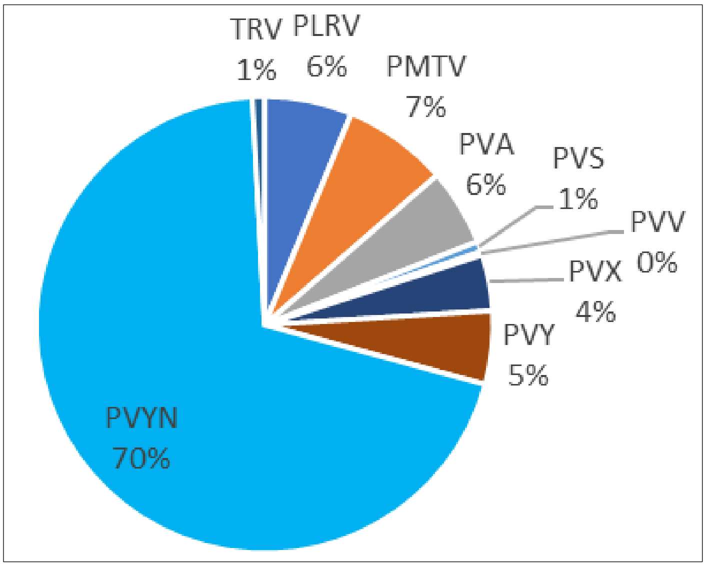

Figure 1. Relative proportion of virus species identified in symptomatic leaves of seed potatoes in 2019.

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.

PVY accumulation was monitored in upper-non-inoculated leaves as an indicator of resistance-breaking trait. 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 cv. Pentland Ivory (Ny_{tbr}-*Nc*-*Nz*) than in cv. Pentland Crown (Ny_{tbr}). The PVY^{EU-NTN} isolate DV76 accumulation levels were significantly lower in cv. P. Ivory as opposed to the two other 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 *Ry* background. 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 cvs. P. Crown and P. Ivory. This 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 strains/molecular variants tested even for the isolate DV71 of the PVY^{O} strain group.

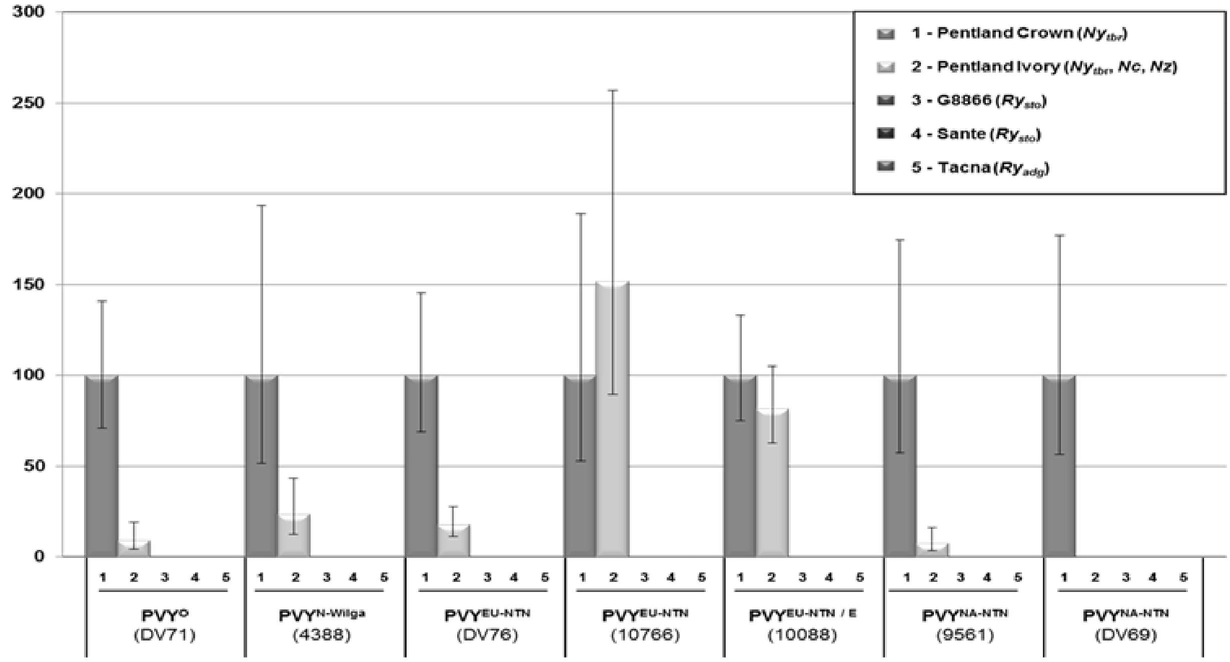

Figure 2. Relative PVY accumulation levels in potato cvs harbouring different resistance genes. PVY accumulation levels for each virus isolate belonging to a specific strain or molecular group for each of the five potato cvs are expressed as a percentage of relative expression levels to Pentland Crown (Mean ± SD).

Impact of mature plant resistance and of foliage re-growth on PVY infection

The effect of mature plant resistance on the ability of PVY$^{EU\text{-}NTN}$ and PVY^{O} isolates to infect potato plants at different developmental stages was further assessed (Figure 3. While a comparable incidence in tuber progeny was found between 1 to 8 weeks post-emergence for both PVY isolates, only PVY$^{EU\text{-}NTN}$ was found in tubers at 9 weeks post-emergence (Figure 4). These results indicate that PVY^{O} and PVY$^{EU\text{-}NTN}$ incidence was comparable while declining for both PVY^{O} and PVY$^{EU\text{-}NTN}$ in plants inoculated during the 7 weeks post-emergence period. PVY$^{EU\text{-}NTN}$ detection in progeny tubers was observed in late infection at 9 weeks post emergence.

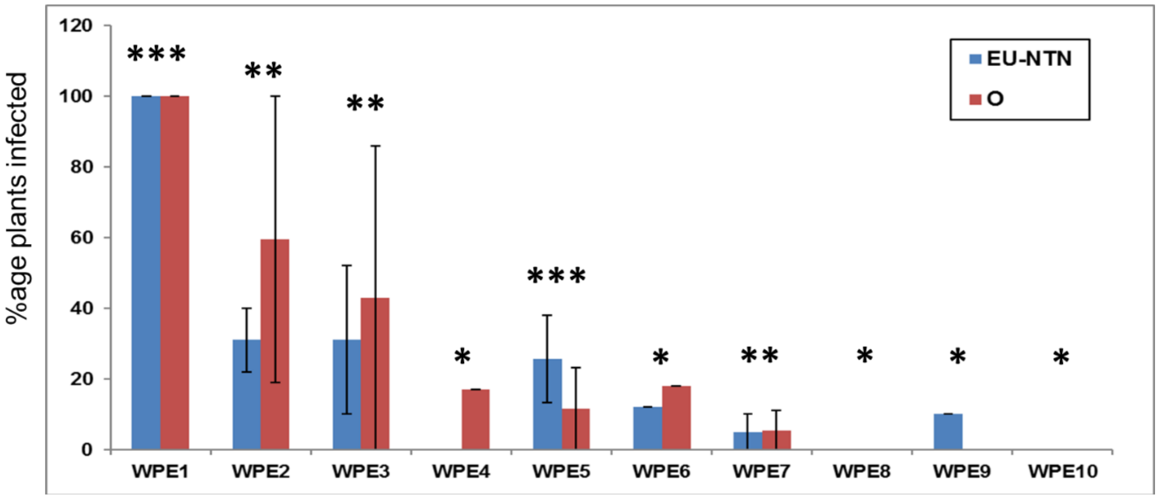

Figure 3. Relative accumulation levels of PVY isolates in infected potato cvs. at different inoculation periods after emergence (Week Post Emergence – WPE). Data are expressed as percentage of infected plants relative to WPE1 (Mean ± SD). Time points were repeated over one (*), two (**) or three (***) years of field trial.

Further, the capacity of PVY to infect newly grown leaves (re-growth) and tubers following flailing was investigated. 75% of plants inoculated at 3 weeks post planting had tubers infected by PVY, while 32% of unflailed plants infected at the same stage (9 to 11 WPP) had infected tubers. Contrastingly, only 2% of flailed plants in which re-growth foliage was inoculated between 9 and 11 WPP were found to have infected tubers (Figure 4).

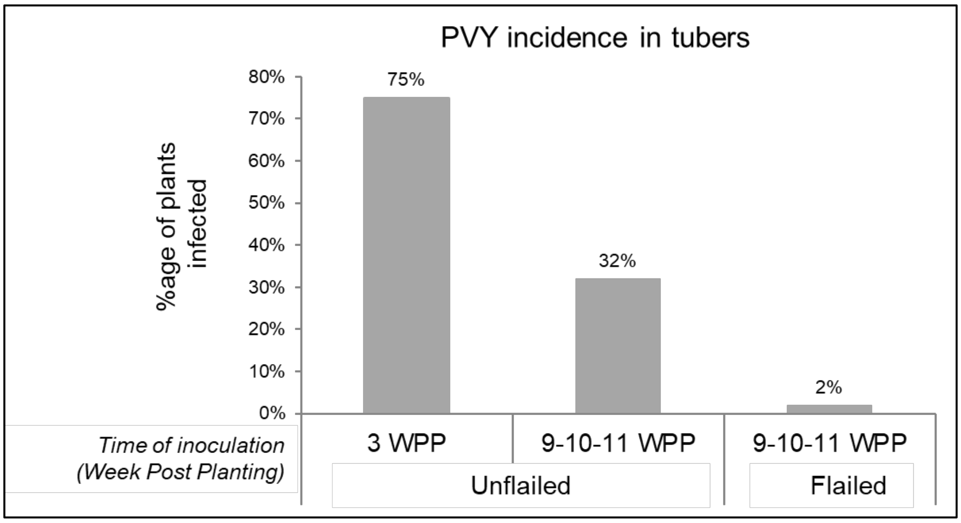

Figure 4. Incidence of PVY$^{EU\text{-}NTN}$ in tubers from plants infected at different time after flailing (1-2-3 weeks post flailing respectively 9-10-11 weeks post planting-WPP). The incidence of PVY in infected unflailed control plants is presented.

DISCUSSION

The survey of virus species in symptomatic leaves of seed potatoes indicates that aphid-transmitted viruses are the most prevalent species. The prevalence of PVY serotype groups shifted towards the PVY^N serotype group 20 years ago and since then has remained the most abundant species. Within the PVY^N serotype, isolates of the phylogenetic group EU-NTN are consistently the most prevalent group as opposed to NA-NTN and EU-N isolates. In contrast to other potato-growing areas (EU, North America and China) (Gray *et al*., 2010), isolates of the PVY$^{N\text{-}Wi}$ strain (PVY$^{O/C}$ serotype), while accounting for the majority of PVY$^{O/C}$ cases, are not prevalent in our environmental conditions, as isolates of the PVY^O or PVY^C serotype account for less than 5% of all PVY cases (Davie *et al.*, 2017).

The aim of our study was to examine the parameters that are driving PVY variant prevalence in Scottish seed crops. The reasons for these changes in the PVY population are likely to be dependent on complex interactions between PVY and its aphid vectors, environmental conditions and PVY ability to overcome host resistance mechanism. PVY$^{EU\text{-}NTN}$ might out-compete PVY^O and PVY$^{NA\text{-}NTN}$ for host cellular functions which could result in a higher incidence in systemic tissue (Davie *et al,* 2017).

The ability of PVY$^{EU\text{-}NTN}$ to successfully infect potato hosts at a relatively late developmental stage (i.e., up to 9 weeks post emergence) suggests that PVY$^{EU\text{-}NTN}$ might counteract mature plant resistance mechanisms more efficiently than PVY^O. This would give another selective advantage for PVY$^{EU\text{-}NTN}$ in initiating successful infection events. Our data currently implies that PVY infection and transmission to daughter tubers of plants displaying re-growth of foliage plants do occur after flailing. However, infection of re-growth and translocation to tubers is less effective than in control unflailed plants of the same age late in the growing season. Previous studies suggested that the prevalent PVY$^{EU\text{-}NTN}$ variant is more efficiently transmitted by aphids to neighbouring plants and daughter tubers in comparison to other PVY variants (Davie *et al*., 2017). Altogether, these characteristics may further explain the mechanisms that control the population dynamics of PVY$^{EU\text{-}NTN}$ over other PVY strains and illustrate the complex nature of PVY interactions with its host and its environment. Further studies are needed to further understand these mechanisms and develop strategies to control PVY and minimise its impact on potato production.

ACKNOWLEDGEMENTS

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PARAMETERS AFFECTING THE EPIDEMIOLOGY AND DETECTION OF VIRUSES INFECTING POTATO

C Lacomme, Y Nova, L Mullholland and J Pickup
SASA, Roddinglaw Road, Edinburgh EH12 9FJ
E-mail: christophe.lacomme@sasa.gov.scot

Summary: While virus incidence remains relatively low in Scottish seed crops, aphid-transmitted viruses represent about 90% of virus cases in symptomatic plants, accounting for 4.7% of seed crop area downgraded this year. Amongst the aphid-transmitted viruses, *Potato virus Y* (PVY) has become the most prevalent species. PVY isolates representing the main phylogenetic groups and strains suggest that they can differentially overcome *Ny*-mediated hypersensitive response mechanisms. However, *Ry*-mediated (extreme) resistance still provides efficient resistance to all PVY isolates tested. The most prevalent $PVY^{EU\text{-}NTN}$ species was able to infect older plants in comparison to PVY^{O}. Re-growth foliage after flailing resulted in a 15-fold lower tuber infection in comparison to unflailed plants. 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. The nature of PVY species and physiological state of the plant has a significant impact on PVY infectability and detection.

INTRODUCTION

Viruses of the genus Potyvirus such as 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 transmit them 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 plant.

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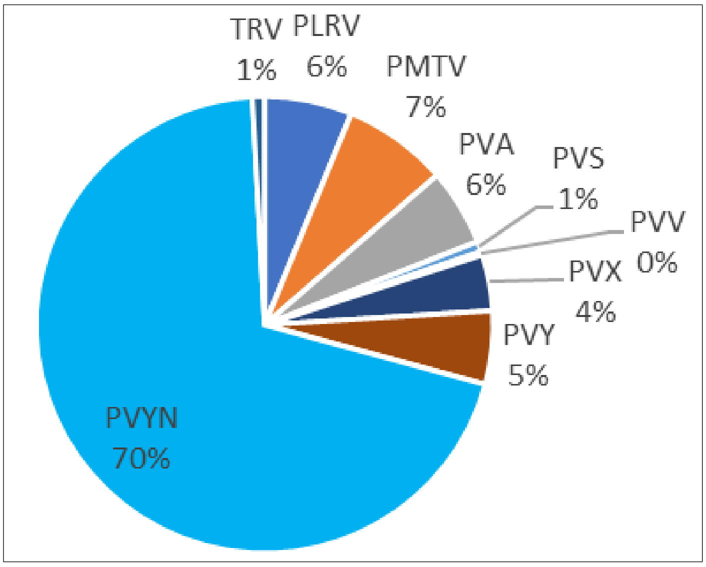

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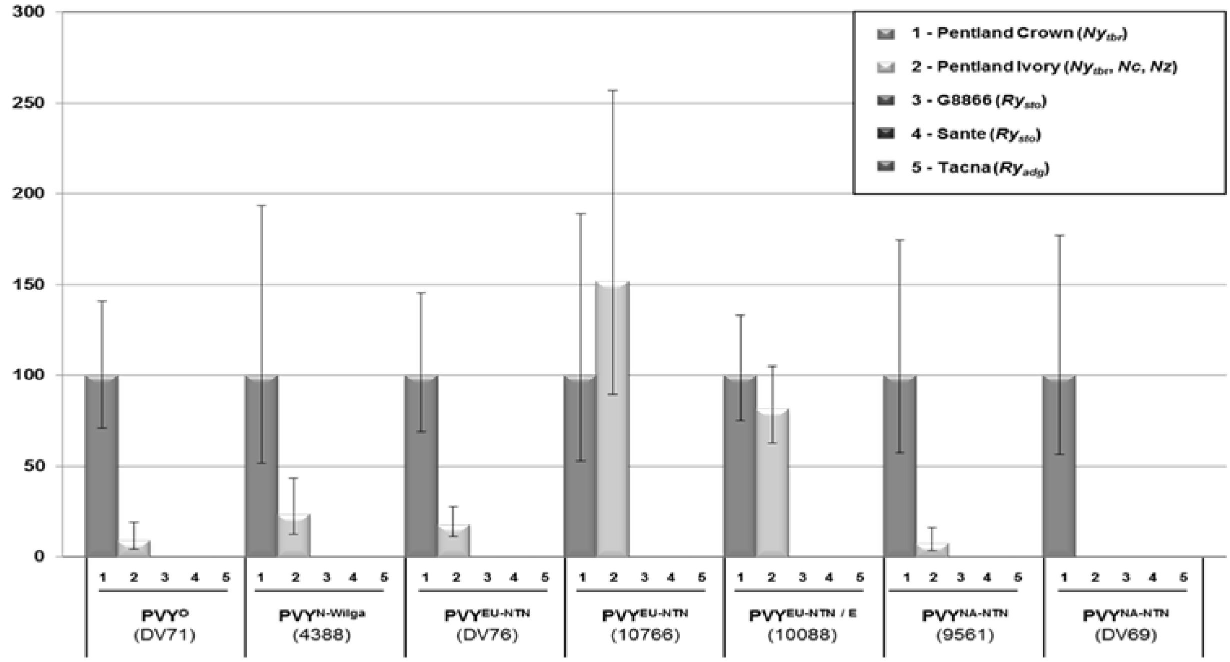

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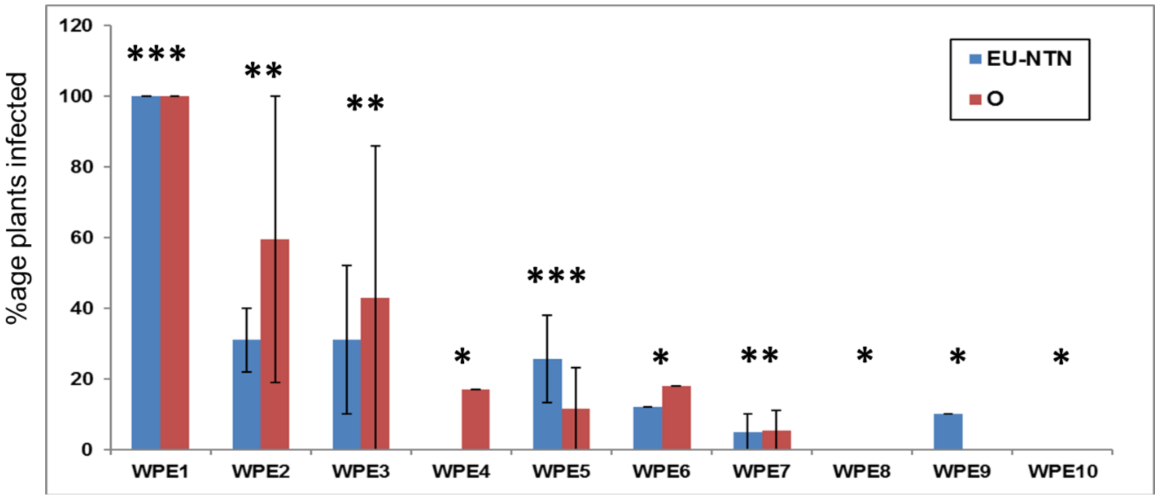

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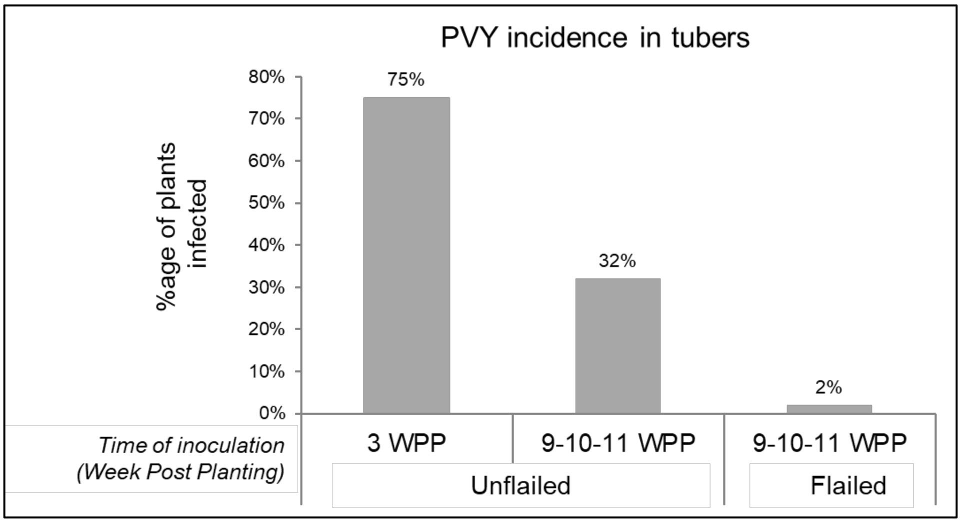

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INCREASES IN SHELFORD POTATO TUBER YIELD RESULTING FROM STABILISING UREA NITROGEN CAN BE MANIPULATED VIA TIMING OF APPLICATION TO INFLUENCE SIZE DISTRIBUTION

S Wilkinson, D J Marks and A K Weston
Levity Crop Science, Myerscough College and University Centre, Preston, Lancashire, UK
E-mail: sally@levitycropscience.com

Summary: Technologies for stabilising urea N in fertiliser, preventing its breakdown to pollutants, and prolonging its availability for plants, have been developed. We have already shown that chemically stabilising ureic amine N in foliar fertilisers applied to potato crops in the field increases yield. Greenhouse trials demonstrated that this was due to increases in root growth and leaf chlorophyll content. Here we report results from UK field trials on *Solanum tuberosum* L. *cv*. Shelford showing that stabilised amine-induced increases in yield can be manipulated to shift tuber size distribution. Overall yield is greatest when four rather than three applications are made over the growing season. However, when applications are limited to three, a greater percentage of the harvested tubers falls within the 60-80 mm size category when a final application, at tuber bulking, remains within the program. When the final bulking application is left out, and a tuber initiation application remains within the program, a greater percentage of the harvest is comprised of tubers within the 40-60 mm size category. The ability to target a particular size category can enable farmers to match fertiliser regime to market; for example large tubers for chipping, or small tubers for seed and salad.

INTRODUCTION

Whilst crop growth is broadly aligned to the amount of nitrogen fertiliser applied to it, evidence is accumulating that the form of the nitrogen (N) in which it is supplied – nitrate, ammonium, urea, amine – can significantly influence growth and yield even when the total amount of N supplied is the same. This is because plants fertilised with a particular form of N are characterised by a specific architectural appearance, or phenotype, generated by a specific set of N-dependent plant regulatory functions. For example nitrate stimulates leafy growth and apical dominance rather than lateral root production (Wilkinson *et al*. 2019a, b). In experimental conditions ammonium fertilisation gives rise to plants with a similar amount of total tissue biomass as those treated with nitrate, but more of this is found underground as root tissue, with less partitioned above ground (Andrews *et al.* 2013). Shorter plants with more roots are more resistant to stresses such as drought or lodging (Wilkinson *et al.* 2019a). Plants with more roots are also able to scavenge a greater volume of soil for the nutrients and water required for above ground growth at later developmental stages. However in the field it is hard to distinguish between these phenotypes, because several N forms are present at any one time, and because conventional fertilisers (ammonium nitrate or urea) are not environmentally stable, such that most of the N applied is degraded to nitrate, regardless of its original form.

We have demonstrated, in glasshouse experiments, that urea amine N gives rise to a third phenotype, or rather a range of phenotypes which alter with developmental stage (Wilkinson *et al.* 2019a, 2019b). This phenotype group (see below) has been difficult to isolate in past experimental analyses, because bacteria that break urea down are ubiquitous in soil and on leaf surfaces, even in compost used in pot-based indoor studies. Urea is converted to CO_2, ammonia and ammonium (and eventually nitrate) within hours to days of its application, whether it is added via soil or in foliar sprays (e.g. Hoult & McGarity, 1986). However, the fertiliser industry has developed techniques to stabilise urea and prevent some of its degradation, such that it is available in this form for longer, and lower levels of polluting breakdown products are emitted (see Wilkinson *et al.* 2019a). We have used a chemical method to stabilize ureic amine such that it persists in this form when applied to plants via soil or leaves, and we found that this third phenotype is readily generated in a range of species including potato. Yields of field grown Sassy (Marks *et al.* 2018), Shelford and Rooster (Wilkinson *et al.* 2019b) were increased in UK and Irish field trials by this technology (termed 'Limin', developed by Levity Crop Science, UK). We demonstrated experimentally that the yield increase was a result of the generation of the specific ureic amine phenotype in Casablanca (Wilkinson *et al.* 2019b), which is characterised as follows, in comparison to un-stabilised urea and/or conventional N fertiliser: increased root production, reduced stem elongation and increased chlorophyll content prior to / during tuberisation; and increased above-ground biomass and chlorophyll content at bulking. Higher yields correlated with root proliferation, slow shoot extension, and increased chlorophyll content prior to tuberisation; and with increased shoot biomass and increased chlorophyll content at bulking. We describe here how this technology can be used in the field to manipulate tuber size distribution within a Shelford crop, by changing the number and scheduling of its applications.

MATERIALS AND METHODS

Solanum tuberosum L. *cv*. Shelford (FL1625 x Hermes) was used in field trials designed to test the efficacy of four N fertilisation programs, on a commercial farm in 2016 in Hampshire, England, UK. The previous crop was wheat; soil type was sandy (no ridging required); beds were ploughed and de-stoned and fertiliser was broadcast at a rate of 180 kg N h^{-1} as urea. The crop was irrigated; and dates of crop treatments, from sowing to harvest; are described in Table 1. Five replicate plots (4.0 m^2) for each of four experimental treatment programs were laid out in a randomised block design: 1) commercial urea controls, 2) foliar stabilised amine nitrogen (SAN) applied four times (SAN x 4), 3) SAN applied three times, including at tuber initiation but excluding at late bulking (SAN x 3 + early), 4) SAN applied three times, excluding at tuber initiation but including at late bulking stage (SAN x 3 + late). Foliar SAN applications were carried out at a rate of 5.0 L ha^{-1} in 200 L water (0.1 mmol m^{-3}).

Maincrop marketable yield data (40-80 mm tubers) is presented as tuber number per hectare, and tuber yield weight per hectare (metric tonnes: t ha^{-1}). Tubers were graded into categories by size: 40-60 mm and 60-80 mm. Means and standard errors of yield data for the four treatment programs (control, SAN x 4, SAN x 3 + early, SAN x 3 + late) are displayed as bar charts. The significance of any difference from the control treatment was calculated using a one-tailed *t*-test for two independent means, and where treatments are significantly different from controls, (denoted by differing letters 'a', 'b', or 'c'), the *p* value is displayed above the appropriate column on the graphic representations of the data.

Table 1. Chronology of field activities from sowing to harvest of crop (*Solanum tuberosum L. cv*. Shelford).

Date	Activity
03.05.2016	Fertiliser broadcast, crop sown
24.06.2016	SAN treatment 1 (omitted for SAN x 3 + late)
08.07.2016	SAN treatment 2
22.07.2016	SAN treatment 3
05.08.2016	SAN treatment 4 (omitted for SAN x 3 + early)
26.08.2016	Harvest

RESULTS

Fig 1 A demonstrates that all SAN treatment programs significantly increase mean marketable tuber number per hectare in comparison to conventionally fertilized controls, and that this is greatest when all 4 applications are included across the season (16.1 %). When only 3 applications are given, excluding the last, numbers are increased by 8.2 % (SAN x 3 + early); and when 3 applications are given, excluding the first, numbers are increased by 10.5 % (SAN x 3 + late). Fig 1 B shows that all 3 SAN treatment programs increase mean marketable yield weight (t ha^{-1}), but that this is only statistically significant, at 5.9%, when all 4 applications are included. When only 3 applications excluding the last are given, the increase is 2.6 %, and with 3 applications excluding the first, the increase is 4.8%.

Figure 2A shows that, when only the smaller size category of 40-60 mm tubers is included in the analysis, tuber numbers are increased significantly in all SAN treatment programs, this being greatest when all 4 applications are included (32.1 %). When only 3 applications are included, excluding the last, numbers are increased by 16.7 %, and when the first application is excluded, numbers are increased by 9.8%. Figure 2B shows that yield weight is increased the most in the SAN x 4 treatment program, by 18.3%, and this is a significant result. When the final application is excluded from the treatment regime (SAN x 3 + early), yield weight is also significantly increased, by 10.4 %. However when the first application is excluded from the program (SAN x 3 + late), total yield weight of smaller tubers is not increased at all, even though there are greater numbers of these than in controls (Fig 2A).

Fig 3 shows the effects of the SAN treatment programs on number and weight of tubers in the larger 60-80 mm range category. Only the treatment program supplying 3 SAN applications that excludes the earliest (SAN x 3 + late) gives rise to an increase in tuber numbers (3A) in comparison to control treatments, of 11.6%, although this is not significant. The SAN x 4 and the SAN x 3 + early treatment programs slightly decrease numbers of tubers in this larger category. The SAN x 3 + late treatment regime significantly increases 60-80 mm tuber numbers compared to the treatment program that includes all 4 SAN applications across the season, by 19.1 %. It significantly increases tuber number in comparison to the program that excludes the late application, by 16.7 %. Figure 3B demonstrates the effects of treatment programs on yield weight of the larger tuber category. Whilst there are no significant differences between all 4 programs, there is an increase in the total weight of large tubers compared to all other treatments, when the first SAN application is excluded. This increase is 11.9% compared to controls, 17.4 % compared to SAN x 4, and 16.5 % compared to the program which excludes the latest application (SAN x 3 + early).

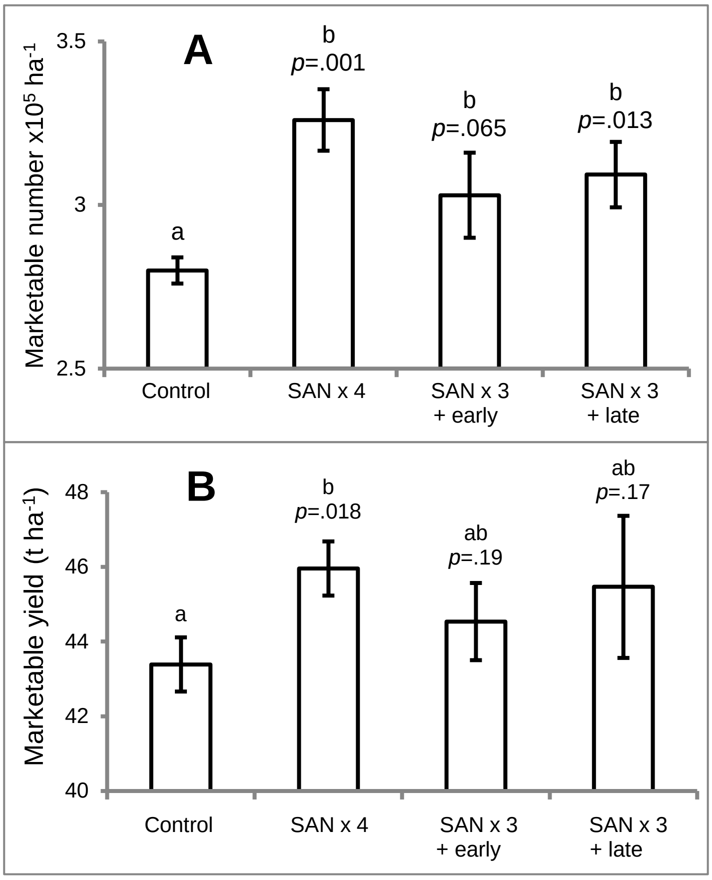

Figure 1. Effect of foliar SAN application program on marketable tuber number (A) and yield (B) compared to conventionally fertilized controls. SAN was applied either 3 (SAN x 3) or 4 times (SAN x 4). Means ± standard errors are depicted; significance (*p*) of differences from the control are shown above columns.

Figure 4 details the composition of the SAN-induced changes in yielding (positive or negative) from the control. The positive change induced by the inclusion of the early SAN application consists entirely of a greater number and total weight of small (40-60 mm) tubers, both in the SAN x 4 and the SAN x 3 + early regimes. However this is slightly off-set by a reduction in the number and total weight of large (60-80 mm) tubers, even when the bulking stage final application is included (SAN x 4). On the other hand the positive change in tuber number resulting from the exclusion of the initial SAN application, and the inclusion of the late application, consists of both large and small tubers. However in terms of yield as weight, 100% of the positive change in the SAN x 3 + late treatment consists of 60-80 mm tubers. The weight of smaller tubers is slightly reduced. Retention of the final SAN application within the SAN x 4 program, could not override an effect of the inclusion of the initial application to set final tuber size at 40-60 mm, in 100% of the SAN-induced yield increase.

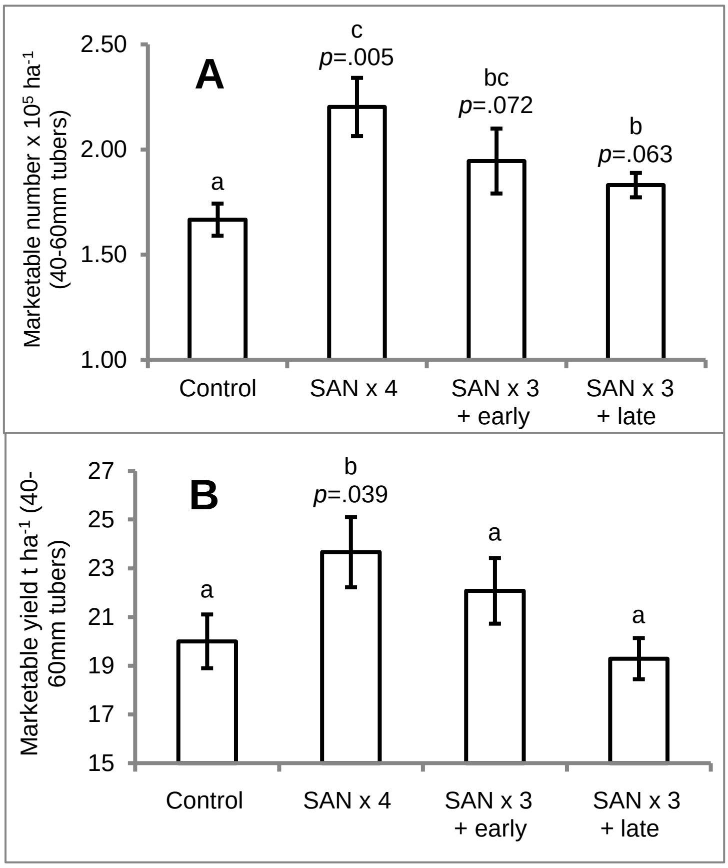

Figure 2. Effect of SAN application program on marketable tuber number (A) and yield (B) within the 40-60 mm tuber size category, compared to conventionally fertilized controls.

DISCUSSION

Manipulation of tuber size distribution within a crop is an important agronomic aim when targeting a specific market (crisping, frying, salad). Both seed potato characteristics and field management practices can affect tuber size and/or size distribution at yield. Seed potato influences include weight, physiological age, and apical dominance (reflected by shoot number). Seed planting density and depth, and timing of haulm destruction can be controlled in the field (e.g. Knowles & Knowles, 2015; Struik *et al*. 1990). Here we describe a novel technique for manipulating tuber size distribution within an increased overall yield, which consists of the use of specified application schedules of stabilized amine nitrogen (SAN) over the course of the growing season. Namely, inclusion of an early, pre-tuberisation application of SAN within the fertilization program gives rise to yields consisting of a high percentage of smaller tubers (40-60 mm); and exclusion of an early application is necessary for the bulking stage application to increase the percentage of large (60-80 mm) tubers. The shift in yield content to large tubers (SAN x 3 + late) does not come at the price of a smaller yield number or weight *per se*; rather, yield is still increased over and above that attained using standard agricultural practice.

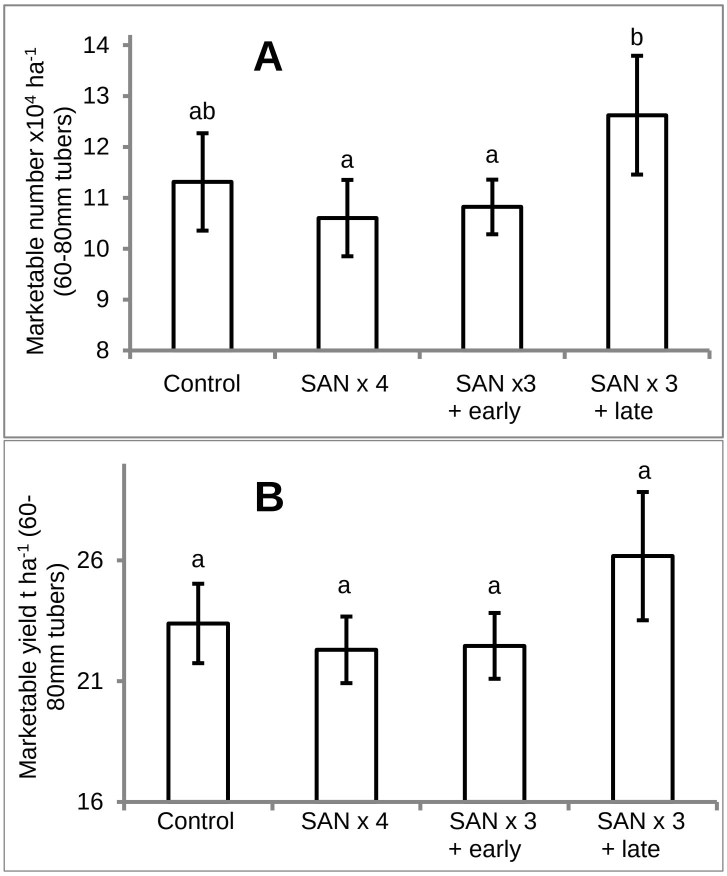

Figure 3. Effect of SAN application program on marketable tuber number (A) and yield (B) within the 60-80 mm tuber size category, compared to conventionally fertilized controls.

Effects of nitrogen fertilization programs designed to test whether tuber size distribution within a potato crop could be influenced have previously been reported. For example, Qsaki *et al.* (1995, field) and Gao *et al.* (2014, greenhouse) demonstrated that final yield in terms of weight was unaffected by nutritional N form when comparing nitrate and ammonium N. Differences in tuber size distribution were nevertheless apparent: nitrate nutrition led to the development of many small tubers; and ammonium nutrition induced the formation of large tubers, although these were less numerous. More stolons (specialized underground shoots) were generated pre-tuberisation under nitrate nutrition, increasing the number of tubers initially set, because this N form is partitioned to, and assimilated within shoot tissue (Qiqige *et al*. 2017). N sourced from ammonium is preferentially used for root growth, such that less N is available for stolon growth, and fewer tubers are formed. At bulking, later in the season, above-ground vegetative growth continued to be stimulated by nitrate in comparison to ammonium nutrition, and shoots attained a greater size. The authors hypothesized that little resource remained to allow all the tubers set earlier in the season to bulk to marketable size. On the other hand, ammonium nutrition increased tuber bulking compared to nitrate treatment, albeit in the lower number of tubers set under this N form, as above-ground growth was comparatively limited later in the season, freeing resource for tuber growth.

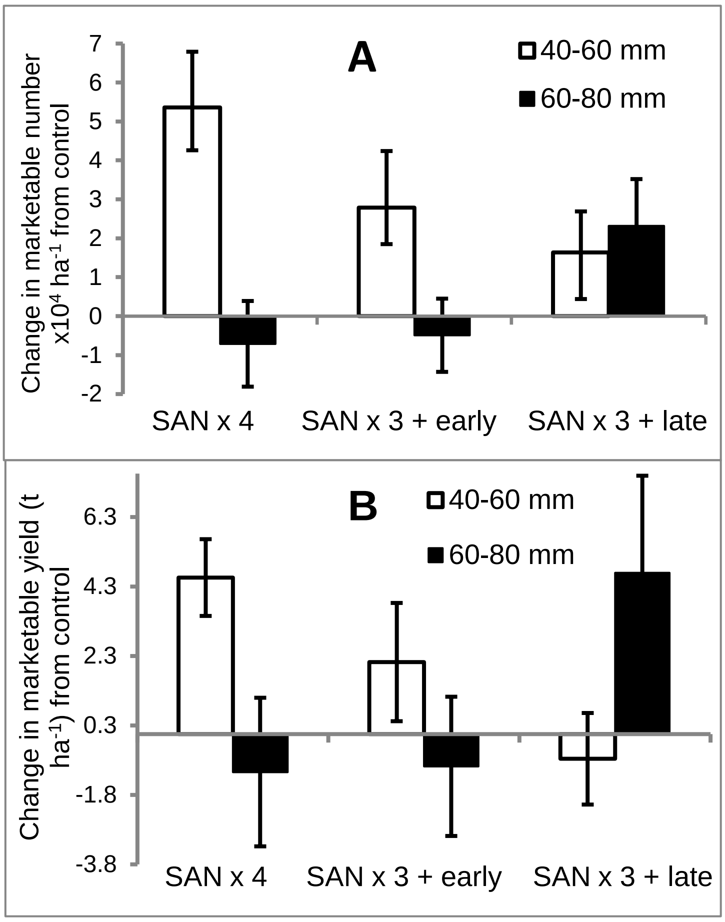

Figure 4. Effect of SAN application program on the change in marketable tuber number (A) and yield (B) from control values, within both the 40-60 mm and 60-80 mm tuber size categories.

We show here that pre-tuberisation stage SAN applications increase numbers of smaller tubers at yield in comparison to conventional fertilization (Figs 1-4), however we propose that this occurs via a different mechanism to the nitrate-induced effect described above. Wilkinson *et al*. (2019b) demonstrated that SAN nutrition increased root biomass and reduced shoot extension at this stage in Casablanca, compared to ammonium nitrate and un-stabilised urea, implying that a reduction in apical dominance was being induced (see also Wilkinson *et al.* 2019a). This reflects some interesting findings described by Dean *et al.* (2018), where seed treatments with plant hormones also altered apical dominance, which in turn affected tuber size. Application of Gibberellic Acid (GA) induced early shoot emergence in Alturas and Payette Russet, also decreasing apical dominance, which shifted tuber size distribution towards smaller classes. However inclusion of the auxin NAA in the seed treatment maintained apical dominance, and prevented the shift in tuber size to the smaller class. However, this begs the question: how do SAN treatments that include an early application provide enough resource to increase both root biomass (Wilkinson *et al*. 2019b) and tuber numbers; and therefore overall yield? The answer lies in the fact that the uptake and processing of urea and amide in root cells is much more resource efficient than assimilation and processing of either nitrate (in shoots), or ammonium (in roots) (see Wilkinson et al. 2019a, b). Thus, whole-plant pools of N-based protein, and chlorophyll content for photosynthetic provision of carbon resource, are increased. As the crop matures and tubers start to bulk, we surmise that the larger root system developed by SAN-treated plants, that persists from the early application (Wilkinson *et al.* 2019a, b), provides more soil-sourced resource in the form of a range of nutrients, as well as N, and water, to the shoots. As a result vegetative tissue also proliferates under SAN treatment, and provides enough resource to bulk all the tubers set earlier in the season, at least to the marketable 40-60 mm size category.

In the case of the SAN x 3 + late program, the improved yield in comparison to all other treatments comprised of a larger percentage of 60-80 mm tubers. We conclude that the absence of a SAN treatment prior to tuberisation means that the seed tubers that expressed an innate tendency for apical dominance (assuming that a portion of these were present even within a relatively uniform seed batch) generated fewer tubers per plant. These will inevitably grow large enough to be part of the 60-80 mm size category as a result of the final SAN application at bulking, as all SAN-treated plants (with or without seed-induced apical dominance) generate more total resource throughout the growing season than plants supplied with other N sources, as described above, as a result of the increased internal nitrogen utilization efficiency of urea amine (Wilkinson *et al.* 2019a, b).

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PESTICIDE USAGE TRENDS ON SCOTTISH POTATO CROPS 2008-2018

G Reay, J Hughes, C Monie and J Wardlaw
Pesticide Survey Unit, SASA, Scottish Government, Roddinglaw Rd, Edinburgh, EH12 9FJ
E-mail: gillian.reay@sasa.gov.scot

Summary: There was little variation in the proportion of Scottish seed and ware potatoes treated with pesticides between 2008 and 2018. Varying pest pressure between years and changes to the composition of active substances used, resulted in minor variations in the overall area treated with pesticides and quantity applied, particularly for insecticides. The ongoing loss of active substances will make the control of pests, weeds and diseases increasingly challenging for the Scottish potato industry. This could potentially reduce future pesticide use as growers adapt their regimes, shifting towards a more integrated approach to control pests, weeds and diseases.

INTRODUCTION

Scotland has a reputation for growing high quality potatoes, particularly seed potatoes. Just over 12,000 ha of seed potatoes and 15,000 ha of ware potatoes were grown in Scotland in 2018 with a total market value of £201 million (Anon, 2019). The aim of this poster is to describe trends in pesticide application to potato crops between 2008 and 2018 and to highlight potential changes in future pesticide use.

METHODS

Pesticide application data for the period 2008 to 2018 were obtained from the dataset collected by SASA during biennial arable pesticide use surveys (Reay, 2009; Reay *et al* 2011; Watson *et al*., 2013; Monie *et al*., 2015; Monie *et al*., 2017, Wardlaw *et al*., 2019). The surveys are conducted by collecting data from a random sample of farms classified by size and geographic region. National estimates of pesticide use are produced from the sample data by applying raising factors based on actual crop areas obtained from the agricultural census.

RESULTS AND DISCUSSION

The overall Scottish potato cultivation area remained fairly static over the last decade, although the proportion of the crop grown for seed production has increased from 39% in 2008 to 44% in 2018. Almost all potato crops are treated with pesticides. The proportion of potatoes treated has shown minor variation over the last ten years (Tables 1 and 2) with an average of 98, 95 and 61% of ware crops and 97, 94 and 90% of seed crops treated with fungicides, herbicides/desiccants and insecticides/nematicides respectively.

Fungicide use patterns

Fungicides are the dominant pesticide group applied to potatoes. The overall spray area (total area treated with pesticides including repeat applications during the growing season) for both seed and ware potatoes has shown minor fluctuations over the decade in response to seasonal disease pressure. Total spray area peaked in 2012, which was an extreme disease pressure year and was lowest in 2018 (Figure 1) influenced by a hot dry summer resulting in reduced disease pressure. Despite these fluctuations in spray area, the quantity applied has been fairly static over the decade with the exception of 2010, where the quantity applied to both seed and ware potatoes (kg/ha grown) dropped significantly driven by a decrease in the use of carbamates such as mancozeb and propamocarb hydrochloride which are applied at high application rates. The use of carbamates increased again in 2012 and 2014. The use of fluazinam has declined over the 10-year period for both seed and ware potatoes due to reduced fluazinam sensitivity in late blight populations. During this period there has been a corresponding increase in the use of carboxylic acid amide herbicides such as dimethomorph and mandipropamid.

Table 1. The area of seed potatoes cultivated and proportion of crops receiving a pesticide application 2008-2018

	2008	2010	2012	2014	2016	2018
Seed area grown (ha)	11,720	13,497	13,002	13,300	12,760	12,092
	Proportion of crop receiving a pesticide application (%)					
Fungicides	100	96	98	100	100	89
Herbicides/desiccants	100	96	95	93	95	89
Insecticides/nematicides	94	94	88	100	79	86

Table 2. The area of ware potatoes cultivated and proportion of crops receiving pesticide application 2008-2018

Survey Year	2008	2010	2012	2014	2016	2018
Ware area grown (ha)	18,116	17,881	16,534	15,211	14,766	15,268
	Proportion of crop receiving a pesticide application (%)					
Fungicides	98	99	99	93	98	98
Herbicides/desiccants	94	93	99	91	98	96
Insecticides/nematicides	66	59	70	54	51	64

Herbicide/desiccant use patterns

Overall herbicide/desiccant spray areas and quantities applied show very little variation between 2008 and 2018 (Figure 2). The data reported in Figure 2 excludes the use of sulphuric acid in 2008, when over 800,000 kg were applied to potatoes for haulm destruction. Following the withdrawal of sulphuric acid in 2009, the use of other desiccants such as diquat and carfentrazone-ethyl increased until 2010, with use remaining stable thereafter. There was a decline in the use of diquat in 2018 ahead of its loss of authorisation in 2020, with corresponding increases in the use of other desiccants such as carfentrazone-ethyl and pyraflufen-ethyl. The use of key pre and post-emergence active substances such as linuron and metribuzin were fairly static over the 10-year period, although there was a significant decline in the use of linuron prior to its final use date in June 2018.

Insecticide/nematicide use patterns

The percentage of ware crops treated with an insecticide is considerably lower than on seed potatoes where the management of virus vectored by aphids is particularly important. Over the last decade 90% of seed potatoes on average were treated with an insecticide/nematicide compared to 61% of ware potatoes. Insecticides are applied far more frequently than nematicides. The spray area of insecticides/nematicides showed slight variation over the 10-year period reflecting temporal differences in pest pressure, with an overall decline of 22% for seed potatoes and 38% for ware potatoes between 2008 and 2018 (Figure 3). In contrast, the quantity applied to ware potatoes (kg/ha grown) declined by 82% between 2008 and 2018, driven mainly by a steady decrease in the use of carbamates such as pirimicarb which were applied at high dose rates. However, there was a large increase in quantity applied to ware crops in 2012 due to an increase in the use of the nematicide ethoprophos which was applied at high rates. The quantity of insecticides/nematicides applied (kg/ha grown) to seed potatoes only decreased by 3% over the decade. The low quantity applied to seed potatoes in 2016 was due to no nematicides being encountered during the 2016 survey. The use of pyrethroid and neonicotinoid insecticides on both seed and ware potatoes remained fairly static over the 10-year period.

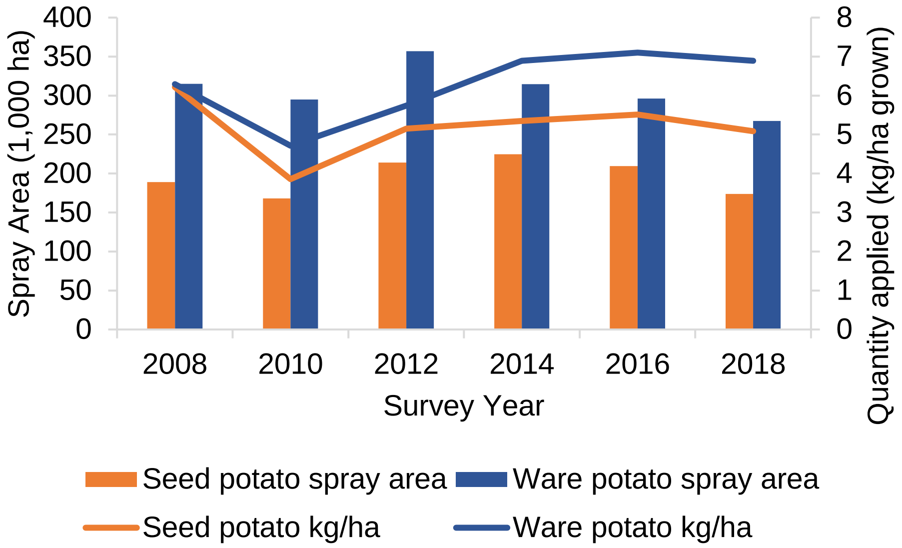

Figure 1. Fungicide spray area and quantity applied to potato crops 2008-2018

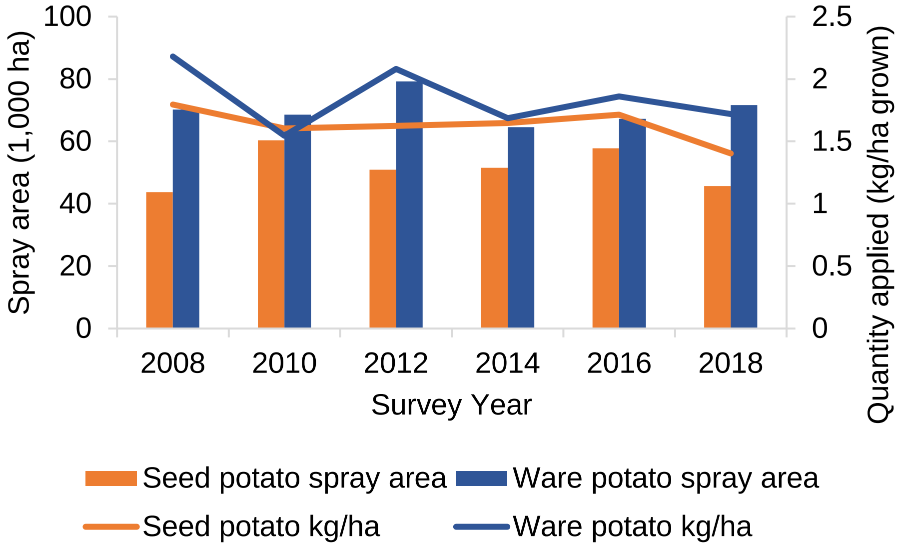

Figure 2. Herbicide/Desiccant spray area and quantity applied to potato crops 2008-2018

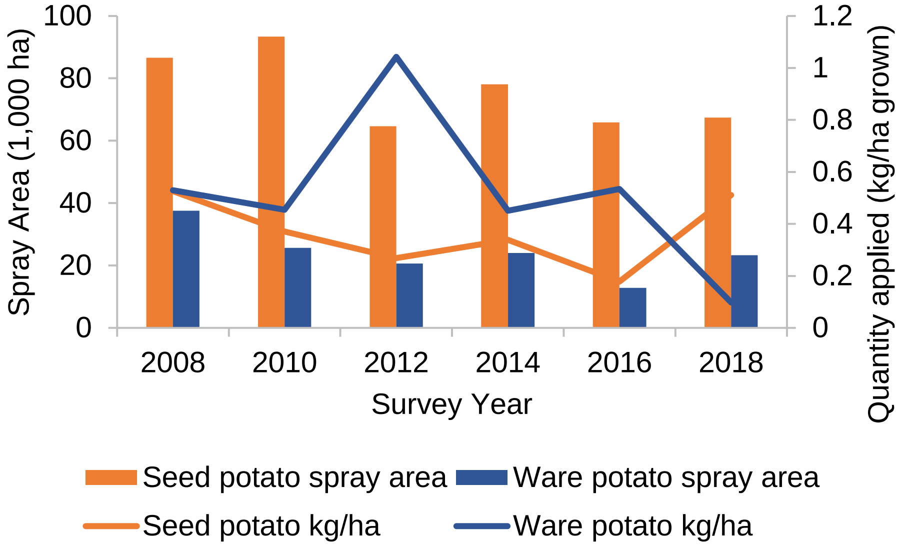

Figure 3. Insecticide/nematicide spray area and quantity applied to potato crops 2008-2018

Future pesticide use

The Scottish Potato industry are facing a challenging period with the loss of key active substances impacting on their ability to control pests, weeds and diseases. Many active substances have been lost already or will be withdrawn shortly. For example, the herbicides linuron and diquat, the insecticides pymetrozine and thiacloprid and the nematicides ethoprophos and oxamyl. The loss of non-pyrethroid insecticides will be particularly challenging for virus management in the seed potato sector due to pyrethroid resistance in the peach-potato aphid. Further key active substances, such as mancozeb, metribuzin and potentially glyphosate, could be at risk as they go through the renewal process under the 1107/2009 directive. Growers will be reliant on fewer active substances increasing the risk of resistance issues. This reduction in availability of pesticides could potentially reduce the overall future use of pesticides. With few new active substances coming onto the market, growers will have to adapt their regimes, shifting towards a more integrated approach to control pests, weeds and diseases. This will potentially include longer rotations, the use of resistant varieties and non-chemical forms of control including the use of biologicals.

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IN VITRO EVALUATION OF TWO BACTERIAL BIOLOGICAL CONTROL AGENTS AGAINST POTATO PATHOGENS

L Feehan, G R D McGrann, A Tsinganos and R C McHugh
SASA, Roddinglaw Road, Edinburgh, EH12 9FJ
E-mail: ros.mchugh@sasa.gov.scot

Summary: Potato production is under constant threat from pathogens in the growing crop and during storage. Approximately 50% of Scottish seed potatoes are treated with fungicides in store, primarily for control of *Fusarium* spp and *Helminthosporium solani* which cause dry rot and silver scurf diseases, respectively. However, increased risk categorisation of pesticides due to health, safety and environmental concerns, combined with potential development of fungicide insensitivity in pathogen populations, has reduced the range of pesticides available, resulting in a need to identify alternative control measures. Here we test the efficacy of the biological control agents (BCA) *Bacillus subtilis* and *Aneurinibacillus migulanus* by *in vitro* dual culture assays against *Fusarium* spp. and *H. solani*. Both BCAs were found to significantly inhibit growth of these pathogens suggesting that they could be used as effective components of integrated pest management.

INTRODUCTION

Scottish seed and ware potato crops are of high economic importance (Potato Council, 2014) due to high phytosanitary standards under the Scottish seed potato classification scheme which sets acceptable tolerances and conditions for mini-tuber, and subsequent seed production (Anon, 2016). In addition, seed stocks for export are subject to strict tolerances for diseases, pests, damage and defects.

Dry rot caused by *Fusarium* spp. can be soil or seed borne and introduced to the tuber through damage at harvest or grading (BPC, 2006). It is the most important fungal rot disease of UK grown potatoes, affecting around 1% of tubers and allowing colonisation by soft rot causing bacteria, furthering economic losses (BPC, 2006). Current control methods include long rotations, minimising damage and fungicide application (United Nations, 2014). Silver scurf caused by *Helminthosporium solani* can be soil or seed borne or spores may be present in dry soil in store (United Nations, 2014). In 2016, 100% of the growing crop was treated with fungicide, primarily for late blight, accounting for 99% of the total fungicide use on potato seed crops. In the same year, 46% of stored potato seed was treated with fungicides, with more than 60% of fungicide usage targeting dry rot (35%) and silver scurf (34%, Monie *et al*., 2017).

Pathogens can develop resistance to chemical active ingredients (ai) of fungicides, rendering them ineffective and approval of use may be withdrawn (Hillocks, 2012). Integrated pest management (IPM) aims to reduce pesticide usage by combining their use with other control methods such as improved cultural and agronomic practices, disease resistant crop varieties or by using biological control agents (BCAs) in conjunction with reduced doses of pesticides. *Bacillus subtilis* QST 713 has been used as a commercial BCA in a number of cropping systems. It can reduce disease through antifungal activity from the lipopeptide metabolites iturins and plipastatins, nutrient competition (Edgecomb and Manker, 2005), and induction of host defence response (Fousia *et al*., 2015). *Aneurinibacillus migulanus* (syn. *Brevibacillus brevis*, *Bacillus brevis*) is another bacterium which has proved to be an effective BCA in field trials (McHugh and Seddon, 2001). The modes of action of *A. migulanus* include antibiosis through production of the cyclic peptide Gramicidin S and biosurfactant activity (Seddon *et al*., 2008). Different strains of *A*. *migulanus* produce variable types and quantities of these antimicrobials such as *A*. *migulanus* E1, which does not produce Gramicidin S (Edwards and Seddon, 2001) but does have biosurfactant activity (Edwards, 1993). This report details the efficacy of two different bacterial BCAs (*Bacillus subtilis* strain QST 713 and *Aneurinibacillus migulanus*) against three fungal pathogens, *F. coeruleum*, *F. sulphureum* and *H. solani*.

MATERIALS AND METHODS

Bacterial cultures

Aneurinibacillus migulanus strains Nagano, NCTC 7096 and E1 were obtained from the Institute of Biological and Environmental Sciences, Aberdeen University. *Bacillus subtilis* was isolated from Serenade® ASO (Bayer Crop Science, UK). All stocks were maintained on Tryptic Soy Agar (TSA, Sigma-Aldrich, UK) in the dark at 30°C.

Fungal cultures

Two isolates of each fungal pathogen were tested: *F coeruleum* P60 and P67, *F sulphureum* P28 and P62, *H solani* P27 and P113. All fungal isolates were obtained from the SASA potato pathology culture collection and were originally isolated from Scottish tubers. All stocks were maintained on Potato Dextrose Agar (PDA, Oxoid, UK) at ambient temperature and light cycles.

Biological control agent dual culture assays

A single colony of *B*. *subtilis* or *A. migulanus* was used to inoculate 10ml Tryptic Soy Broth (TSB, Sigma-Aldrich, UK) and incubated overnight at 30°C, 180rpm. A streak of this culture was inoculated in a straight line onto PDA 30mm from the edge of a 90mm petri dish (Corning, UK) using a 10µl loop (Thermofisher, UK) and incubated at 30°C overnight for *B*. *subtilis* and for 72 hours for *A*. *migulanus*. A 3mm plug of a pathogenic fungal isolate, excised using a cork borer, from the leading margin of a pure culture on PDA was used to inoculate the opposite edge of the plate at an equivalent distance. Control plates were inoculated using the same method but with a streak of TSB in place of the BCA. Plates were incubated at room temperature with ambient daylight cycles. Radial growth of isolates was measured at the experimental end point: day 21 for *Fusarium* spp., day 42 for *H solani*, a slower growing pathogen.

Statistical analysis

Three independent experiments were conducted for each BCA and pathogen combination. Each experiment included three replicate plates of each BCA and pathogen combination. Variation in pathogen growth was assessed with a general linear model (GLM) using GenStat software v19. The GLM assessed the contribution of day of measurement and treatment (BCA or control) factors on the observed variation in pathogen radial growth**.**

RESULTS

The effect of biological control agents against *in vitro* growth of *Fusarium* spp.

Bacillus subtilis had a strong inhibitory effect on all dry rot causing isolates tested. Both *F, coeruleum* isolates showed 73% inhibition, *F. sulphureum* isolates P28 and P62 showed 61% and 48% inhibition respectively. *A. migulanus* Nagano also had a strong inhibitory effect on all the isolates tested: 60% for both *F. coeruleum* isolates and 53% and 49% inhibition of *F. sulphureum* isolates P28 and P62 respectively. *A. migulanus* NCTC 7096 had some inhibitory effect against *F. coeruleum* of 30% and 35% against isolates P60 and P67 respectively. A less significant response of less than 10% was observed against both *F. sulphureum* isolates. *A. migulanus* E1 had no inhibitory effect on any of the dry rot isolates tested.

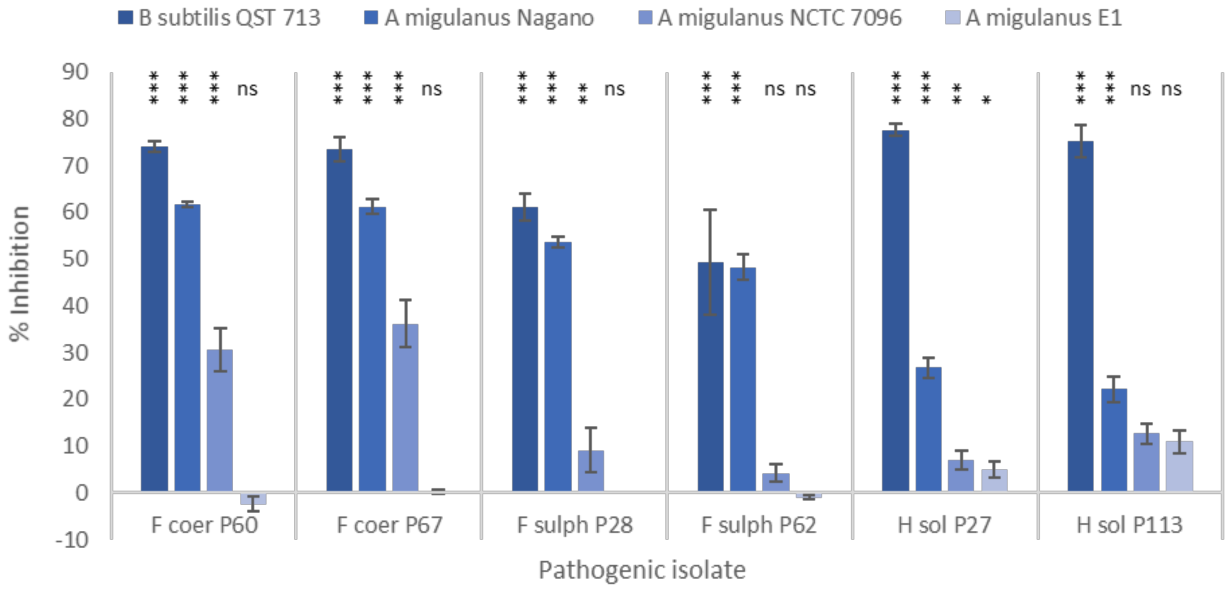

Figure 1. Percentage growth inhibition of pathogenic isolates by *B. subtilis* and *A. migulanus* isolates at experiment end point. Error bars represent standard error. *** = p<0.01, ** = p<0.05, * = p<0.1, ns = not significant.

The effect of biological control agents against *in vitro* growth of *H solani*

B. subtilis had a strong inhibitory effect on both *H. solani* isolates: 78% and 75% for isolates P27 and P113 respectively. *A. migulanus* Nagano had a weaker but still significant effect on both isolates: 27% and 22% on isolates P27 and P113 respectively. *A. migulanus* NCTC 7096 had a less significant inhibitory effect of 7% and 12% on isolates P27 and P113 respectively. As with the dry rot pathogens, *A. migulanus* E1 had no significant inhibitory effect on P113 but an inhibitory effect of 5% on P27 was significant (P < 0.1).

DISCUSSION

Both *A migulanus* and *B subtilis* possess *in vitro* antifungal activity against two major fungal pathogens of potatoes, with *A. migulanus* Nagano having a stronger antifungal effect on dry rot and silver scurf pathogens compared to isolates NCTC 7096 and E1. Alenezi *et al*., (2016a) observed similar effects on a number of fungal and oomycete pathogens and *A. migulanus* Nagano has been shown to reduce red band needle blight disease severity in *Pinus contorta* where NCTC 7096 had no effect (Alenezi *et al*., 2016b). The consistency of response to *B. subtilis* observed may reflect its commercial use as a BCA. Whilst both BCAs show promise for use in protecting potato crops from dry rot and silver scurf, *in vitro* effects of BCAs are not always replicated *in planta* or in field trials (Edwards and Seddon, 2001; Lahlali *et al*., 2011) and further work is required *in vivo* to confirm their efficacy as foliar and tuber treatments for potato. To be truly useful for the potato industry potential BCAs would need to be effective against a number of pathogen species that can negatively affect tuber yields and quality. Experiments are currently underway to assess the *in vitro* and *in planta* efficacy of *B. subtilis* and *A. migulanus* against a wider range of fungal pathogens that challenge potato growing crops and tubers in storage.

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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
low volume LV
maximum max
maximum residue level MRL
metre(s) m
metres per second m/s
milligram(s) mg
less than <
more than >
not less than |<
not more than |>

variety (wild plant use) var.
volume **V**
water dispersible granule WG
weight *wt*
weight by volume *wt/v*
weight by weight *wt/wt*
wettable powder WP
mega (x 10^{6}) M
kilo (x10^{3}) k
milli (x10^{-3}) m
micro (x10^{-6}) *μ*
nano (x10^{-9}) n
pico (x10^{-12}) p