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Developing sustainable management methods for clubroot

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1. Abstract

The project aimed to progress sustainable and cost-effective management of clubroot, through an enhanced understanding of the diversity of UK clubroot populations, and the prevalence of varietal resistance breaking strains. Secondly, the project aimed to assess the potential for targeted treatment by mapping the severity of clubroot in affected fields.

The project results have highlighted the prevalence of clubroot in the UK, the diverse nature of the pathogen population and the patchy nature of the disease in affected fields. Twenty-five fields were assessed using a standardised European Clubroot Differential Host set and 20 different pathotypes were identified, in almost equal proportion. Strains of clubroot that can overcome the 'Mendel' resistance to clubroot, which is present in resistant varieties like Crome, Mentor and Cracker, were found to be present throughout the UK. Soils from seventy-five fields were tested in total, using the oilseed rape varieties Mendel (resistant) and Tolken (susceptible) in bioassays. Of these, almost half (49%) had disease severities of more than 10% in the resistant variety, as a % of what was noted in the susceptible variety, and 15% of sites gave results of over 30% disease severity.

Mapping clubroot severity in commercial crops, at 50m grid squares, and testing the soil and examining plants at three timings in the season, illustrated the patchy nature of the disease. However, yield maps of these sites highlight that the pathogen is just one cause of low-yielding patches in fields. The disease mapping demonstrated the potential for the targeted management tools, such as liming or any new but relatively costly methods that might later be developed. It would also allow for hygiene precautions to be taken around infected patches and for patches to be differentially treated when drilling susceptible crops – for example leaving bare or seeding with grass. Disease mapping also has potential to help with decision-making; a field with a low level of infection distributed evenly in the field would be managed differently to a field with a single hot spot. However, such fields might give a similar test result under current testing systems, which could assign a single risk level for the whole field. Fields in this project were mapped using qPCR, bioassays and by visually assessing disease levels developing in the field. It was evident that soil tests, particularly molecular tests, do not correlate well with in-field disease development, so this is an area where further refinement is needed.

The practical advice arising from this project is that, because of the prevalence of clubroot in the UK and the widespread occurrence of resistance breaking strains, growers should keep accurate crop records of clubroot occurrence, location and intensity and note where varietal resistance has been deployed in fields to aid long-term planning and help prevent spread.

The use of resistant varieties should not be used as the sole management tool because of the high frequency of Mendel-breaking strains in the UK. The use of non-susceptible crops in rotational

courses is the most sustainable long-term solution, and should be used in partnership with other options such as resistant varieties or liming. Where resistant varieties are used, crops should be monitored carefully to assess the levels of clubroot that develop so that, if levels of infection start to increase, farm strategy can be changed. Inoculum increase through volunteers and weeds is a recognised risk, so managing volunteers and susceptible weeds within and between oilseed rape crops, especially on farms with a record of clubroot and or on tight rotations, is critical. Purchasing certified seed ensures that susceptible plant numbers are minimised in a resistant variety seed batch, so resistant varieties should not be home-saved for seed.

Growers should be mindful of other susceptible crop choices when planning rotations – spring rape is susceptible and cover crop mixes often contain susceptible species. Ideally, long-term planning should be based on the long-term profitability of a field and not on a single season's predicted margin. Field mapping should be considered in order to inform management choices; around isolated patches, basic hygiene measures to reduce spread can be taken and there is potential to target available tools at infected areas. These might include liming, the use of resistant varieties or the use of fallow / grass in a patch of known infection. Since clubroot persists for up to 20 years, knowledge of patches would remain useful for several seasons after a mapping exercise.

2. Introduction

Clubroot is the most intractable and damaging problem affecting oilseed rape in the UK, causing significant, or even complete, crop losses when present (Dixon, 2009). The disease can persist in soils for up to 20 years, so cannot be managed practically through extending normal arable rotations (Wallenhammer, 1996). A recent AHDB project (No. 3373) (Burnett et al., 2013) sampled soils on UK arable farms where clubroot was suspected to be constraining yield and found that over 50% of oilseed rape land in the UK was contaminated with clubroot. Clubroot-free land is therefore a diminishing resource. The disease is favoured by warm autumns and winters and so in addition to close rotations, climate warming may be a factor in increased clubroot incidence and severity (Gossen et al., 2014)

More than 100 pathotypes of *P. brassicae* have been recorded based on the response to a series of different brassica host species called the European Clubroot Differential lines (ECD) (Buczacki et al., 1975). Pathotype variation differs not only at a global scale but also at a field scale reflecting the heterogenetic nature of *P. brassicae* populations.

Clubroot management remains a challenge for growers and any current tools offer only partial control. Available soil treatments (lime, Calcium cyanamide and soluble boron) were studied over several sites and seasons (McGrann, 2015) and gave highly variable reductions in clubroot. The most effective treatment (high dose lime at 8 t/ha) gave a mean reduction of 25% in clubroot severity.

Varietal resistance remains the most effective method of clubroot control, although resistance to clubroot is currently based on a single resistance gene (termed ‘Mendel’) and this resistance source is not effective against all pathotypes of the disease (McGrann et al., 2015). In areas where resistant crops have been deployed repeatedly ‘Mendel’ resistance has become eroded and those ‘resistance breaking strains’ of the pathogen that are virulent on ‘Mendel’ varieties have inevitably become more prevalent. Fundamental research for new sources of resistance is ongoing and the work described here will align and add field relevance to that search. There are large knowledge gaps around the UK clubroot population, and no up-to-date information on common strains and their distribution.

The project reported here aimed to establish the distribution and prevalence of pathotypes within the UK *P. brassicae* population in order to provide immediate information on areas where races that are pathogenic on ‘Mendel’ resistant varieties are present in the UK. This would better inform variety and rotational choices for growers and would also inform work on breeding for novel sources of resistance.

Soil treatments with lime, calcium or boron gives partial control, but strategies used in vegetables, such as raising field pH to over 7.5, are not practical over whole fields in the context of arable

rotations as other crops within the rotation might be severely compromised. Targeted treatment of affected patches offers a potential approach and might be more cost effective than treating whole fields. The project set out to map fields to establish the impact and distribution of patches of the disease within crops. This is the approach being taken in Europe and Canada where the disease is also prevalent. The practical applications of this might be that, through the use of soil sampling and precision farming technologies, spot treatment liming (or application of alternative but potentially expensive biological controls) would be possible. Mapping fields and targeting application of control treatments to only affected areas of fields would be more practical and cost effective than treating whole fields. There would be immediate practical gains to the grower in targeting treatment or resistant varieties at the high-risk areas, and also at the simple level of taking basic hygiene measures to avoid spreading patches and leaving them fallow or grassed.

This project aimed to advance our understanding of the variation of this pathogen in the UK and add field relevance to searches for novel resistance and to produce practical management messages for growers. We are grateful for the input of stakeholders who attended two workshops in the course of the project, funded by the P3 initiative at Sheffield University and helped shape protocols.

Figure 1. Example clubroot symptoms in oilseed rape

The key aims of the project are set out below:-

1. Determine prevalence and distribution of resistance breaking *P. brassicae* strains in the UK
2. Quantify the rate of selection for resistance breaking *P. brassicae* strains in clubroot field populations at previously studied sites
3. Establish, through field mapping, the impact and spread of patches of clubroot contamination in soils
4. Investigate the cost efficacy of treatment through field mapping

3. Materials and methods

3.1. Clubroot populations studies

This work package mapped the clubroot pathotypes present in the UK to determine the distribution and prevalence of resistance breaking pathotypes which can infect in the presence of the 'Mendel' gene. Current UK clubroot pathotypes were differentiated using the ECD set. At a 2014 international workshop it was agreed that sources containing the 'Mendel' resistance would be included in the standard pathotype screen for the first time. As well as characterisation of field populations, the work also aimed to prepare reference isolates of clubroot to deliver into breeding programmes. Clubroot from each site was bulked in Chinese cabbage and then tested for the presence/absence of Mendel virulence. Single-club clubroot isolates were isolated from a subsample of 10 soils.

3.1.1. Determining the prevalence and distribution of resistance breaking strains in the UK

To structure the sample of sites, as far as possible, sites were selected on geographical-scale line transects (at least approximately), N-S and E-W. Through local consultants, seventy-five fields from across the UK were identified that were at high risk of clubroot. Soil was collected from each of these fields so that bioassays could be completed to determine the prevalence of resistance breaking strains through growing varieties of oilseed rape with and without Mendel resistance to determine the proportion of the potential clubroot population that was capable of overcoming the Mendel resistance. Further methodology for completing bioassays is included in section 3.1.3.

3.1.2. Indicate the diversity of strains in the UK clubroot population

From the seventy-five fields sampled above (section 3.1.1), soil from a random sub-sample of 25 fields was selected for testing using differentials of the European Clubroot Differential Set (ECD). The seed material was kindly supplied by Prof. Geoff Dixon, from the University of Warwick. The ECD set was initially described by Buczacki et al., (1975), and consists of fifteen lines, split into three chromosome groups (Table 1). Each host tested within a group is assigned a Denary number, so that the hosts from each group which prove within a bioassay test to be susceptible to a particular population can be summed together, with each combination providing a unique number code which describes the population tested.

Table 1. European Clubroot Differential Set (ECD) host species with their denary values.

ECD genotype	ECD score	Differential	Denary value
20 chromosome group	ECD 01	subsp. *rapifera*. Line aaBBCC	1
Brassica rapa	ECD 02	subsp. *rapifera*. Line AAbbCC	2
	ECD 03	subsp. *rapifera*. Line AABBcc	4
	ECD 04	subsp. *rapifera*. Line AABBCC	8
	ECD 05	var. *pekinensis*. Chinese cabbage cv. Granaat	16
38 chromosome group	ECD 06	var. *napus*. Dc101 Nevin	1
Brassica napus	ECD 07	var. *napus*. Dc119 Giant Rape	2
	ECD 08	var. *napus*. Dc128 selection from Dc 119	4
	ECD 09	var. *napus*. Dc129 New Zealand resistant rape	8
	ECD 10	var. *napobrassica*. Dc130 Wilhelmsburger	16
18 chromosome group	ECD 11	var. *capitata*. Badger Shipper	1
Brassica oleracea	ECD 12	var. *capitata*. Bindsachsener	2
	ECD 13	var. capitata. Jersey Queen	4
	ECD 14	var. capitata. Septa	8
	ECD 15	var. *acephala* subvar. Laciniata. Verheul (Kale)	16

3.1.3. Bioassay methodology

Bioassays were completed using soils sampled to determine which clubroot populations were able to overcome host resistance in optimal controlled environment conditions. Field soil samples were processed so large stones and plant material were removed before being thoroughly mixed and used to fill plastic seed trays with drainage holes (20 x 14.5 x 5.5 cm). Positive and negative controls, consisting of soil sampled from a heavily infected site (positive control) and John Innes No. 2 potting compost (negative control), were set up with every batch of tests.

To determine the prevalence of resistance breaking strains, twenty-five seedlings of Chinese cabbage cv. Granaat, the susceptible oilseed rape cultivar Tolken, and the clubroot resistant oilseed rape cultivar Mentor were sown in infected soils from each field sampled.

To determine the diversity, trays were sown with the 15 host species of the ECD set as outlined in Table 1. The seedlings were grown for 6 weeks under glasshouse conditions with soil temperature kept above 21^{O}C and the soils kept moist to encourage infection. The plants were assessed and

scored for clubroot infection using a four category scale where 0 = uninfected, 1 = slight clubbing, 2 = moderately clubbed and 3 = severely clubbed. A 0-100 severity index was then calculated by weighting the incidence of plants in the three positive categories by a factor of one, two or three respectively using the following formula: Index = ((1*slight) + (2* moderate)+ (3* severe)) * (100/3*number of plants assessed).

Figure 2. Bioassay in progress, ADAS Boxworth, Cambridgeshire.

3.1.4. Understanding the stability of clubroot strains at previous tested sites

Two sites that were used as field trial sites and so were pathotyped in a previous clubroot study (Defra project no HH3227TFV, Harling, 2007) were revisited and retested to establish changes in clubroot levels and any shifts in the pathotypes present. The first site tested was from the SRUC Craibstone Farm site where long-term clubroot beds have been sited for varietal resistance screening in the Woodland's long-term experiment, established in the 1920s. The second planned site was at Barnsmuir Fife which has been in raspberry production since the Defra project ended. The testing methodology was as described in 3.1.2.

3.1.5. Deliver reference isolates to breeding programmes

Initial experiments to develop near-single spore isolates of clubroot were based on a single *Plasmodiophora brassicae* isolate from Cupar, Scotland (McGrann *et al*., 2015). A recently published method for this procedure based on isolation of single sporangiosori from infected root hairs

(Diederichsen *et al*., 2016) was tested due to reportedly higher success rates compared to previous published protocols. Winter oilseed rape (WOSR) cv. Fashion seeds were grown in John Innes No. 3 compost inoculated with *P. brassicae* resting spores to give a final concentration of approximately 10^4 spores g soil^{-1}. Plants were harvested one or two weeks after sowing and roots examined for the presence of single sporangiosori. Sporangiosori were observed at both harvest dates with more root hairs visibly infected at two weeks (Figure 3). Sporangiosori appeared to be predominantly located in very fine roots hairs which meant that excising these structures from the plant under a binocular microscope at 400X magnification was not possible without damaging the root hair. A student bursary project funded by AHDB (Appendix 7.3) assisted with method development.

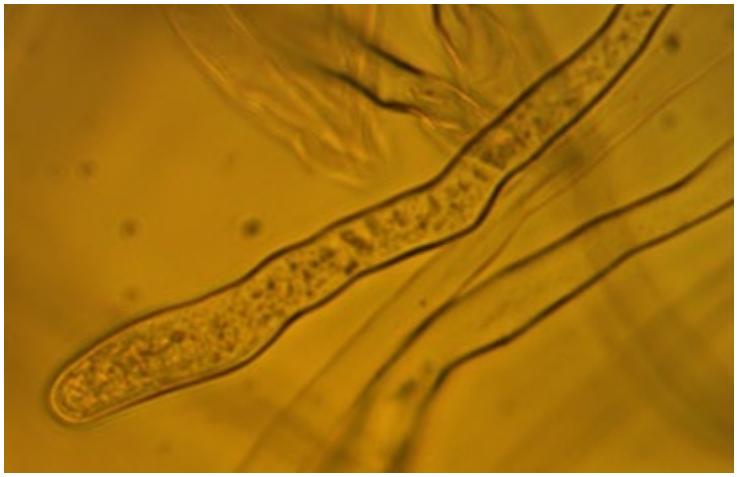


Figure 3. *Plasmodiophora brassicae* sporangiosori in winter oilseed rape cv. Fashion root hair, two weeks after planting in clubroot infested soil.

Due to the difficulty of carrying out single spore isolation an alternative method was agreed with AHDB to perform single club isolation instead. Resting spores were prepared from infected galls as previously described (McGrann *et al*., 2015), quantified using a haemocytometer and diluted to a concentration of 10 resting spores 5 mL^{-1}. Ten three-day old winter oilseed rape seedlings cv. Fashion were placed in the tube with the diluted resting spore solution and incubated in the dark at room temperature before being planted in John Innes compost at a rate of one inoculated seedling per pot. After eight weeks growth in an ambient temperature glasshouse inoculated plants were washed free of soil and examined for signs of root galling indicative of clubroot infection. Galls were harvested and stored at -20 ^{o}C.

3.2. Establish, through field mapping, the impact and spread of patches of clubroot contamination in soils

Clubroot is patchy in its distribution and the importance of this was studied through detailed sampling in a small number of infected fields. Fields were selected in contrasting geographic regions in Scotland and England. The sampling was coupled with quantification of inoculum through visual assessment, bioassays and a quantitative PCR method, following the published methods of the Wallenhammar group (Wallenhammar et al., 2012) so that the UK results could be interpreted in a global context as this methodology is used by international researchers (Strehlow et al., 2014). Field mapping of clubroot severity and incidence was linked with precision farming collaborators so that a natural output of this work package is that treatment can be targeted at high risk areas. Mapping involved taking 30 samples in grid pattern per field at three time points per season. The GPS position of each subsample was recorded and samples retained separately. Sites were selected in consultation with industry partners on farms where yield mapping was undertaken. Mapping was linked to currently available systems for mapping pH across fields so that clubroot treatment through manipulation of lime application could be achieved. All samples were tested for pH.

3.2.1. Commercial field mapping

A total of 16 commercial fields were used in this study to map the distribution and severity of clubroot in a field over a growing season (Table 2). In each field the presence and severity of clubroot infection was estimated by visual assessment of 10 plants carried out at 30 points distributed on a grid pattern across the field. Each point was geo located for mapping purposes using a hand-held GPS device with a horizontal accuracy of approximately 3m. At each point soil samples were collected for subsequent pH measurement and *P. brassicae* DNA quantification (*Cf*. 3.2.2). In addition, a visual destructive assessment was completed on 10 plants per point for club root severity (0-3 scale) which was converted to a 0-100 index, with repeat destructive club root assessments occurring at the same sample locations later in the season. NDVI (normalized difference vegetation index) mapping data was sourced from Data Farming (maps.datafarming.com.au), with maps downloaded as geo tiff files where data was available near to the clubroot infield assessment timings, which varied depending on cloud cover and timing of satellite passes. Field maps of clubroot severity index, treatment economics and NDVI were visualised within this project using QGIS version 2.18.10.

Table 2. Commercial sites mapped for clubroot.

Site	Year	Region	Field name	Description of epidemic
1	2016	Warwickshire	Warwick	Highly infected site
2	2016	Shropshire	Bridgnorth	Highly infected site
3	2016	Angus	Market	Moderately infected field
4	2016	Angus	Podge	Very low disease
5	2016	Herefordshire	Ivington	Moderately infected field
6	2016	Staffordshire	Staffs	Moderately infected field
7	2017	Staffordshire	Staffs	Moderately infected field
8	2017	Herefordshire	Pencombe	Highly infected site
9	2017	Herefordshire	Sutton	Moderate-high disease
10	2017	Herefordshire	Little Dilwyn	Moderately infected field
11	2017	Arbroath	Boysack	Low-moderate infection
12	2017	Earlston	Whinney Hill	Low disease
13	2018	Staffordshire	Congieve	Low-moderate infection
14	2018	Herefordshire	Murder	Moderate-high infection
15[1]	2018	Angus	Slade50	Very low disease
16	2018	Angus	Brae Face West	Very low disease

[1] This field was unfortunately ploughed after the winter sampling due to poor establishment and high herbivory damage

3.2.2. Molecular test methods

In addition to visual assessment in the field, the presence of *P. brassicae* DNA in the soil was assessed at every sampling point in each field. Large stones and plant material were removed prior to manually mixing the soil. Sub-samples of 0.5 g were taken for DNA extractions. Sample preparation was carried out using the FastPrep-24 5G system (MP Biomedicals, Eschwege, Germany) followed by DNA extraction using the Fast DNA Spin Kit for soil (MP Biomedicals, Eschwege, Germany) following manufacturer's instruction. Total DNA was quantified using the QuBit3 Fluorometer (Invitrogen, Carlsbad, CA, USA), and diluted to 10 ng uL^{-1}. A standard curve was obtained by a 1/5 serial dilution of 10 ng of *P. brassicae* DNA. The qPCR reactions were carried out on an Aria Mx Real Time PCR system (Agilent technologies, Santa Clara, CA, USA) using the SybrGreen Jump Start™ Taq system (Sigma, Dorset, UK) following manufacturer's instructions. Reactions were carried out as described in Wallenhammar *et al.* (2012) in triplicate. DNA extraction and quantification were carried out twice for each sampling point.

3.2.3. Economic analysis of the cost efficacy of treatment through field mapping

Clubroot index values from the spring assessment (Feb - April) were used to calculate yield losses from clubroot and the cost efficacy of treatment following the yield loss estimates in Burnett et al., 2013. Later assessments were not used as it was generally noted that infected plants had died by this late time and the levels of clubroot, as quantified by in-field plant assessments, therefore appeared to fall. Burnett et al., 2013 estimated yield loss as 0.03 t/ha per 1% clubroot severity. The benefit of treatment was also based on the results of the trial series reported in Burnett et al., 2013 where the most effective soil amendment was lime ($CaCO_3$). Liming in this trial series provided a mean of 25% control across all sites, and this level of control and commensurate yield benefit was used in the economic analysis.

In the economic analysis presented here liming was costed in at 4 t/ha using a cost of £20 per tonne of lime, including spreading costs. An informal survey of 23 UK agronomists was used to determine this costing and revealed a wide range of liming costs, ranging from less than £10 per tonne up to £35 per tonne. This was often dependent on the local availability of lime so, for example, those with ready access to lime available as a by-product of the sugar industry (LimeX) tended to pay least, those with local mined sourced tended to be mid-range and a few sites such as the south west of Scotland were at the highest end of the price spectrum. Worth noting is the aggregates tax paid on mined lime. The price of oilseed rape was taken as £335.50 per tonne which was the November 2018 price from the AHDB Market Data (AHDB, 2018). The price of oilseed rape has risen since the final harvest of trial sites in this project but was also considerably lower at points over the three-year duration of the project so the price at the finishing point of the project was deemed a logical and topical price to select.

4. Results

4.1. Clubroot populations studies

4.1.1. Determining the prevalence and distribution of resistance breaking strains in the UK

Over the three-year study, 75 fields were sampled across the UK to determine the prevalence of resistance breaking strains. Sites were selected for sampling which were high risk of clubroot, however 12% of those sampled did not produce clubroot visible galls within this test. Of the sites where clubroot was present in the soil, 21% of sites did not develop clubroot symptoms at all within the resistant variety. Within 62% of the field sites tested, clubroot did develop in the Mendel resistant variety, however the proportion of the clubroot population of the Mendel resistance breaking strain was below 30% of the population at these sites, which is not considered significant. In 17% of the sites, between 30% and 100% of the clubroot population was able to produce clubroot symptoms on the Mendel resistant variety. Full details of the frequency of various intensities of resistance breaking

strains of clubroot are shown below (Figure 4). If certified seed is taken as being of 90% purity then the 10% infection level was deemed a reasonable tolerance if the seed used in bioassay testing was at the minimum purity standard and all 'non-Mendel' plants were susceptible.

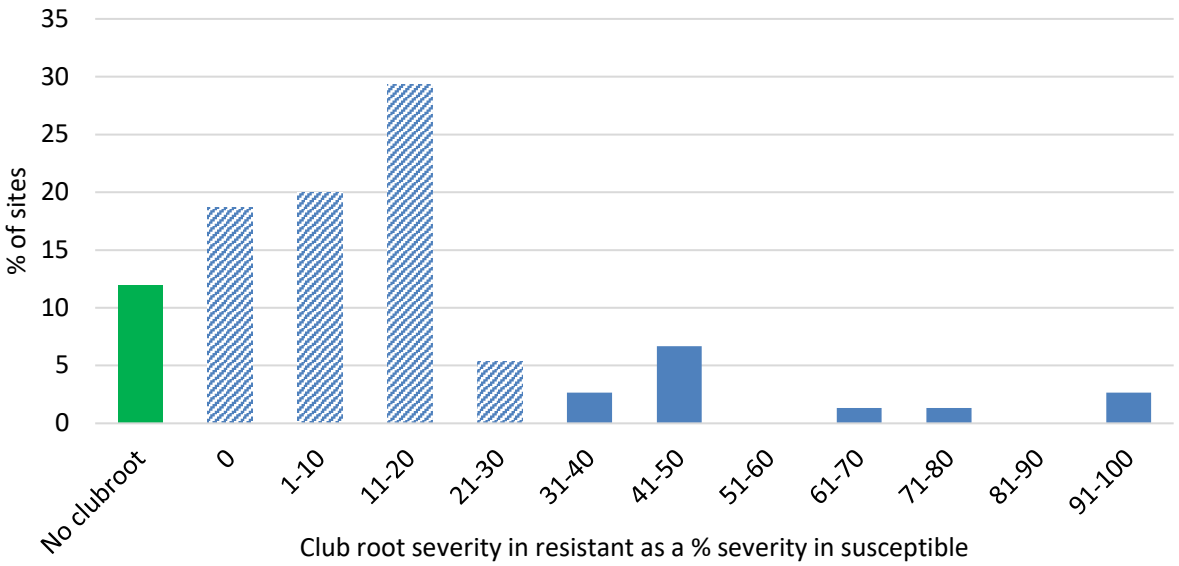


Figure 4. The percentage of fields sampled with differing proportions of Mendel insensitive clubroot populations. The proportion of the population able to overcome the Mendel resistance is represented on the X axis, with the percentage of sites falling into each category is represented on the Y-axis. Total number of fields sampled = 75. Individual site data is published in Appendix 7.1, Table 5.

The distribution of samples taken for the bioassays and the average proportion of Mendel resistance breaking strains in sampled counties is shown in Figure 5. Clubroot severity and levels of sensitivity to resistance genes is likely to vary from field to field however a degree of insensitivity was observed in all but two regions (each of which had very limited data with only one sample tested with clubroot infection). Further data is shown in Appendix 7.1, Table 5.

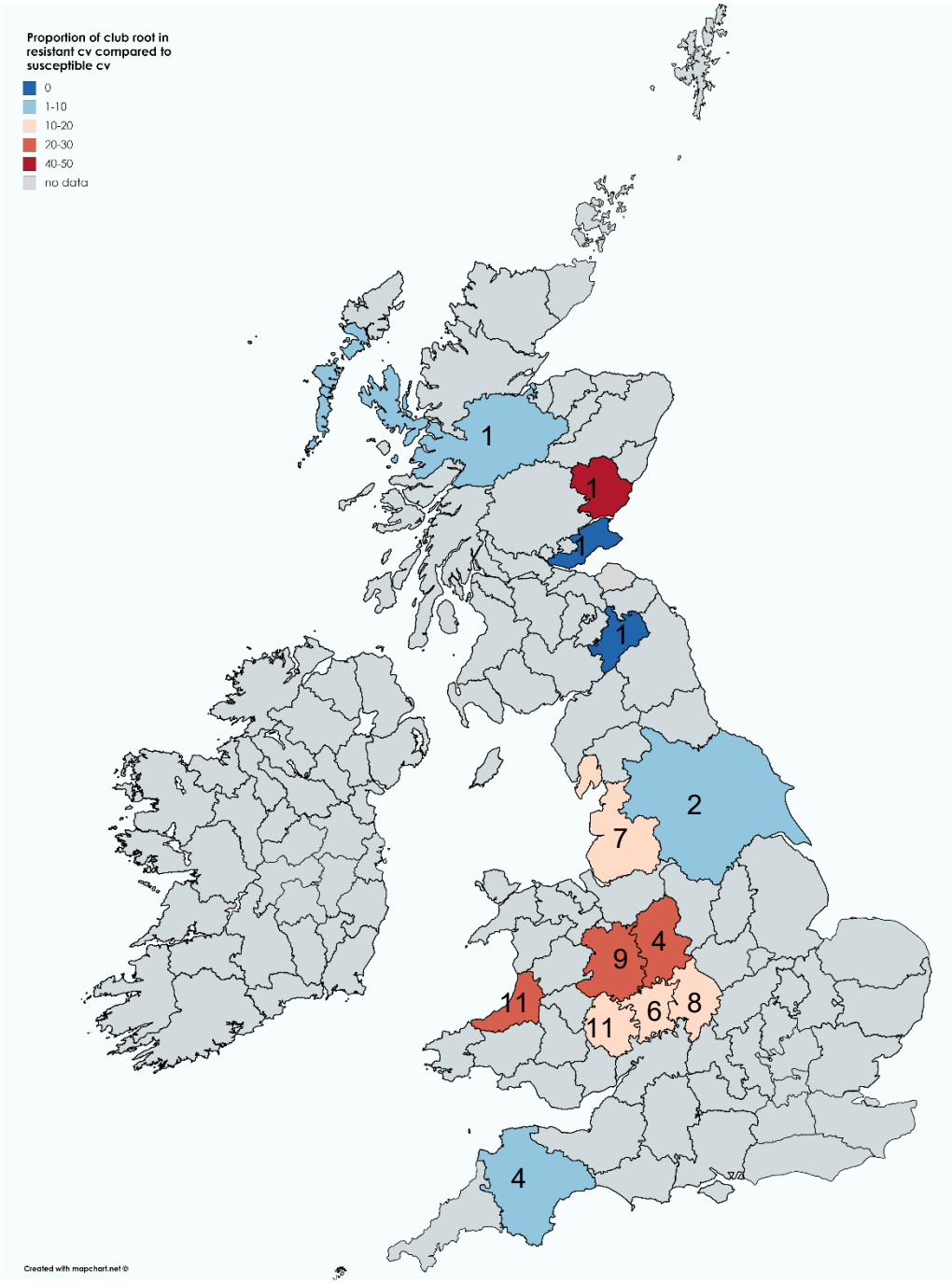


Figure 5. Mendel resistance breaking clubroot populations in the UK. The numbers indicate how many samples were taken in the county which were infected with clubroot, the colour shows the average proportion of resistance breaking strain across all the fields sampled within the county. Samples were not taken from grey coloured regions.

4.1.2. Indicate the diversity of strains in the UK clubroot population

A subsample of 25 fields from those tested in the + / - Mendel resistance bioassay work were selected at random in order to further investigate clubroot populations in the UK using hosts from the European Clubroot Differential lines (ECD). Results from this are presented in Figure 6, which show a diverse clubroot population with 20 different pathotypes identified within this project.

Further data is shown in Table 6 of Appendix 7.1.

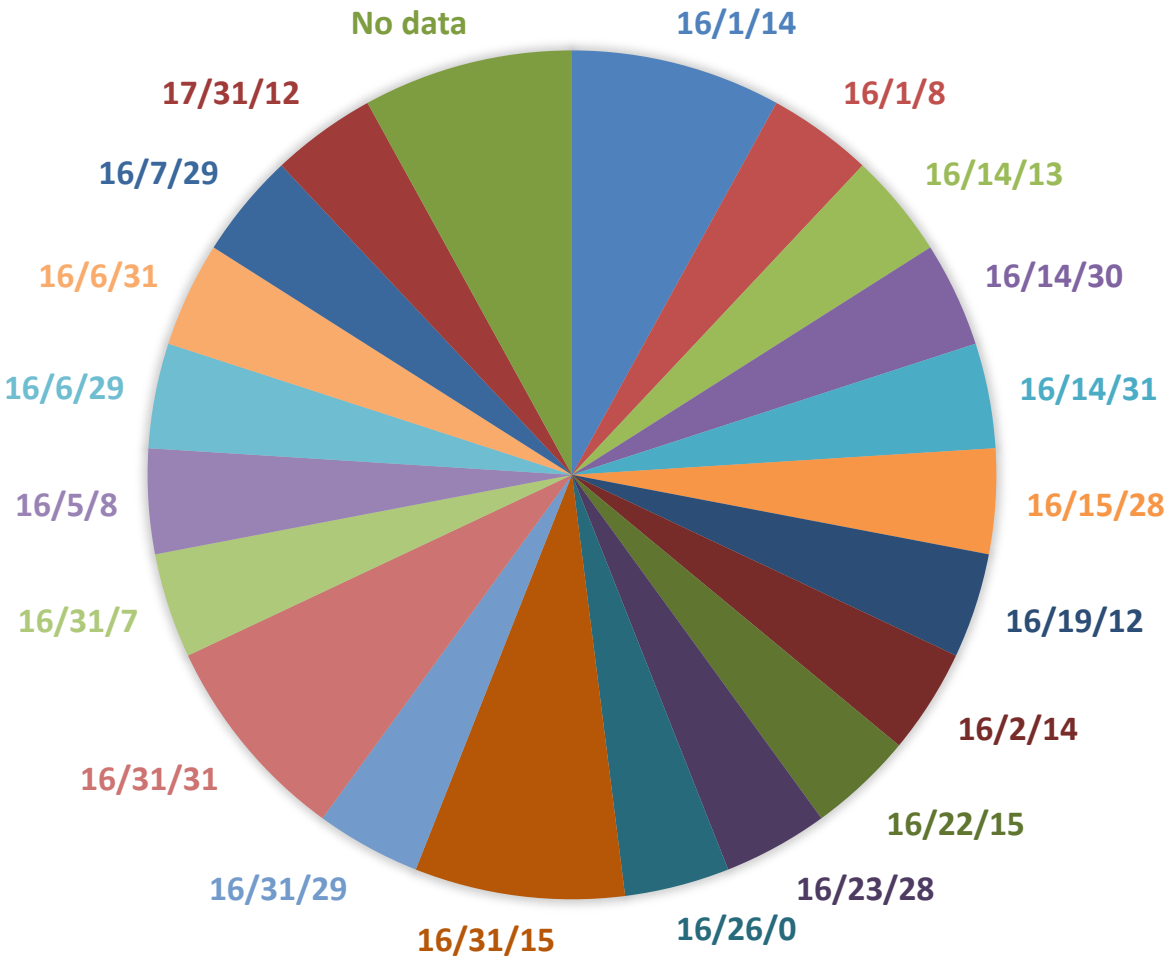


Figure 6. Pathotypes in the UK as determined from growing host species from the European Clubroot Differential lines (ECD) in infected soil from 25 fields across the UK between 2015-2018.

4.1.3. Understanding the stability of clubroot strains at previous tested sites

Two sites that were pathotyped in a previous clubroot study (Defra project no HH3227TFV, 2007) were revisited and retested to establish changes in clubroot levels and any shifts in the pathotypes present including within field pathotype heterogeneity. The Fife site at Barnsmuir had been in raspberry production since 2007 and no clubroot was detected in a bioassay of the site and so the plan to pathotype this site was abandoned. The second site at SRUC Craibstone was pathotyped using the ECD tests detailed in section 3.1.2 and found to be the same as in 2007 testing - 16/31/7. This site has had a range of brassica hosts and non-susceptible crops as well as radishes and given the varied rotation it is perhaps surprising that the pathotype has remained apparently stable over this period.

4.1.4. Deliver reference isolates to breeding programmes

Small galls were visible on a number of plants inoculated with resting spores and disease scores ranged from 1-3 using a five scale rating based on the severity of galling with 0 = no galling; 1 = small clubs present, most of fibrous root still healthy; 2 = galls visible around tap root and crown; 3 = moderately severe galling with healthy roots still visible; 4 = severe galling with few healthy fibrous roots present; 5 = severe galls with root system now rotten (McGrann *et al*. 2015). The galled roots were washed and clubs were harvested and stored. The single club collection (Table 3) has now been made fully available to breeders and was delivered into the OREGIN project. http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=9879

Table 3. Detail of the single club collection delivered into the OREGIN project

Year	Source	Location	Crop	Pathotype	Number of clubs stored
2017	SRUC	Aberdeen	Oilseed rape	17/31/31	16
2018	SRUC	Turiff	Swedes	*	18
2018	SRUC	Turiff	Oilseed rape	*	14
2017	SRUC	Boysack	Oilseed rape	*	15
2014	Barnsmuir	Cupar	Vegetable brassicas	16/2/30	8
2014	Gaudley bank	Cupar	Vegetable brassicas	16/31/31	18
2018	SRUC	Aberdeen	Oilseed rape	*	2
2018	SRUC	Craibstone	Various	*	3

*Not pathotyped

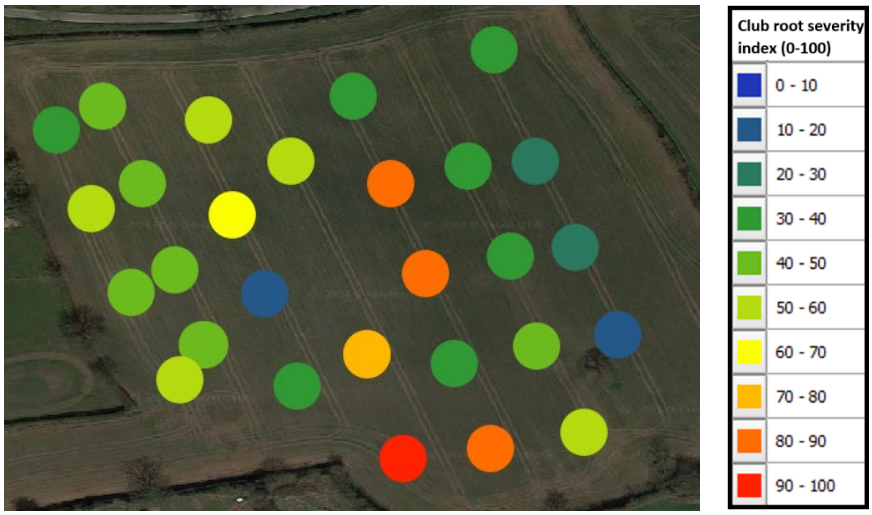
4.2. Establish, through field mapping, the impact and spread of patches of clubroot contamination in soils

4.2.1. Commercial field mapping

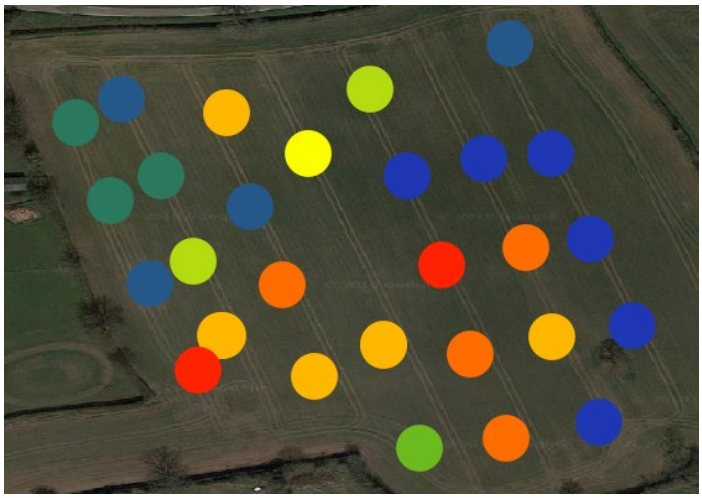
The mapped clubroot severities (from in field plant assessments) from each of the intensely monitored fields are presented below (Figure 7 – Figure 21). A range of disease severities were observed during the project, with some fields observed having very low levels of clubroot e.g. Brae Face West in 2018 (Figure 20), to the other extreme with very severe clubroot severities at Bridgnorth in 2016 (Figure 8).The disease development over time varied between fields and seasons. No consistent pattern of infection was observed, with patch size shape and location varying on a field by field basis, with patches not necessarily being identified near a field entrance. In addition, through the season the severity of clubroot did not consistently become more severe, with levels of infection in some fields staying relatively static or even declining towards the end of the season as infected

plants died out. The patch size within a season did not increase by a detectable amount, and with the longevity of clubroot in soils this means that information on patches of infection is likely to remain useful over a number of seasons. Where available in monitoring years 2017 and 2018 spring clubroot severities were overlaid on NDVI (Normalized Difference Vegetation Index, which quantifies the density of green on a patch of land) maps from similar time points, to show the correlation between clubroot infection and NDVI decline where clubroot was known to be the main cause of stunting or plant loss within a field (maps presented in Appendices; 7.2.2 NDVI maps).

In field clubroot results 2016

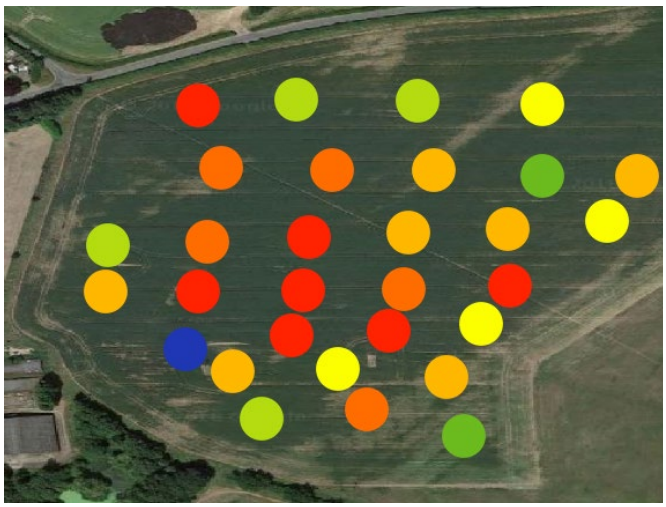


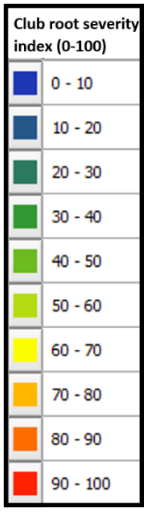
Clubroot severity index, February



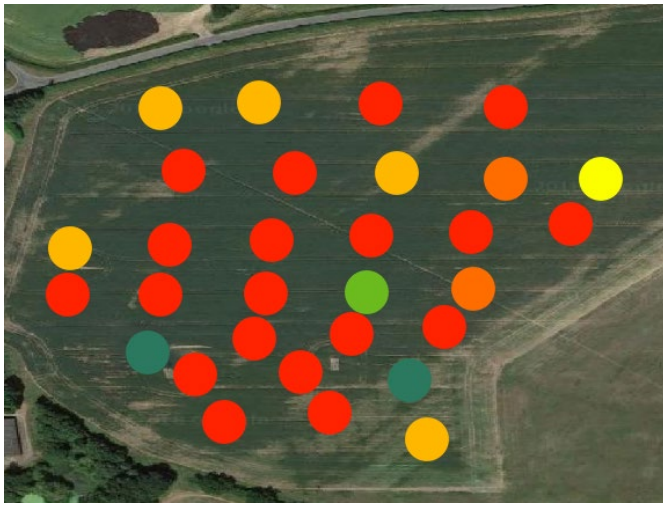
Clubroot severity index, June

Figure 7. Intense field monitoring, Clubroot severity index over time, Field: Warwick, 2016.

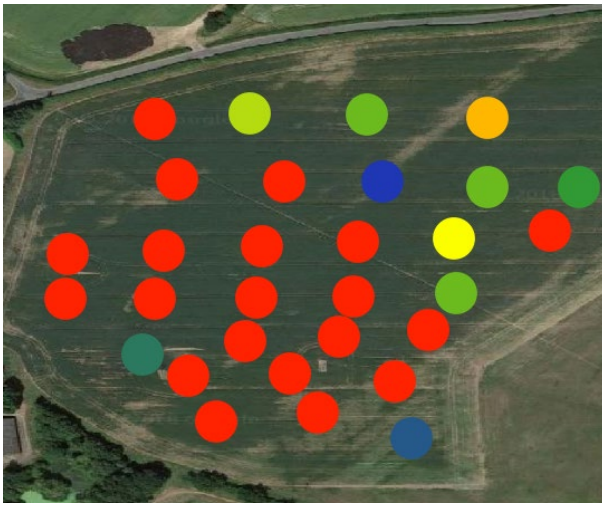




Clubroot severity index, February

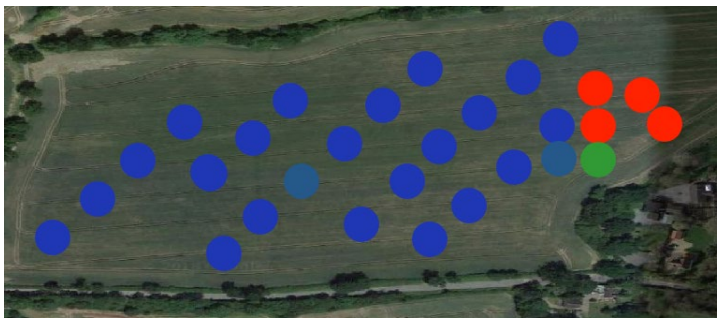


Clubroot severity index, April

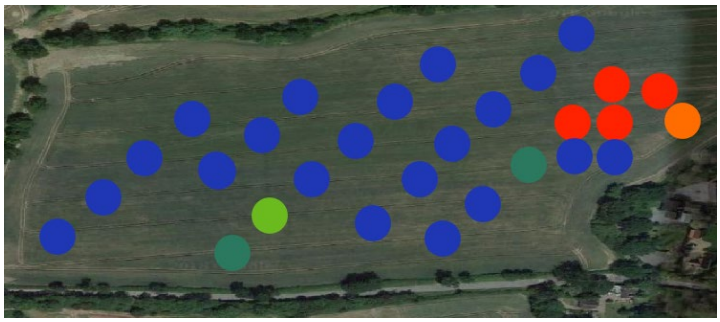


Clubroot severity index, July

Figure 8. Intense field monitoring, Clubroot severity index over time, Field: Bridgnorth, 2016.



Clubroot severity index, February



Clubroot severity index, June

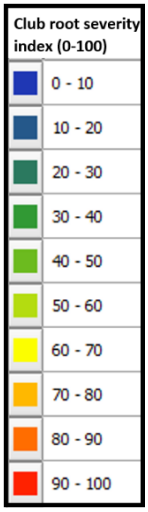
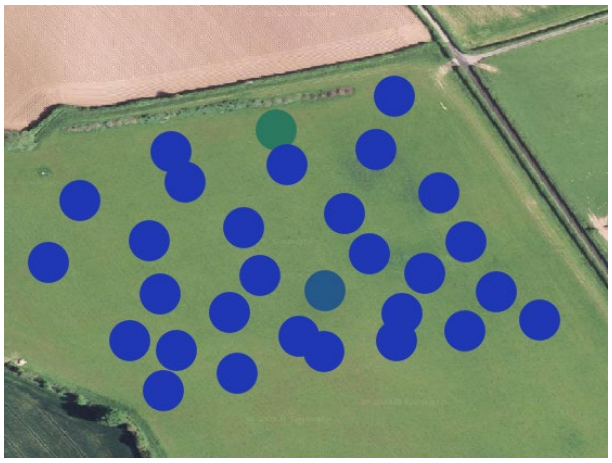
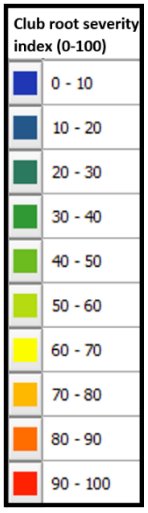
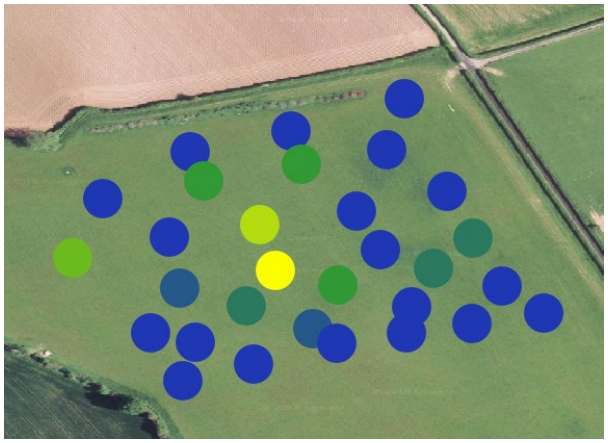


Figure 9. Intense field monitoring, Clubroot severity index over time, Field: Staffordshire, 2016.

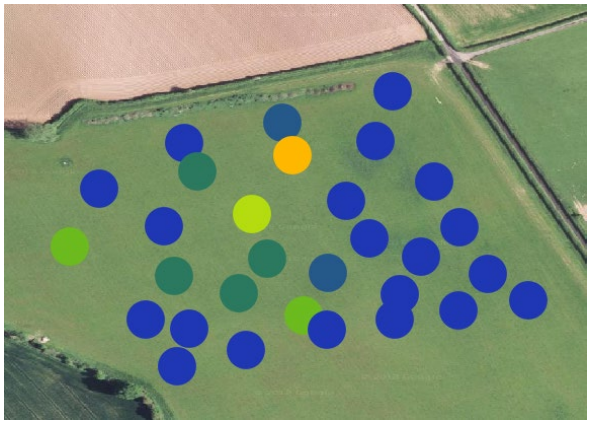


Clubroot severity index, December



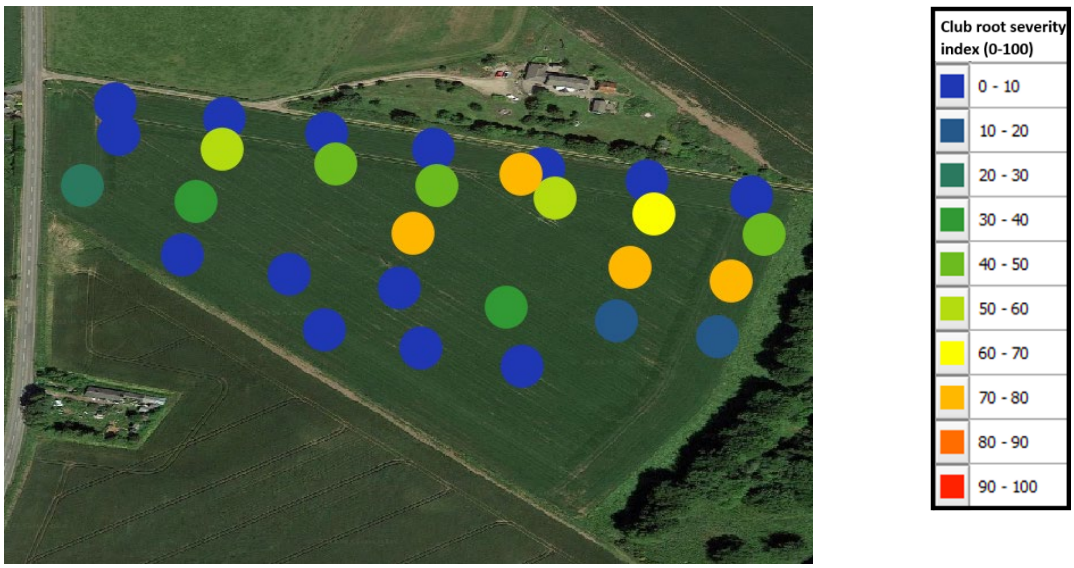


Clubroot severity index, February

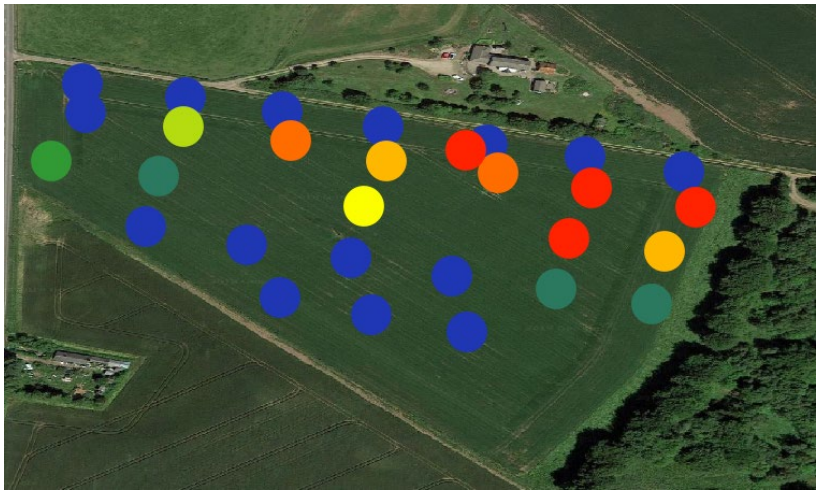


Clubroot severity index, June

Figure 10. Intense field monitoring, Club root severity index over time, Field: Ivington, 2016.

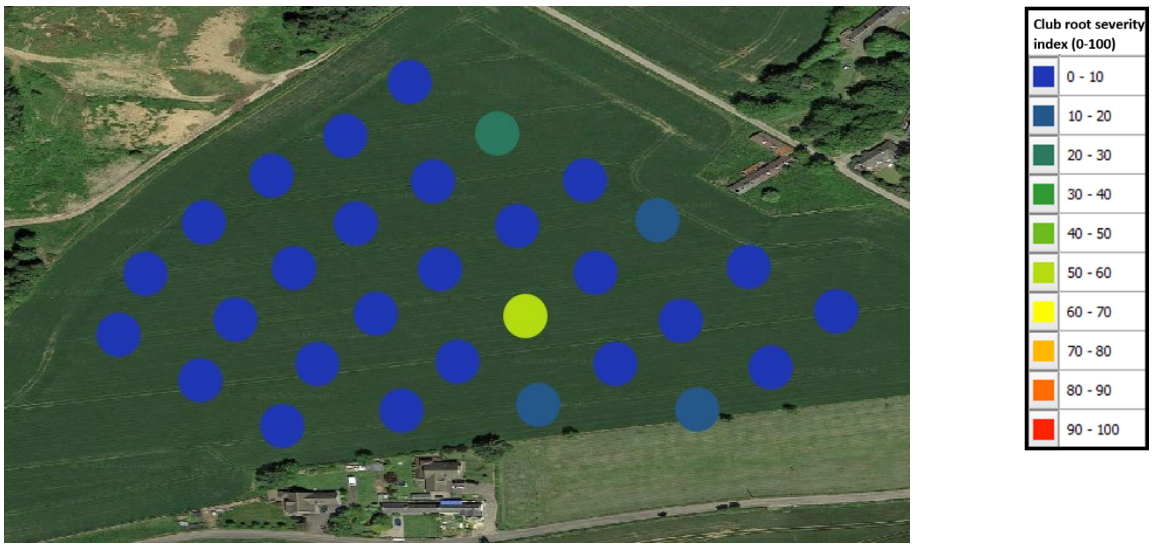


Club root severity index, May

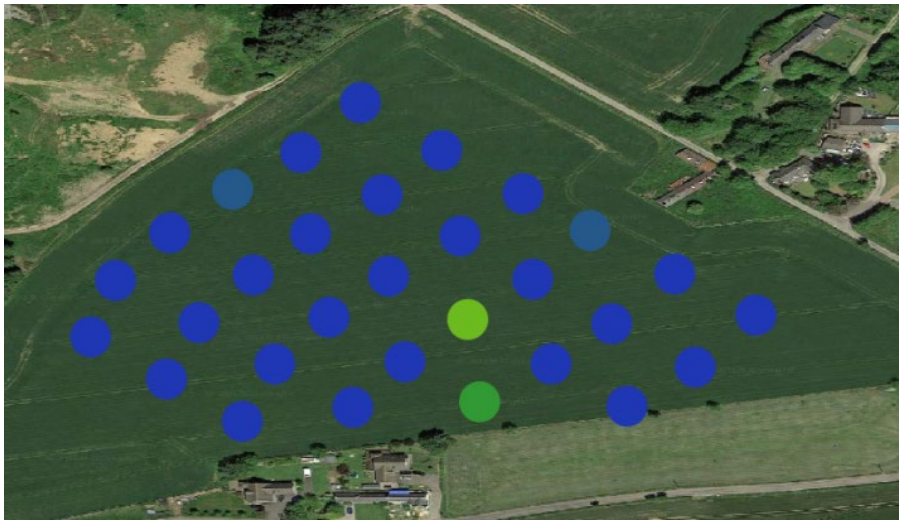


Club root severity index, July

Figure 11. Intense field monitoring, Clubroot severity index over time, Field: Market field, 2016.



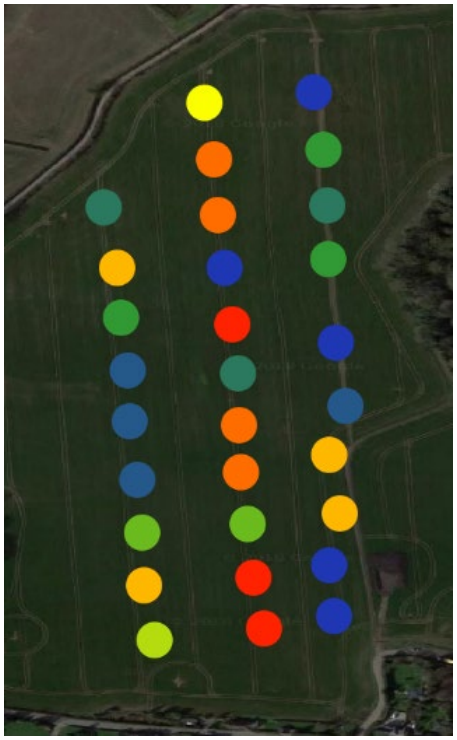
Clubroot severity index, May

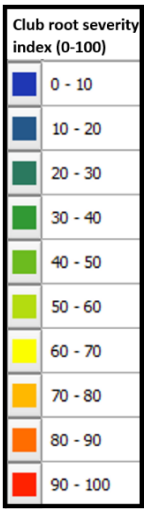


Clubroot severity index, July

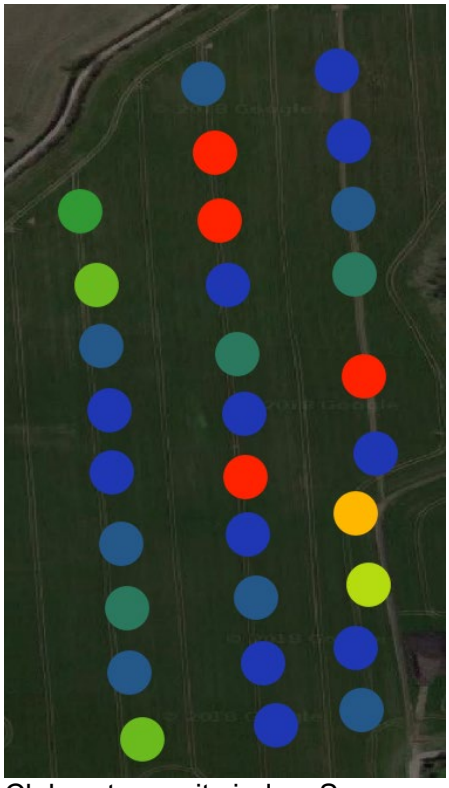
Figure 12. Intense field monitoring, Club root severity index over time, Field: Podge field, 2016.

In field clubroot results 2017



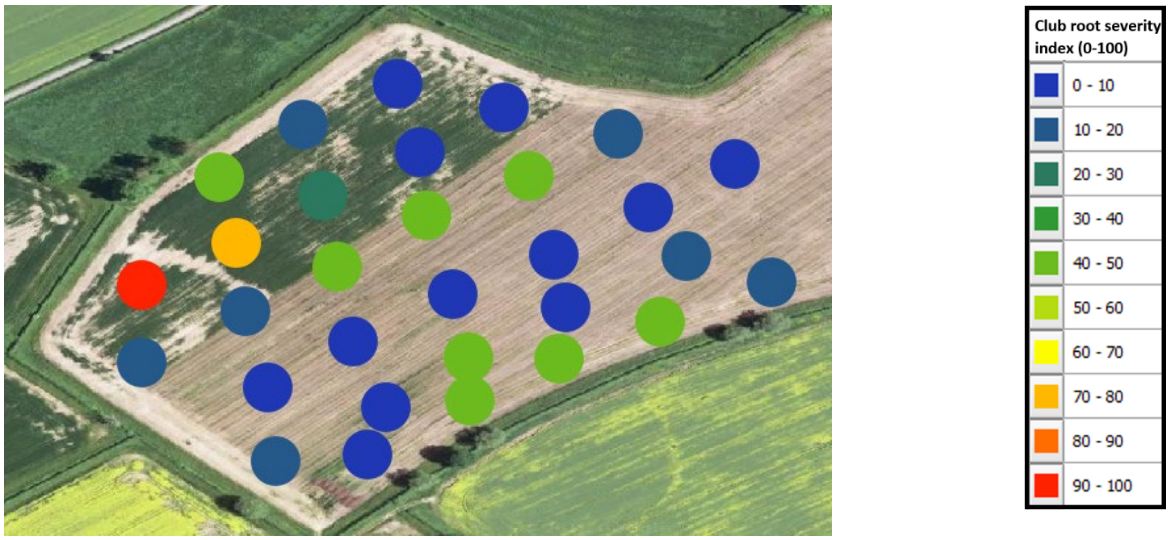


Club root severity index, Winter

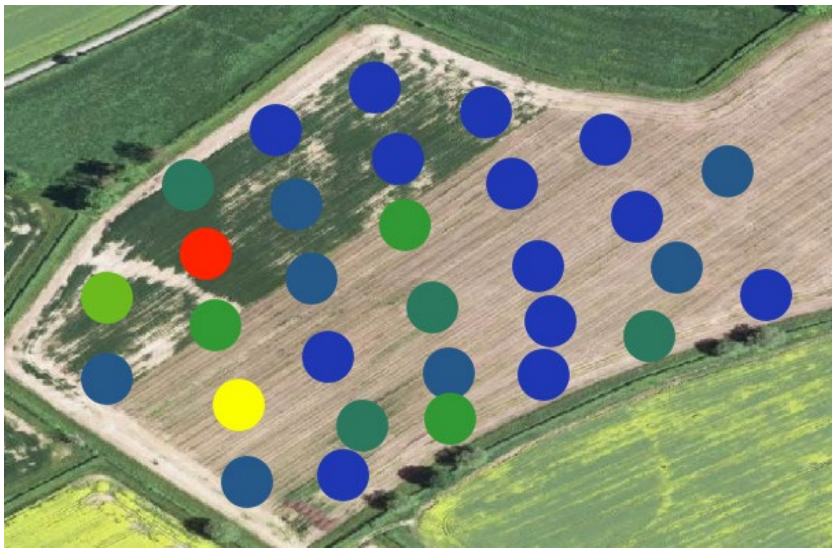


Clubroot severity index, Summer

Figure 13. Intense field monitoring, Clubroot severity index over time, Field: Staffordshire, 2017.

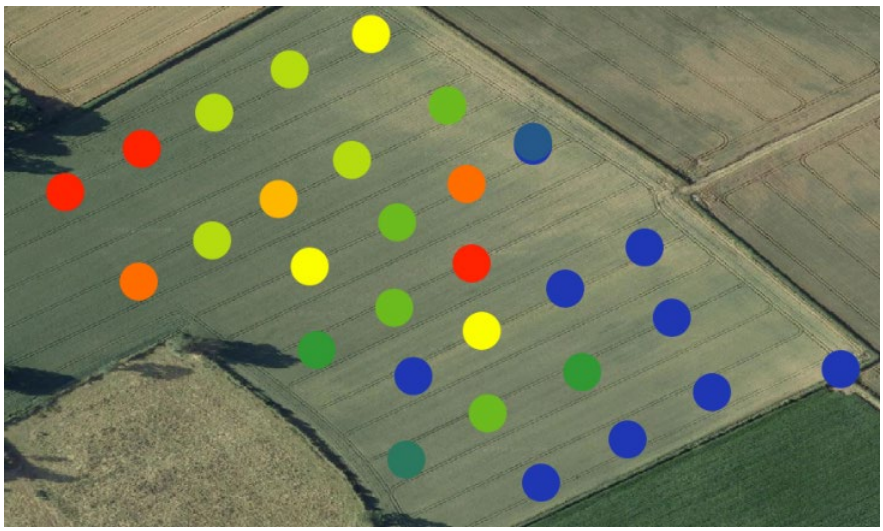


Clubroot severity index, Winter

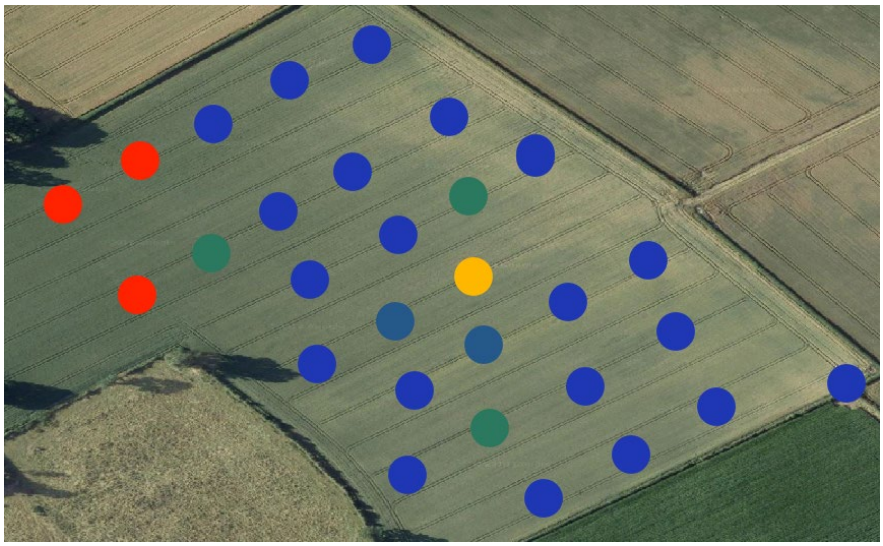


Clubroot severity index, Summer

Figure 14. Intense field monitoring, Clubroot severity index over time, Field: Little Dilwyn, 2017.

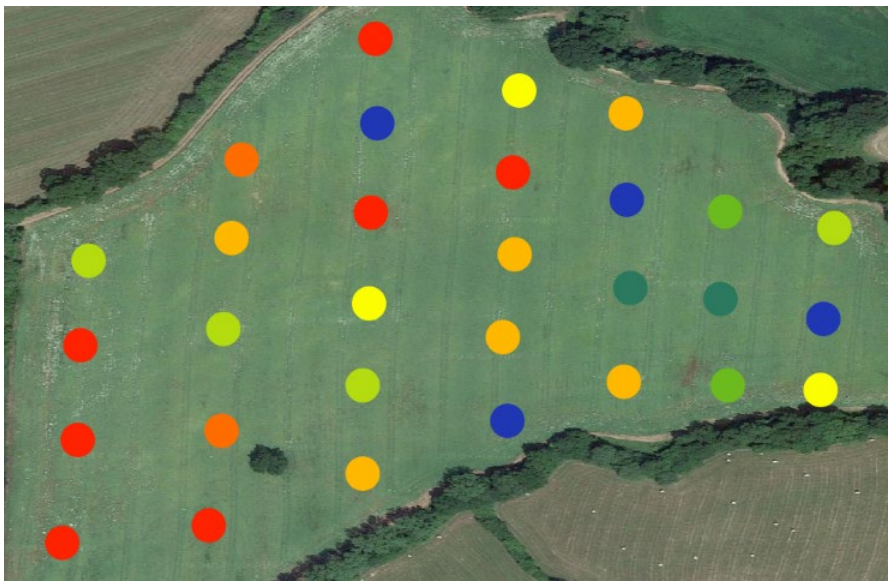


Clubroot severity index, Winter

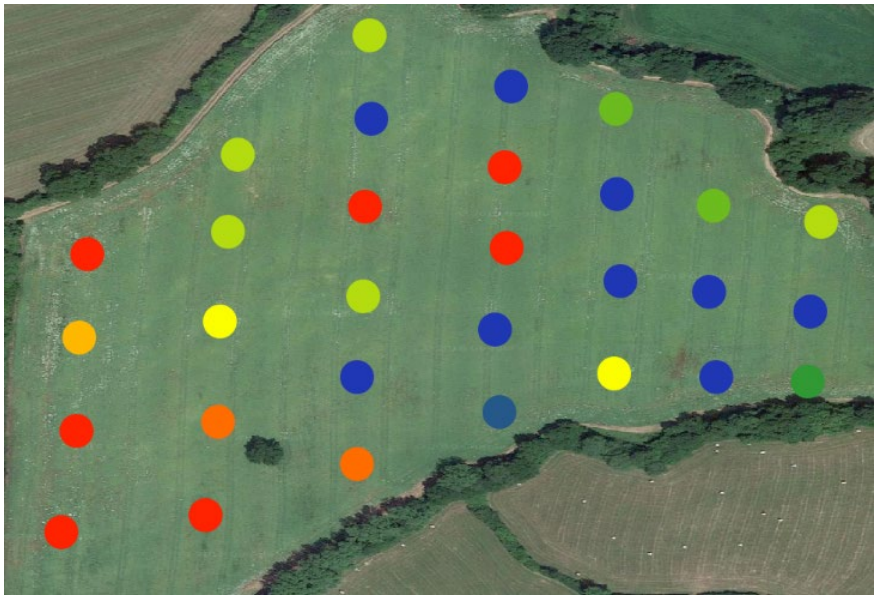


Clubroot severity index, Summer

Figure 15. Intense field monitoring, Clubroot severity index over time, Field: Pollys Field, 2017.

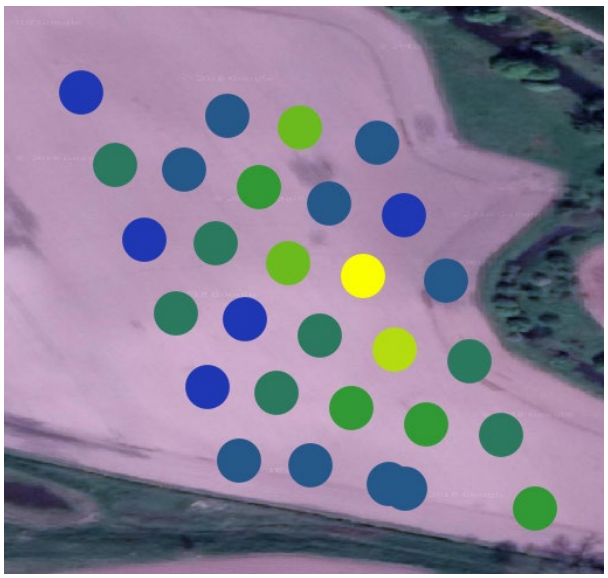


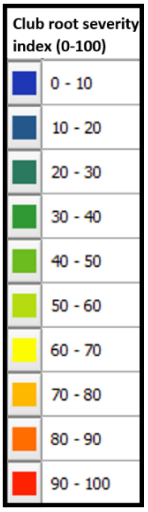
Clubroot severity index, Winter



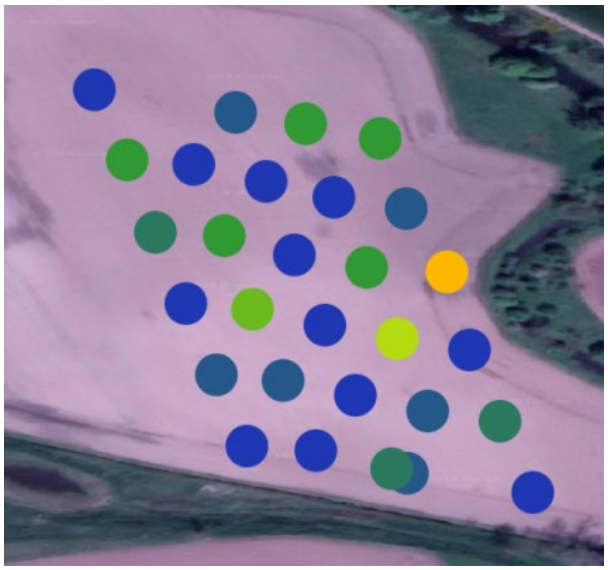
Clubroot severity index, Summer

Figure 16. Intense field monitoring, Club root severity index over time, Field: Penecombe Field, 2017.





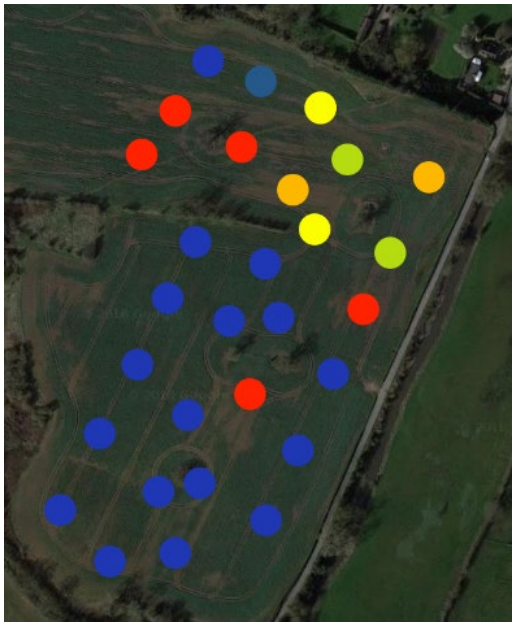
Club root severity index, Spring



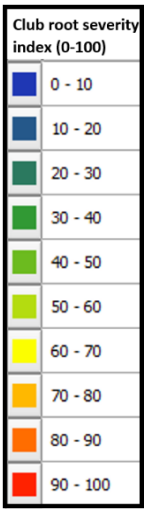
Club root severity index, Summer

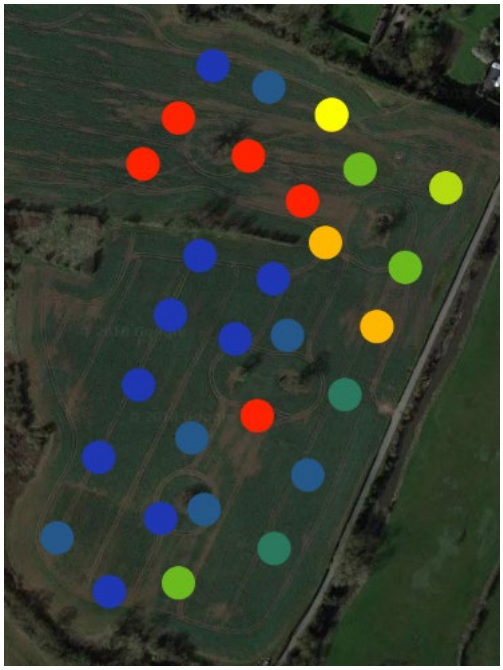
Figure 17. Intense field monitoring, Club root severity index over time, Field: Boysack Field, 2017.

In field clubroot results 2018

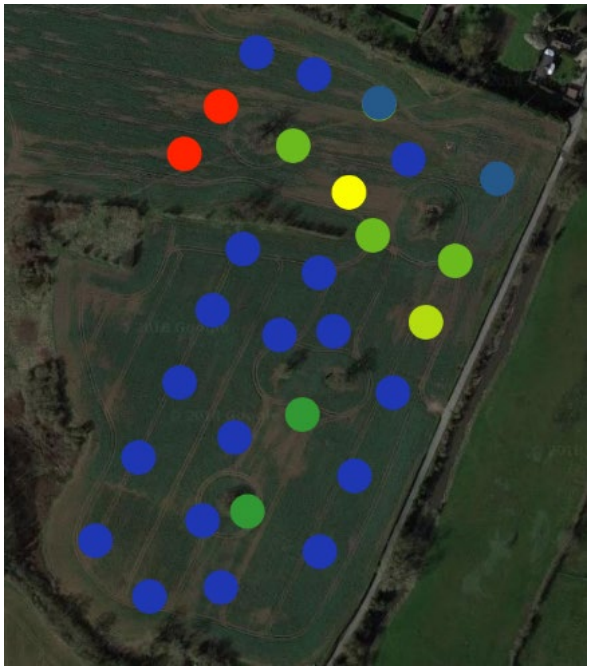


Clubroot severity index, Winter



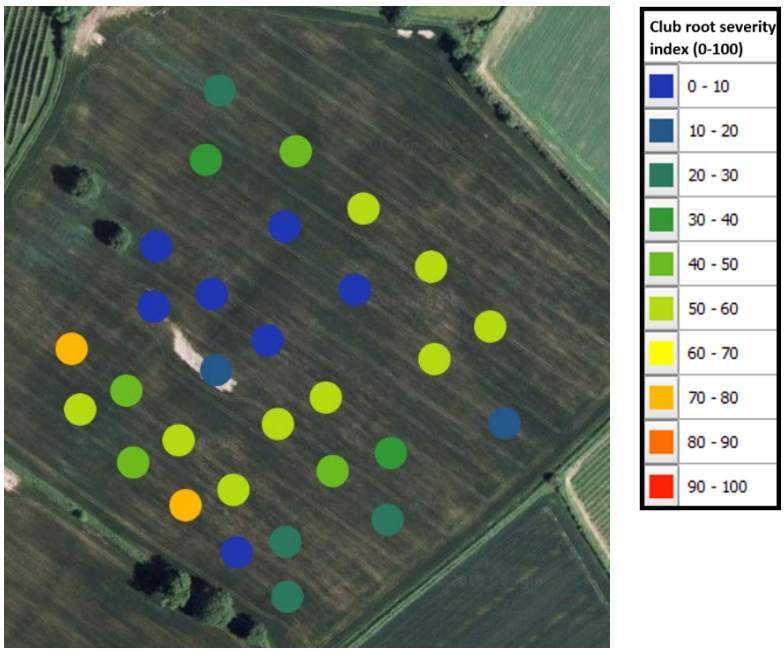


Clubroot severity index, Spring

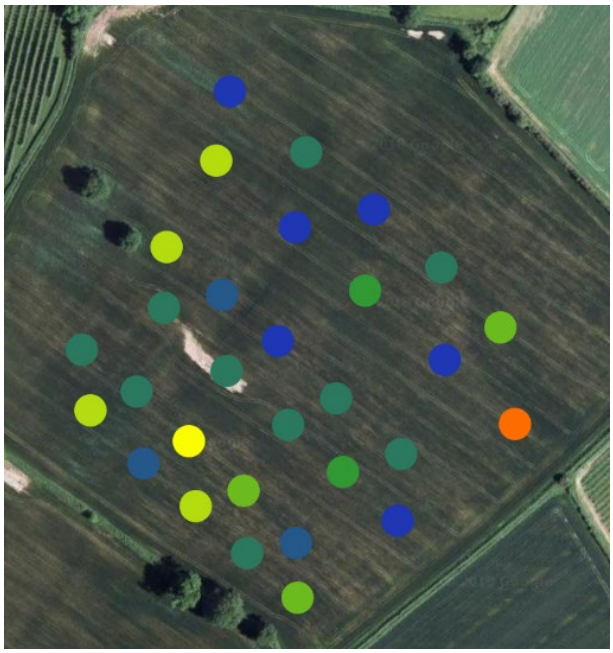


Club root severity index, Summer

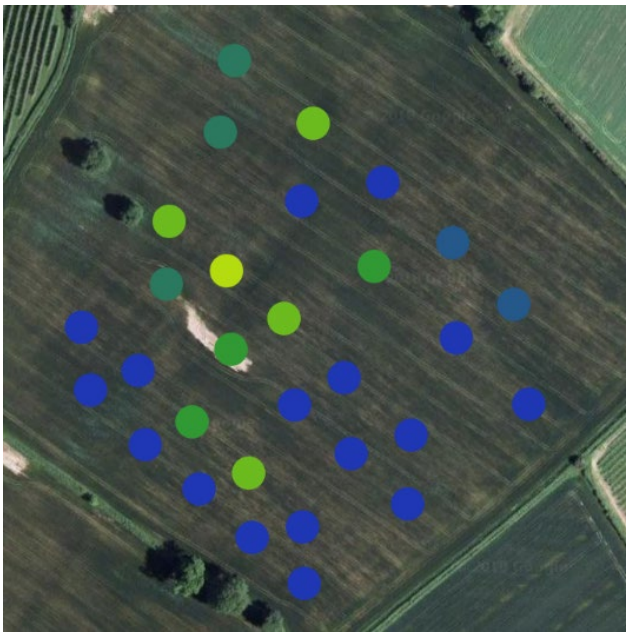
Figure 18. Intense field monitoring, Club root severity index over time, Field: Congrieve, Staffordshire, 2018.



Clubroot severity index, Winter

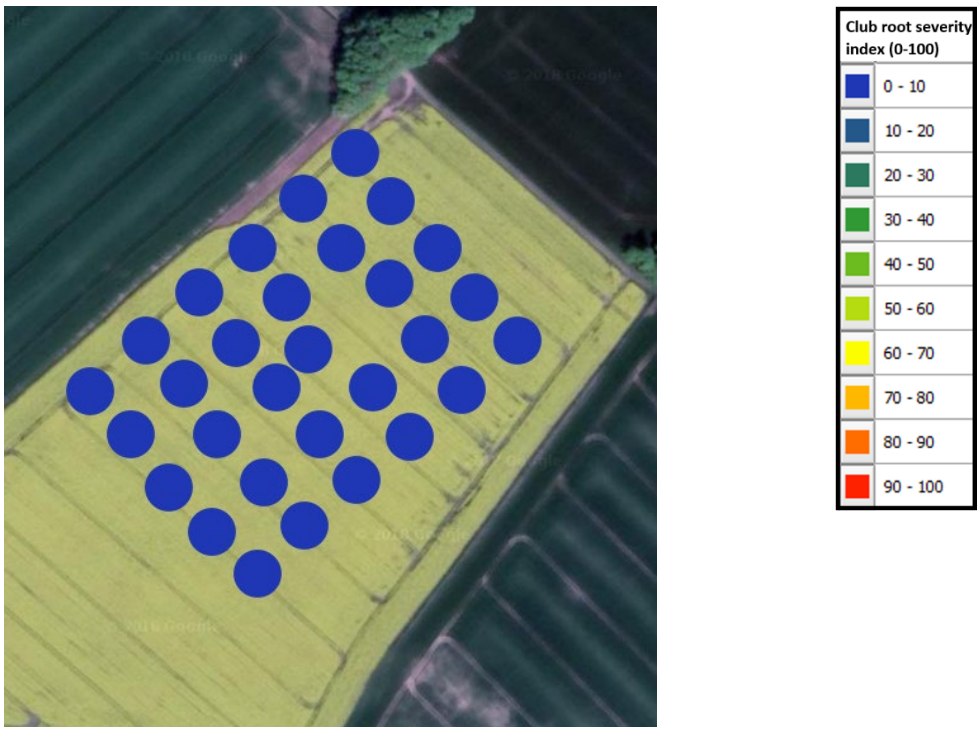


Clubroot severity index, Spring

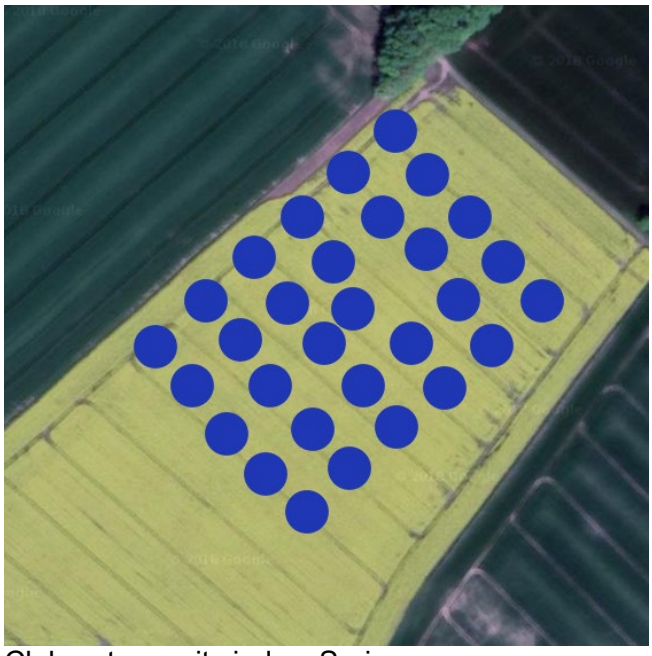


Clubroot severity index, Summer

Figure 19. Intense field monitoring, Club root severity index over time, Field: Murder, Herefordshire, 2018.

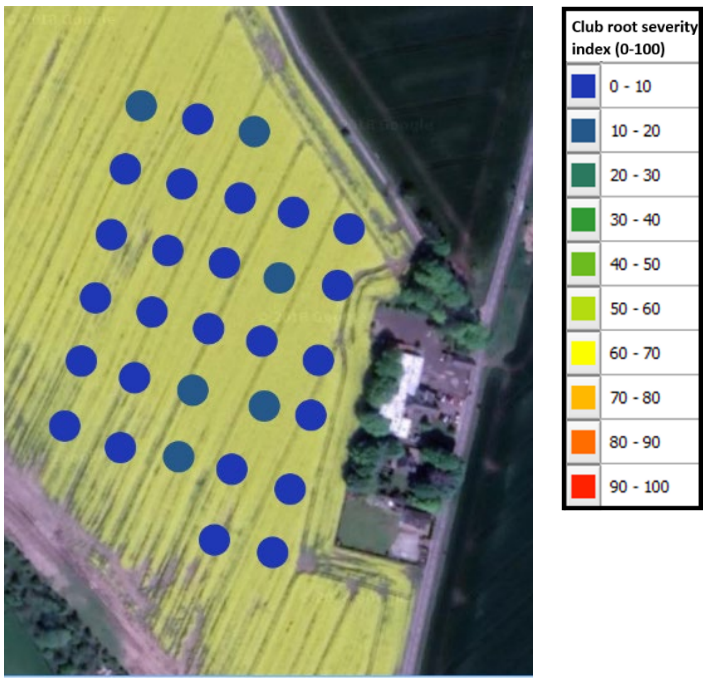


Clubroot severity index, Winter



Clubroot severity index, Spring

Figure 20. Intense field monitoring, Clubroot severity index over time, Field: Brae Face West, Arbroath, 2018.



Clubroot severity index, Spring

Figure 21. Intense field monitoring, Clubroot severity index over time, Field: Slade 50, Arbroath, 2018.

The effect of soil pH on disease severity was assessed for all the commercial fields. Soil pH was measured for every sampling point in each field and compared to the disease severity obtained by *in situ* visual assessment. Overall no correlation was found between clubroot severity and soil pH, yet in few fields such as Pencombe and to a lesser extend Staffordshire, clubroot severity decreased with increased pH (Figure 22. Distribution of clubroot severity depending on soil pH. Each dot corresponds to one of 30 sample points taken across the field. Data shows the distribution of clubroot severity in eight different commercial fields.). In the Boysack and Whinney Hill field, soil pH did not appear to have an influence on disease severity whereas for Little Dilwyn clubroot severity seems to increase slightly with increasing pH. The range of pH measured in the Murder and Congrieve fields was very narrow and no clear correlation was noted in these fields. It appears that increasing pH may not always result in lower clubroot incidence in certain fields, which is consistent with previous findings showing the inconsistent results from soil treatments. The variability of responses observed may be due to varying soil types in the different fields sampled, and the varying levels of clubroot infection pressure between fields and within fields.

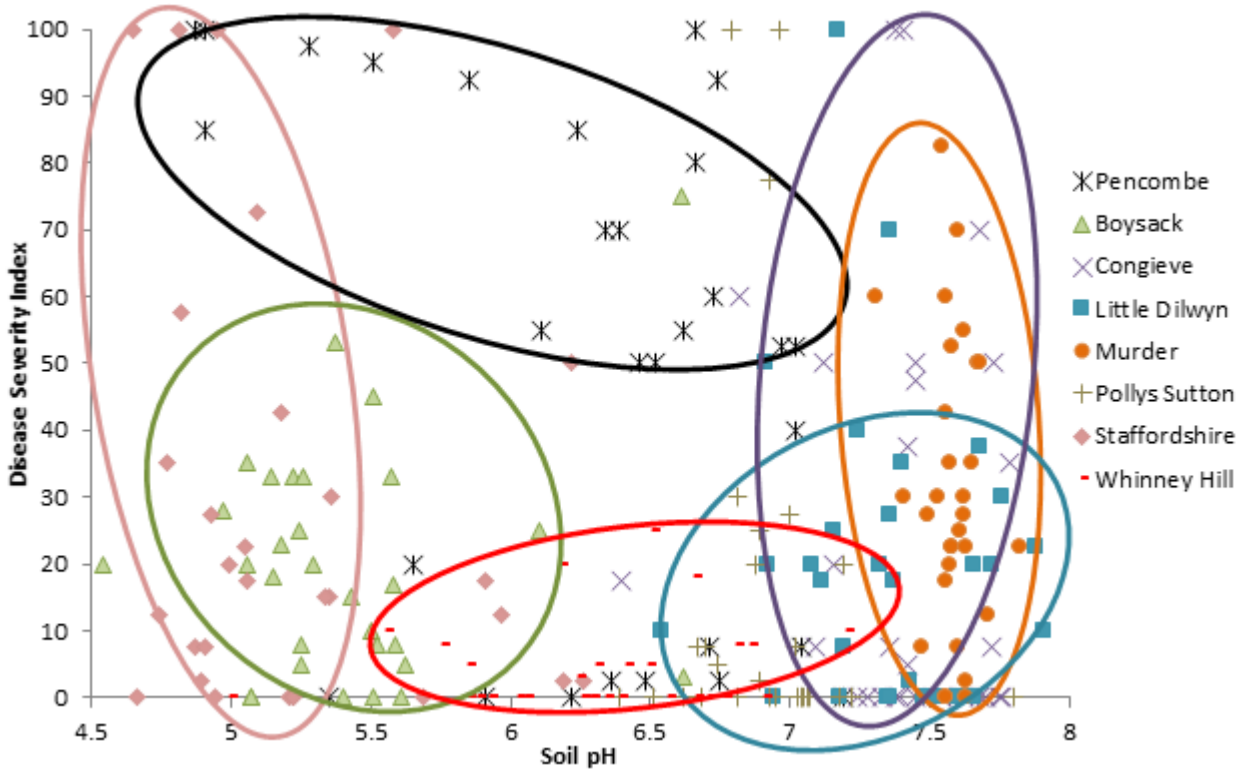


Figure 22. Distribution of clubroot severity depending on soil pH. Each dot corresponds to one of 30 sample points taken across the field. Data shows the distribution of clubroot severity in eight different commercial fields.

4.2.2. Molecular test results

The results given by the visual assessment carried out *in situ* were not in agreement with those given by the molecular-based detection of clubroot in the soil. No significant correlation or trend between the results given by the two techniques was found. In several occurrences, the visual assessment returned a negative result whereas the molecular tests were positive (Figure 23. Frequency of occurrence of positive and negative molecular test in each category of clubroot disease severity). The molecular-based technique is highly sensitive and could have detected very low quantities of DNA that would not necessarily result in disease development. By contrast, in several cases heavily infected roots were observed in the field, yet the presence of *P. brassicae* DNA was not detected by qPCR. (Figure 23. Frequency of occurrence of positive and negative molecular test in each category of clubroot disease severity). The main limitation of the molecular-based technique resides in the low amount of soil used for DNA extraction as only 0.5 g of soil is needed in the method tested here. Considering the well-known sporadic nature of the disease, the presence of *P. brassicae* DNA in the soil is expected to be extremely localised, therefore despite hand-mixing the risk of collecting soil that does not contain *P. brassicae* DNA is relatively high. Increasing the amount of soil used for DNA extraction may help improve the reliability of this method; however it increases the risk of extracting potential PCR reaction inhibitors such as humic acid and these inhibitors may be why there are a high proportion of false negatives.

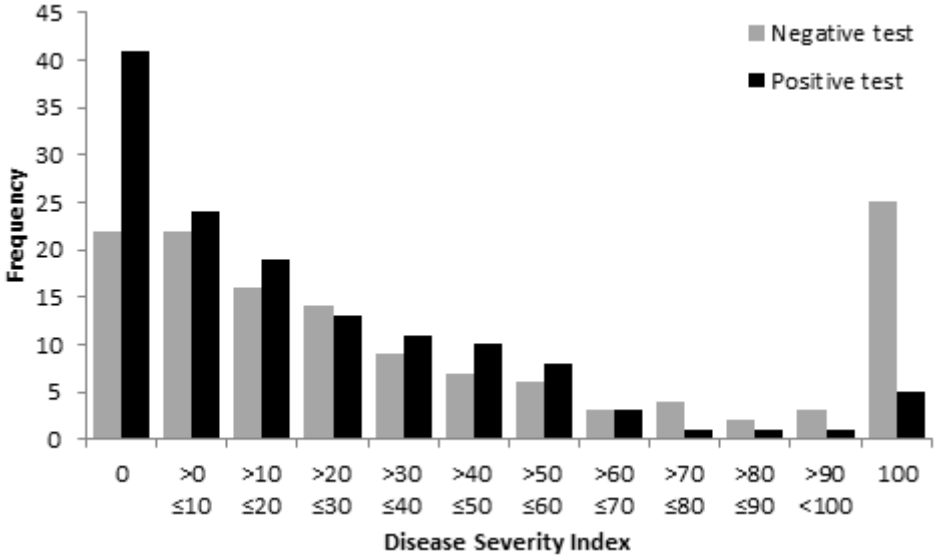


Figure 23. Frequency of occurrence of positive and negative molecular test in each category of clubroot disease severity.

4.2.3. Bioassay results

The results of the bioassay partially correlated with the in-field visual assessment. Despite exhibiting different levels of disease severity, the bioassay appears in most cases to detect the disease when the *in-situ* assessment had detected it (Figure 24). A fuller set of bioassay and in-field crop assessment charts is shown in Appendix 7.2.4. The link between bioassay results were a good predictor of crop disease levels in several fields – for example Market field (Figure 53; Appendix 7.2.4) and Little Dilwyn (Figure 55; Appendix 7.2.4). However, some variability was also observed across the different fields as in several occurrences the bioassay did not show any sign of infection whereas the field observation exhibited a moderate disease level (Figure 25).

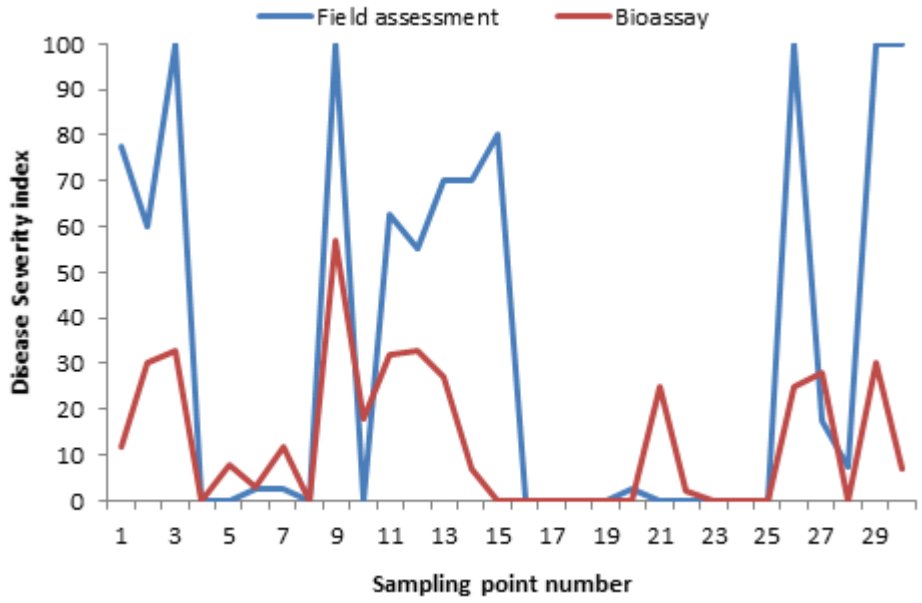


Figure 24. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Congrieve field, Staffordshire in 2018.

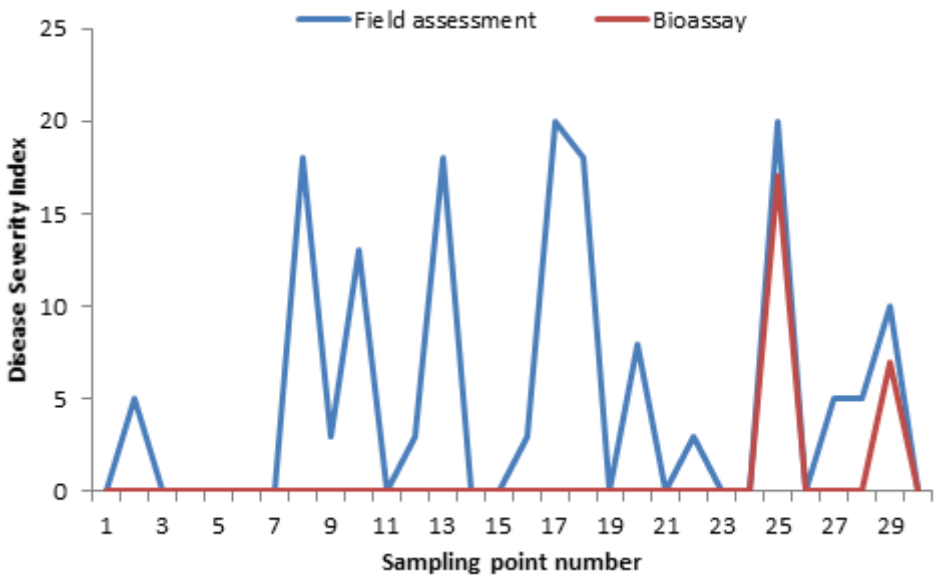


Figure 25. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Slade50 field, Angus in 2018.

4.2.4. Economic analysis of the cost efficacy of treatment through field mapping

Analysis showed that whole field approaches to treatment tended to give lower economic responses to treatment compared to the potential for targeted approaches, where only clubroot infested patches are treated (Table 4). The breakeven point for treatment using this analysis was 32% clubroot severity, which was the cut-off used to determine the cost of treating only patches with this approximate level of infection. Economic margin maps are shown in Appendix 7.2.1, Figures 26-41.

Table 4. Margin over treatment costs for whole field lime application compared to treatment applied to areas mapped as having an economically damaging level of clubroot as assessed in Feb-Apr (cost of liming treatment £80 / ha (@ £20/t spread), treatment efficacy 25% and price of rape £335/t).

Site no	Field name	Year	Margin over treatment cost per hectare if whole field treated (£/ha)	Margin over treatment cost per hectare if field mapping used to identify areas >32% clubroot (£/ha)
1	Warwick	2016	47.01	51.03
2	Bridgenorth	2016	142.06	142.36
3	Market	2016	-7.65	29.7
4	Podge	2016	-64.60	52.1
5	Ivington	2016	-44.98	3.94
6	Staffs	2016	-38.48	23.15
7	Staffs	2017	-7.03	31.15
8	Pencombe	2017	47.91	71.01
9	Sutton	2017	-35.55	21.00
10	Little Dilwyn	2017	-29.46	11.88
11	Boysack	2017	-56.23	0
12	Whinney Hill	2017	-56.23	0
13	Congieve	2018	19.18	50.05
14	Murder	2018	-2.84	20.36
15[1]	Slade50	2018	-67.56	0
16	Brae Face West	2018	78.49	0

[1]This field was ploughed in due to poor establishment – field mapping in February before the crop was ploughed in showed that there were no areas with clubroot severity over the threshold severity of 32% so on this criterion no areas would have been treated.

5. Discussion

The results from this project have demonstrated the unexpected diversity of clubroot strains within the UK, which may reflect the long and varied history of susceptible brassica growing and the diversity of wild plant flora that are susceptible. This variability may represent an enhanced risk of rapid adaptation in infected fields. The frequency in occurrence of resistance-breaking strains of clubroot found in this project and the wide distribution over the UK was an important finding, with significant implications in terms of clubroot management. Previous work on clubroot in the UK (McGrann et al, 2015) identified varietal resistance as the most consistently effective method of management so it was inevitable it would be heavily used in infected fields. The wide-spread occurrence of Mendel-breaking clubroot strains illustrates the need for an enhanced range of control options and more sustainable methods of management to be developed.

Knowledge of the UK clubroot population, and the common strains within it, is important intelligence to breeders in identifying and screening for effective, novel sources of genetic resistance. The diversity of strains identified in the UK presents something of a challenge to breeders in developing novel host-resistance mechanisms effective against all strains and so the development of other physiological traits in breeding programmes that might preserve yield in the presence of disease is likely to be of enhanced importance.

The results from the project highlight the vulnerability of relying on a single resistance mechanism to manage clubroot and a focus of following research should be on the identification of novel resistance sources and their rapid incorporation into breeding programmes. Although single club isolates of clubroot obtained in this project have been made available to breeders through the OREGIN project (Defra funded), an expanded number of reference isolates and a greater understanding of the key strains to target will assist in tailoring breeding programmes to UK needs. The exploitation of complimentary yield maintaining traits should also be incorporated into breeding programmes.

The project has also highlighted the need for control measures, of whatever sort, to be sustainable over the longer term. There are large gaps in knowledge around the persistence of clubroot in UK soils and the relative impact of rotational choices and management interventions on inoculum levels. This knowledge, combined with economic modelling, would allow for the development of decision aids to assist growers in planning the most profitable and sustainable crop and management choices over the longer term.

Regular testing of fields for clubroot, and more detailed field mapping, will be an effective tool for growers in making informed rotational and management choices; however, current bioassay and qPCR methods are still somewhat compromised either for reasons of expense (both types of testing) or reliability as molecular test results do not always correlate well with the levels of clubroot that

develop in a field. Cheaper testing methods that made field mapping and multiple tests per field affordable would potentially overcome some of the limitations of testing single bulked samples per field, where for a patchy disease with relatively large sporeballs it is almost inevitable that the very small soil weights used in tests will either hit a 'spike' of inoculum or miss it altogether. Clearly, tests that are more accurate should also be a key aim for future research, as should be the development of extraction and testing methods which remain reliable over different soil types and which differentiate viable from non-viable inoculum.

The patchy nature of clubroot within fields was identified in the mapping of commercial fields, undertaken in this project. These patches tended to be fairly stable and although spread through cultivations is likely over time, knowledge of the location and intensity of clubroot patches within fields is likely to be informative to growers in subsequent crops, given the long-term persistence of the problem (up to 20 years, Dixon, 2009) with a half-life (in Swedish soils) of 3.7 years (Wallenhammar, 1996). At a basic level, the identification of patches allows for hygiene measures to be taken around them when moving kit and the potential for targeted treatment options is an additional benefit. These measures could be as simple as avoiding drilling infested patches when a susceptible crop is planted so as to avoid multiplying the inoculum within the patch. This approach for fields with only few confirmed patches, sometimes around gateways, might preserve the efficacy of Mendel-resistant varieties for deployment as a tool further down the rotation and would counter the risk that early deployment in a lightly infected field erodes the efficacy of this tool in future deployments where it is more needed.

Other targeted treatment options could include spot liming. Although a relatively cheap option (as shown by the economic analysis project) liming is only partially effective and liming whole fields to high pHs may not be suitable for other crops in the rotation. Calcium cynanamide is another soil amendment, previously tested (Burnett et al, 2013) which offers some partial control. It gave difficulties in conforming to NVZ regulations when applied over whole fields because at effective rates nitrogen application levels to fields were exceeded, so again spot treatment to identified patches would overcome this restriction. Spot drilling of resistant varieties would be another feasible option if growers are technically equipped and might allow for higher yielding non-resistant varieties to be drilled in the surrounding field. Provided varieties selected have similar maturity dates, mixed seed lots at harvest do not present an issue to buyers who bulk different varieties for crushing; although this assertion should of course be explored at an individual level with merchants before enacting. Other potential novel treatments, such as the use of elicitors, seed treatments or biological controls are likely to come at enhanced costs so again the ability to target them would be desirable.

Mapping fields is also a key tool in long term decision making for fields. As stated before, the development of decision support models that capture the patchy nature of the problem in the decision-making process is vital in assisting growers to make the optimal long-term decisions for a field, from both an economic and sustainability perspective.

The key advisory messages for growers arising from this project are: -

- Keep accurate crop records of clubroot occurrence, location and intensity.
- Note where varietal resistance has been deployed in fields to aid long term planning and help prevent spread.
- Where resistant varieties are used, monitor the crop carefully and assess the levels of clubroot present. If levels of infection start to increase, change strategy.
- Increase the frequency and detail of testing at sites with higher frequencies of susceptible crops in a rotation. Mapping fields will identify hot spots and help management planning.
- Buying certified seed ensures that susceptible plant numbers are minimised in a resistant variety seed batch. Do not home save resistant varieties.
- Manage volunteers and susceptible weeds within and between oilseed rape crops. Allowing weed growth will allow clubroot populations to bulk up so manage weeds as early as possible in the season.
- Be mindful of other susceptible crop choices when planning rotations – spring rape is susceptible and cover crop mixes often contain susceptible species.
- Long term planning should be based on the long-term profitability and sustainability of a field and not on a single season’s predicted margin.

6. References

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7. Appendices

7.1. European differential set

Table 5. Club root bioassay results for the Chinese cabbage, +/- Mendel resistance bioassay tests, individual field results.

Sample year	Region	Chinese cabbage	+ Mendel resistance	- Mendel resistance	Proportion of club root population insensitive to Mendel resistance (%)*
		Club root severity index (0-100)			
2016	Herefordshire	44.4	4.2	76.0	5.5
2016	Herefordshire	5.6	0.0	10.7	0.0
2016	Herefordshire	92.9	0.0	97.2	0.0
2016	Herefordshire	28.6	2.7	21.2	12.6
2016	Shropshire	57.1	10.7	98.7	10.8
2016	Shropshire	71.1	5.3	78.7	6.8
2016	Shropshire	26.1	12.0	100.0	12.0
2016	Staffordshire	61.9	25.3	100.0	25.3
2016	Wales	12.7	0.0	2.7	0.0
2016	Wales	47.2	4.2	86.1	4.8
2016	Wales	3.2	2.7	5.6	48.0
2016	Wales	33.3	8.0	86.1	9.3
2016	Wales	0.0	1.3	1.4	92.0
2016	Warwickshire	48.5	4.3	95.8	4.5
2016	Warwickshire	19.3	0.0	12.0	0.0
2016	Warwickshire	80.0	8.3	82.7	10.1
2016	Warwickshire	55.6	5.3	89.3	6.0
2016	Worcestershire	3.3	0.0	0.0	*
2016	Worcestershire	56.4	14.7	82.7	17.7
2016	Worcestershire	0.0	2.8	0.0	*
2017	Herefordshire	93.3	32.0	97.3	32.9
2017	Herefordshire	97.3	49.3	100.0	49.3
2017	Herefordshire	73.3	5.3	66.7	8.0
2017	Herefordshire	90.7	21.3	85.3	25.0
2017	Herefordshire	74.7	12.0	98.7	12.2
2017	Herefordshire	100.0	8.0	92.0	8.7
2017	Herefordshire	74.7	12.0	98.7	12.2
2017	Shropshire	30.7	12.0	18.7	64.3
2017	Shropshire	14.7	8.0	8.0	100.0
2017	Shropshire	86.7	9.3	44.0	21.2
2017	Shropshire	45.8	2.7	17.3	15.4
2017	Shropshire	56.0	6.7	33.3	20.0
2017	Shropshire	38.7	0.0	40.0	0.0
2017	Staffordshire	98.7	13.3	96.0	13.9
2017	Staffordshire	90.7	17.3	97.3	17.8
2017	Staffordshire	90.7	29.3	89.3	32.8
2017	Wales	79.2	25.6	63.3	40.5
2017	Wales	96.0	1.3	84.0	1.6

2017	Wales	85.3	17.3	100.0	17.3
2017	Wales	74.7	13.3	80.0	16.7
2017	Wales	81.3	10.7	89.3	11.9
2017	Wales	97.3	10.7	100.0	10.7
2017	Warwickshire	0.0	5.3	6.7	80.0
2017	Warwickshire	8.0	0.0	4.0	0.0
2017	Warwickshire	26.7	4.0	24.0	16.7
2017	Worcestershire	37.3	10.7	44.0	24.2
2017	Worcestershire	80.0	16.0	88.0	18.2
2017	Worcestershire	0.0	0.0	2.7	0.0
2017	Worcestershire	40.0	2.7	36.0	7.4
2017	Worcestershire	95.8	8.0	50.7	15.8
2017	Worcestershire	0.0	0.0	0.0	*
2018	Angus	69.2	22.7	46.4	48.9
2018	Devon	60.8	0.0	42.7	0.0
2018	Devon	53.3	5.3	46.7	11.4
2018	Devon	30.7	2.7	29.3	9.1
2018	Devon	73.3	9.5	71.7	13.3
2018	Dumfries & Galloway	0.0	*	0.0	*
2018	Fife	36.0	0.0	37.3	0.0
2018	Inverness	60.0	4.0	94.7	4.2
2018	Lancashire	45.2	0.0	42.6	0.0
2018	Lancashire	62.2	0.0	21.6	0.0
2018	Lancashire	53.3	10.0	100.0	10.0
2018	Lancashire	22.2	6.3	12.8	48.8
2018	Lancashire	46.7	0.0	1.3	0.0
2018	Lancashire	53.7	8.0	97.3	8.2
2018	Lancashire	74.5	5.3	71.4	7.5
2018	Lancashire	2.7	0.0	0.0	*
2018	Roxburghshire	42.1	0.0	44.9	0.0
2018	Warwickshire	8.7	2.7	0.0	*
2018	Warwickshire	83.3	8.0	70.6	11.3
2018	Yorkshire	52.1	4.0	31.4	12.8
2018	Yorkshire	38.1	0.0	10.5	0.0
2018	Yorkshire	0.0	0.0	0.0	*
2018	Yorkshire	0.0	0.0	0.0	*
2018	Yorkshire	17.3	4.2	0.0	*

$* \; {Mentor}/_{Tolkin} \; x \; 100$

Table 6. Results by individual field showing region and ECD population.

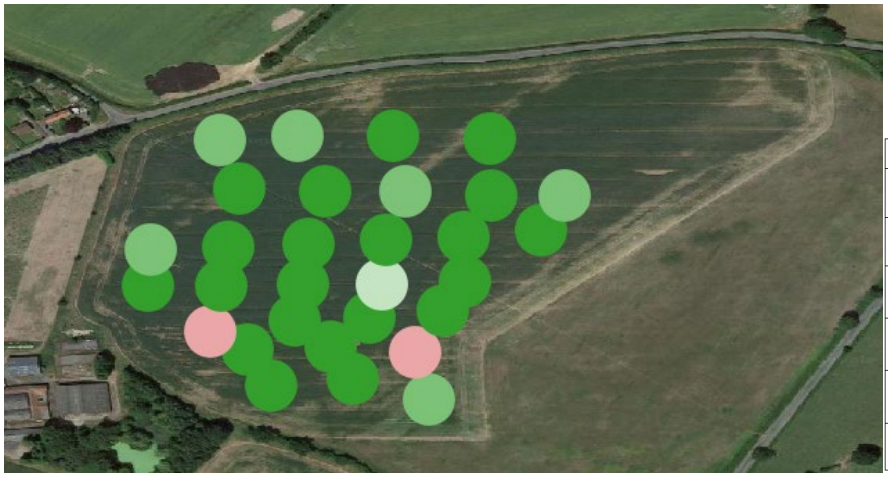
Sample year	Field ID	Region	European Clubroot Differential Set (ECD) population
2016	1	Warwickshire	16/2/14
2016	2	Herefordshire	16/26/0
2016	3	Herefordshire	16/14/30
2016	4	Staffordshire	16/22/15
2016	5	Herefordshire	16/31/15
2016	6	Shropshire	16/23/28
2016	7	Warwickshire	16/14/13
2016	8	Worcestershire	16/7/29
2016	9	Scotland	17/31/12
2017	10	Herefordshire	16/31/31
2017	11	Herefordshire	16/19/12
2017	12	Staffordshire	16/6/31
2017	13	Scotland	16/31/29
2017	14	Wales	16/15/28
2017	15	Staffordshire	16/14/31
2017	16	Herefordshire	16/6/29
2017	17	Wales	16/31/31
2017	18	Worcestershire	No data
2018	19	Yorkshire	16/5/8
2018	20	Devon	16/31/15
2018	21	Lancashire	16/1/14
2018	22	Lancashire	16/1/14
2018	23	Warwickshire	16/1/8
2018	24	Yorkshire	No data
2018	25	Scotland	16/31/7

7.2. Field mapping

7.2.1. Economic yield maps

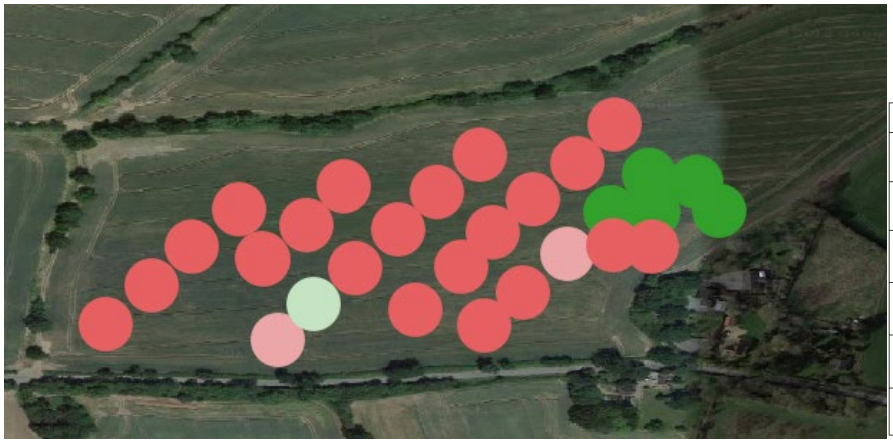
Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 26. Margin over liming cost, Field: Warwick, 2016.



Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 27. Margin over liming cost, Field: Bridgnorth, 2016.

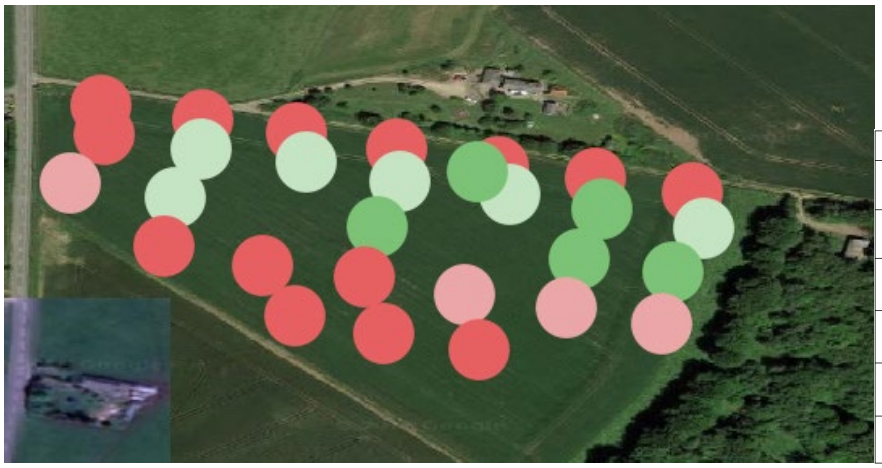


Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 28. Margin over liming cost, Field: Staffordshire, 2016.

Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 29. Margin over liming cost, Field: Ivington, 2016.

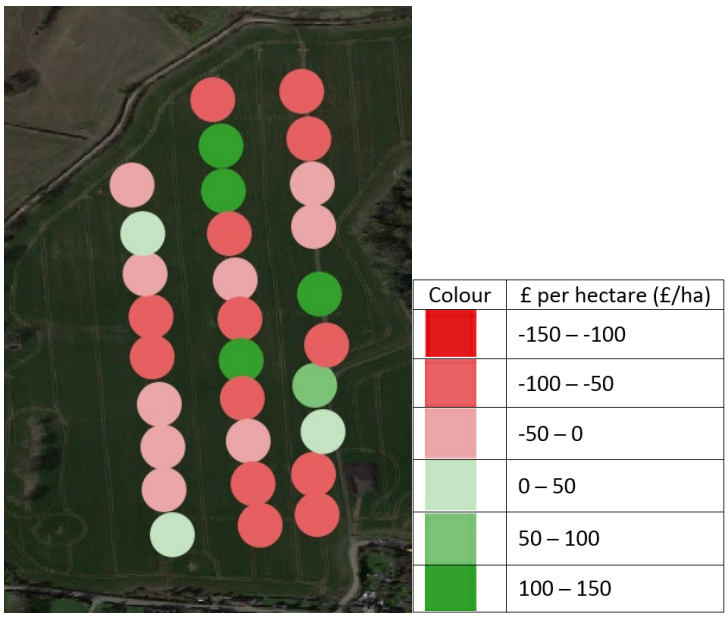


Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 30. Margin over liming cost, Field: Market Field, 2016.

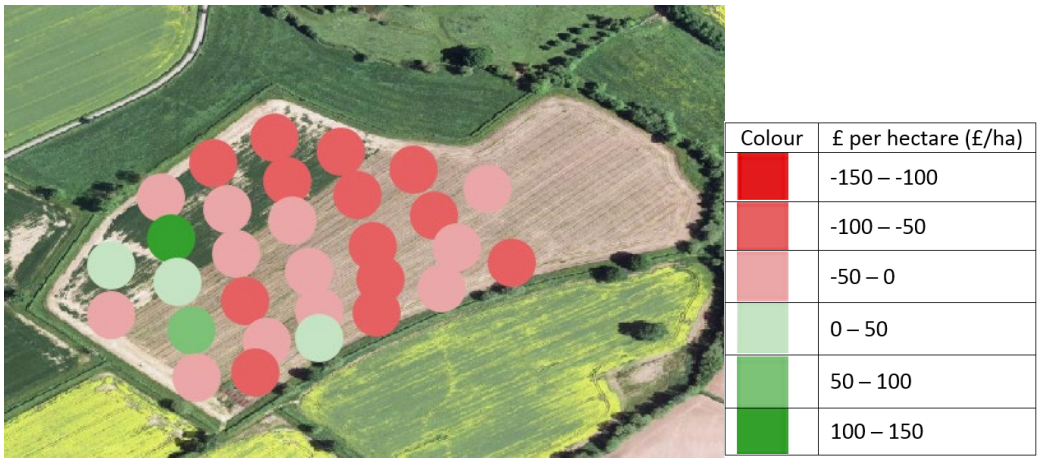
Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 31. Margin over liming cost, Field: Podge field, 2016.



Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 32. Margin over liming cost, Field: Staffordshire, 2017.



Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

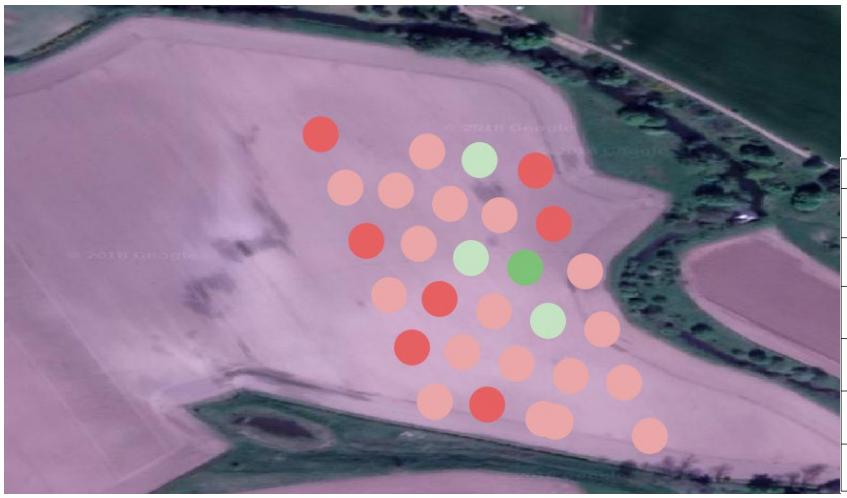
Figure 33. Margin over liming cost, Field: Little Dilwyn, 2017.

Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 34. Margin over liming cost, Field: Polly's field, 2017.

Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 35. Margin over liming cost, Field: Penecombe, 2017.



Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

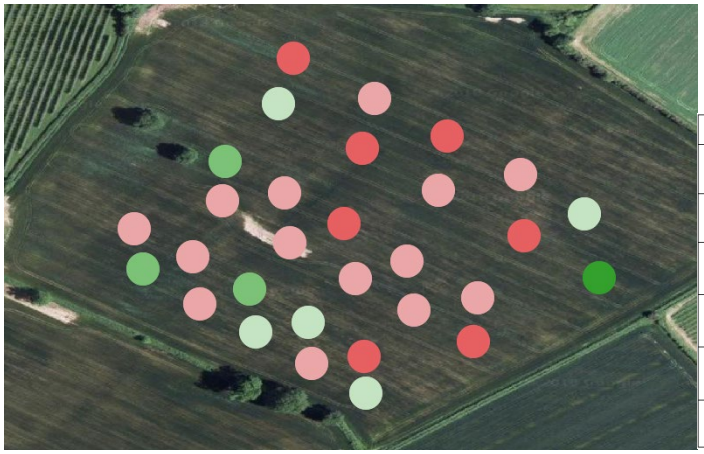
Figure 36. Margin over liming cost, Field: Boysack, Forfar, 2017.

Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 37. Margin over liming cost, Field: Whinney Hill, Earlston, 2017.

Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 38. Margin over liming cost, Field: Congrieve, 2018.

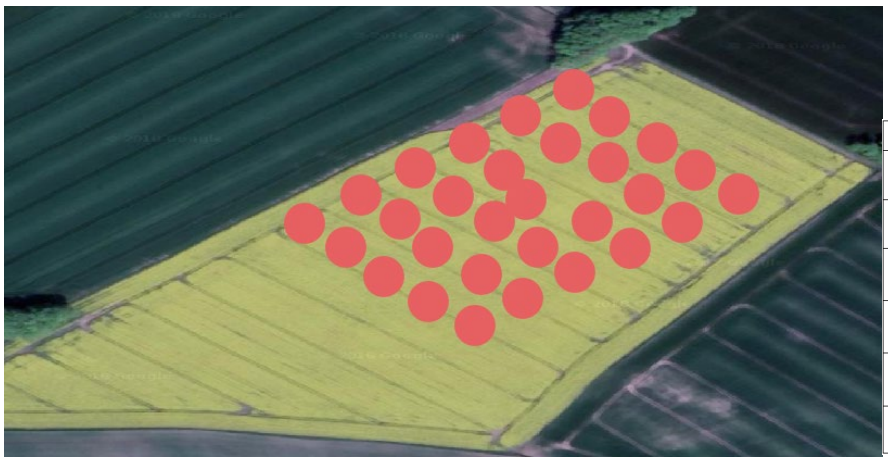


Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 39. Margin over liming cost, Field: Murder, 2018.

Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 40. Margin over liming cost, Field: Slade 50, Arbroath, 2018.



Colour	£ per hectare (£/ha)
	-150 – -100
	-100 – -50
	-50 – 0
	0 – 50
	50 – 100
	100 – 150

Figure 41. Margin over liming cost, Field: Brae Face West, Arbroath, 2018.

7.2.2. NDVI maps

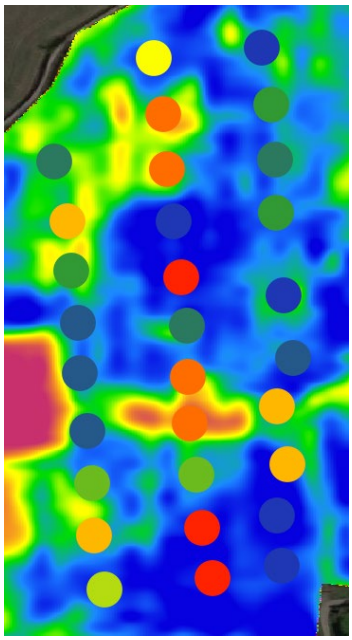


Figure 42. Intense field monitoring, Clubroot severity and NDVI overlay, April assessment. Field: Staffordshire, 2017.

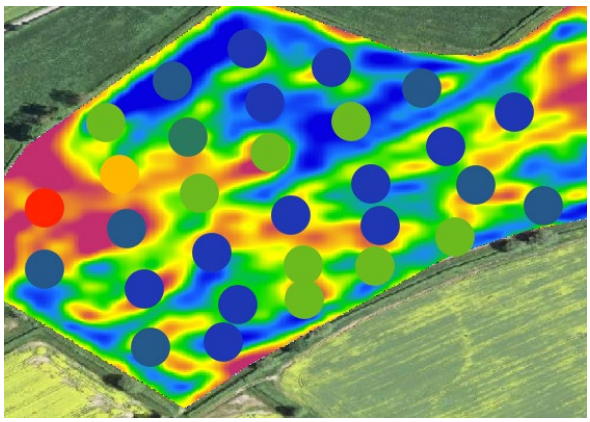


Figure 43. Intense field monitoring, Clubroot severity and NDVI overlay, March assessment. Field: Little Dilwyn, 2017.

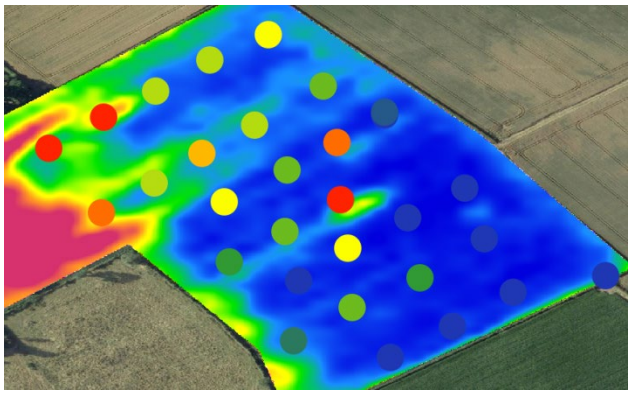


Figure 44. Intense field monitoring, Clubroot severity and NDVI overlay, February assessment. Field: Pollys Field, 2017.

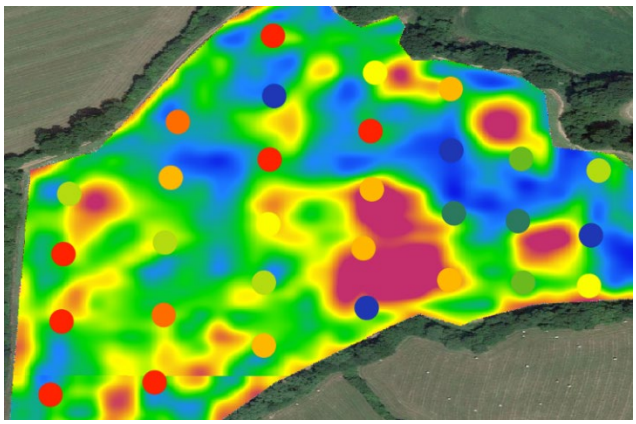


Figure 45. Intense field monitoring, Clubroot severity and NDVI overlay, February assessment. Field: Penecombe Field, 2017.

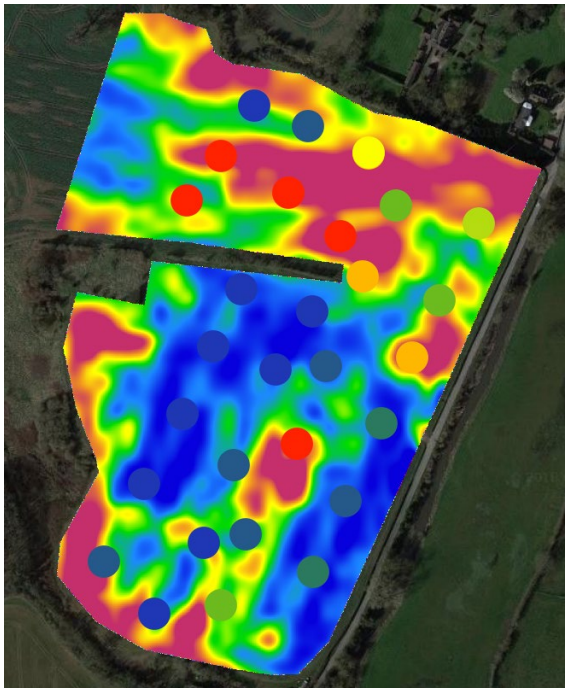


Figure 46. Intense field monitoring, Clubroot severity and NDVI overlay, April assessment. Field: Congrieve field, Staffordshire, 2018.

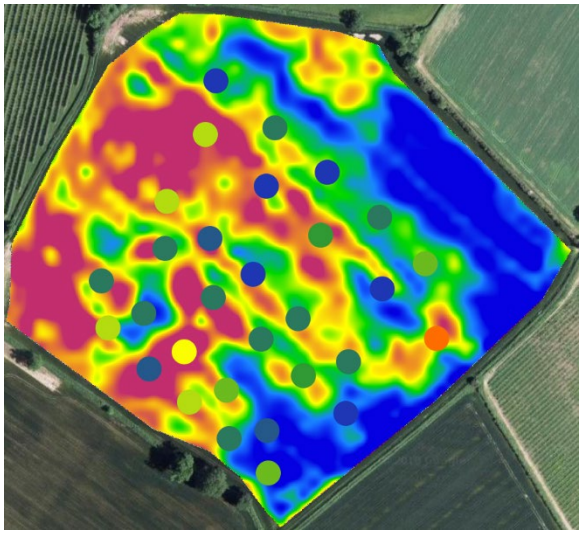


Figure 47. Intense field monitoring, Clubroot severity and NDVI overlay, April assessment. Field: Murder field, Staffordshire, 2018.

7.2.3. Yield maps

Figure 48. Commercial yield map. Field: Staffordshire, 2016.

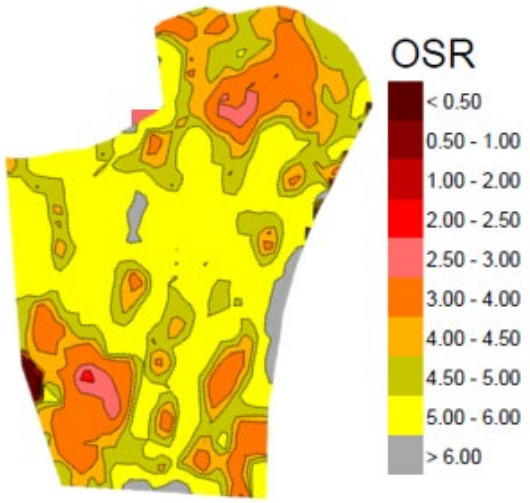


Figure 49. Commercial yield map. Field: Little Dilwyn, Herefordshire, 2017.

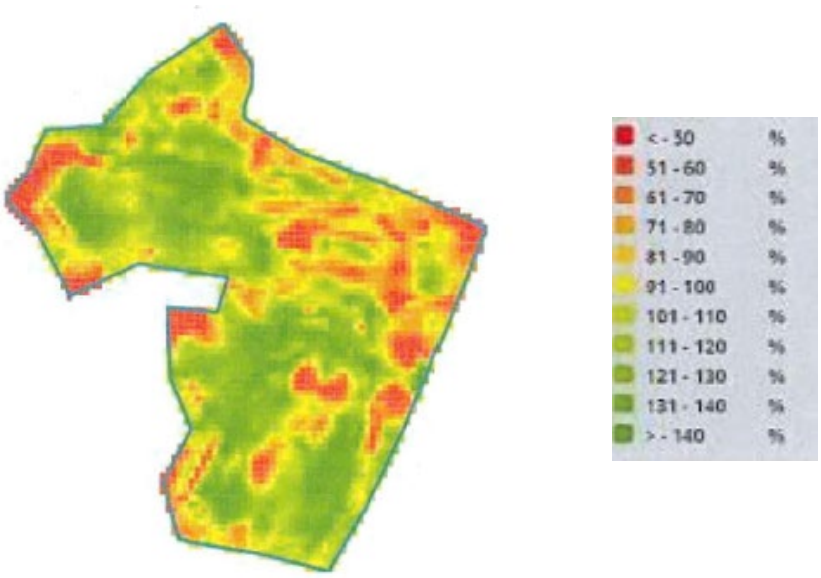


Figure 50. Commercial yield map. Field: Congrieve, Staffordshire, 2018.

7.2.4. Bioassay results

Soil bioassay results have been compared to disease levels in the field crop in the spring at each sampling point in the field, expressed as % severity using the indices described in section 3.1.3 and 3.2.1 respectively.

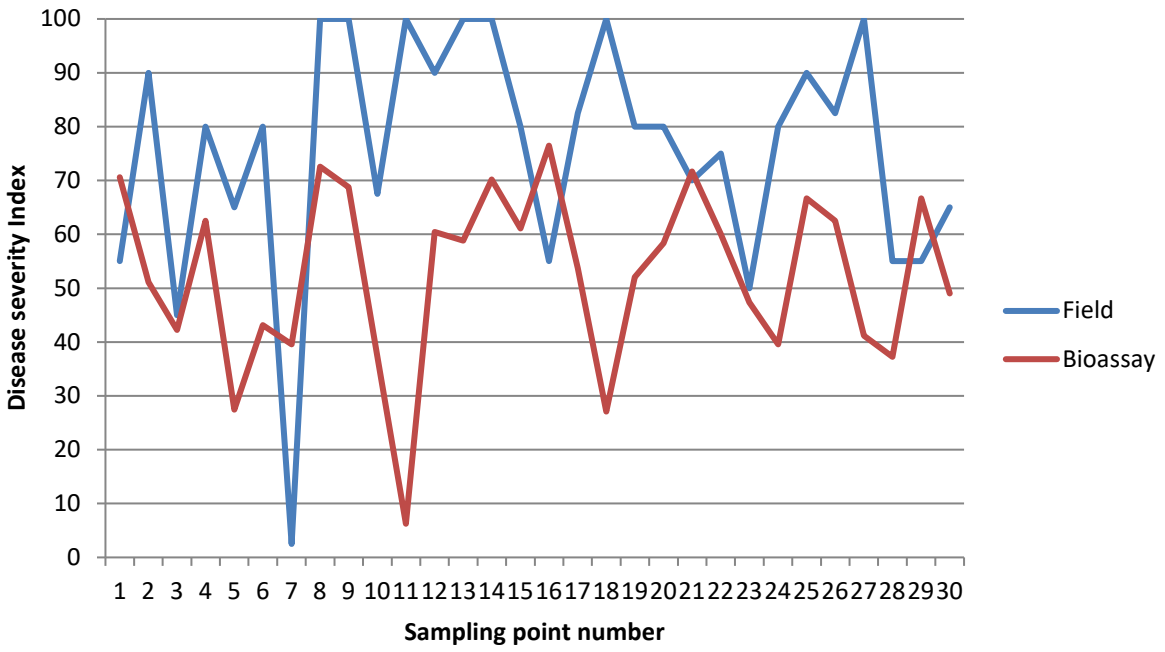


Figure 51. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Bridgenorth field, 2016.

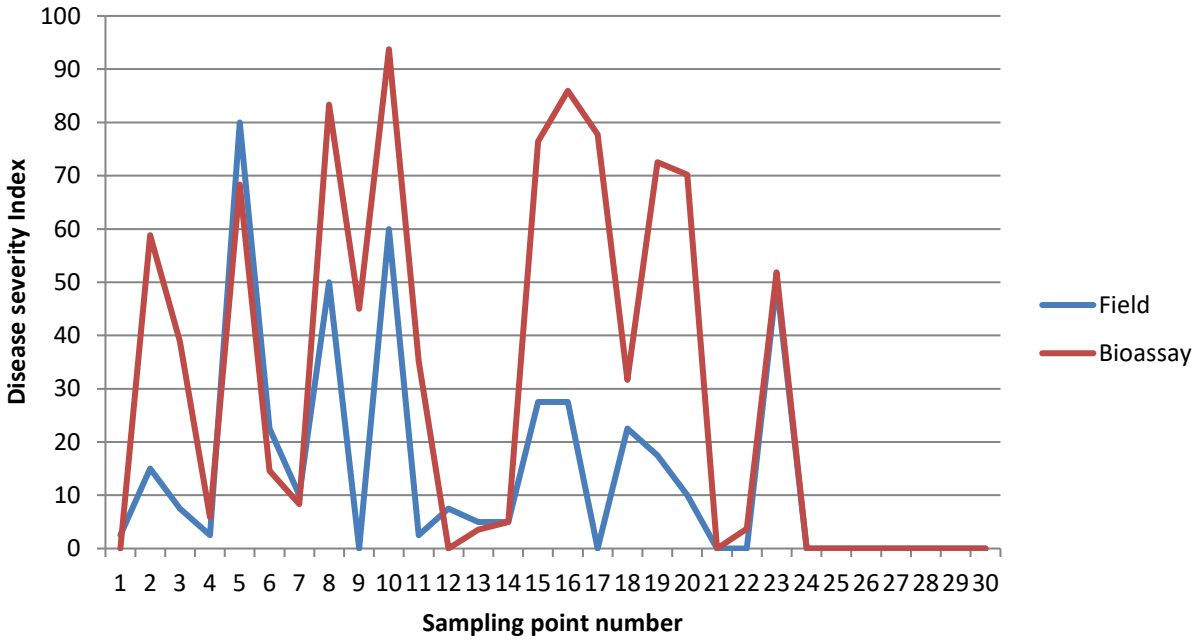


Figure 52. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Ivington field, 2016.

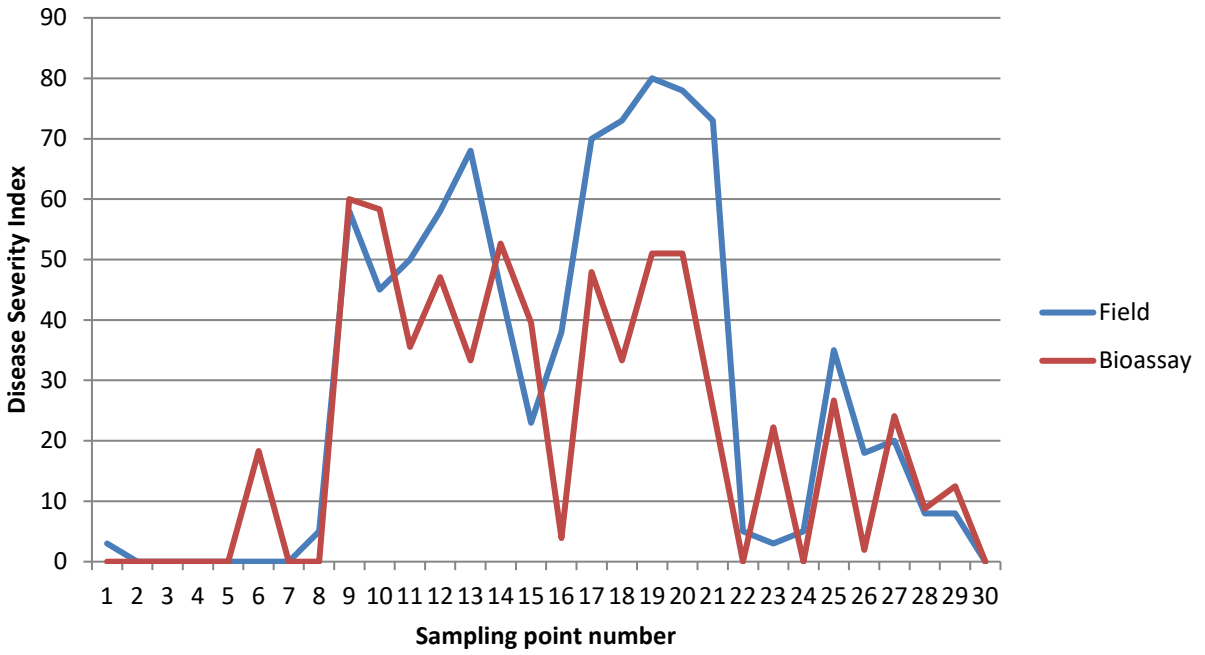


Figure 53. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Market field, 2016.

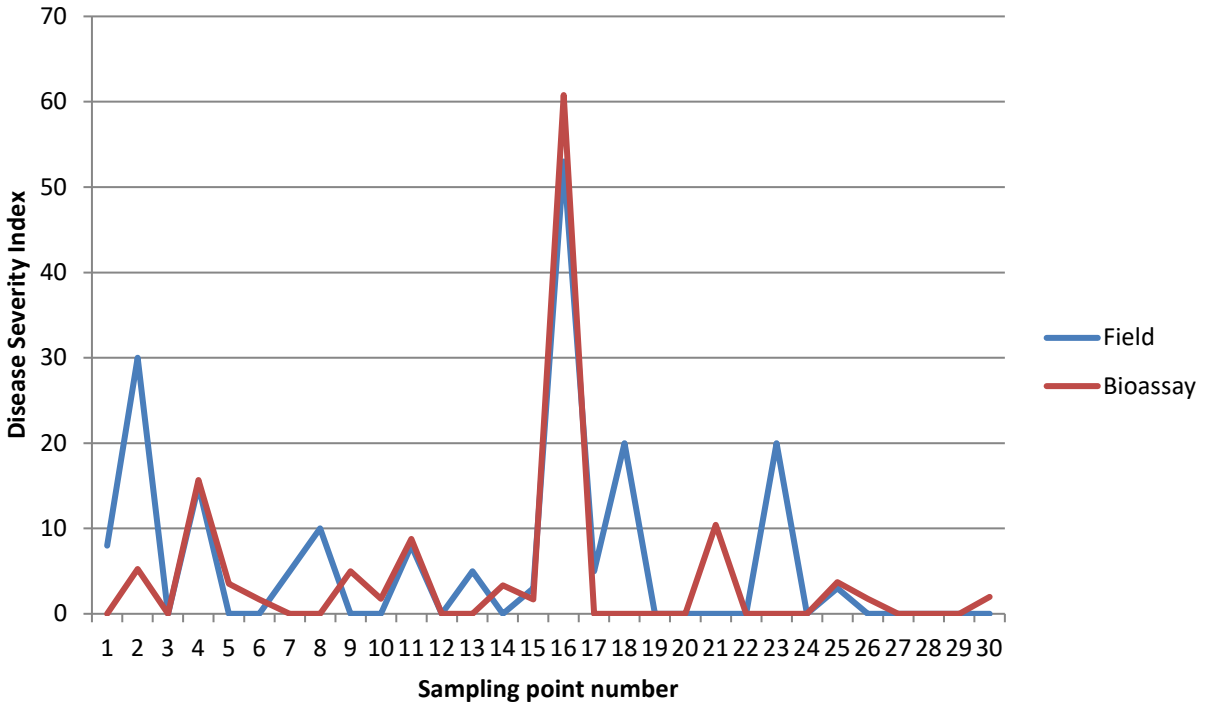


Figure 54. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Podge field, 2016.

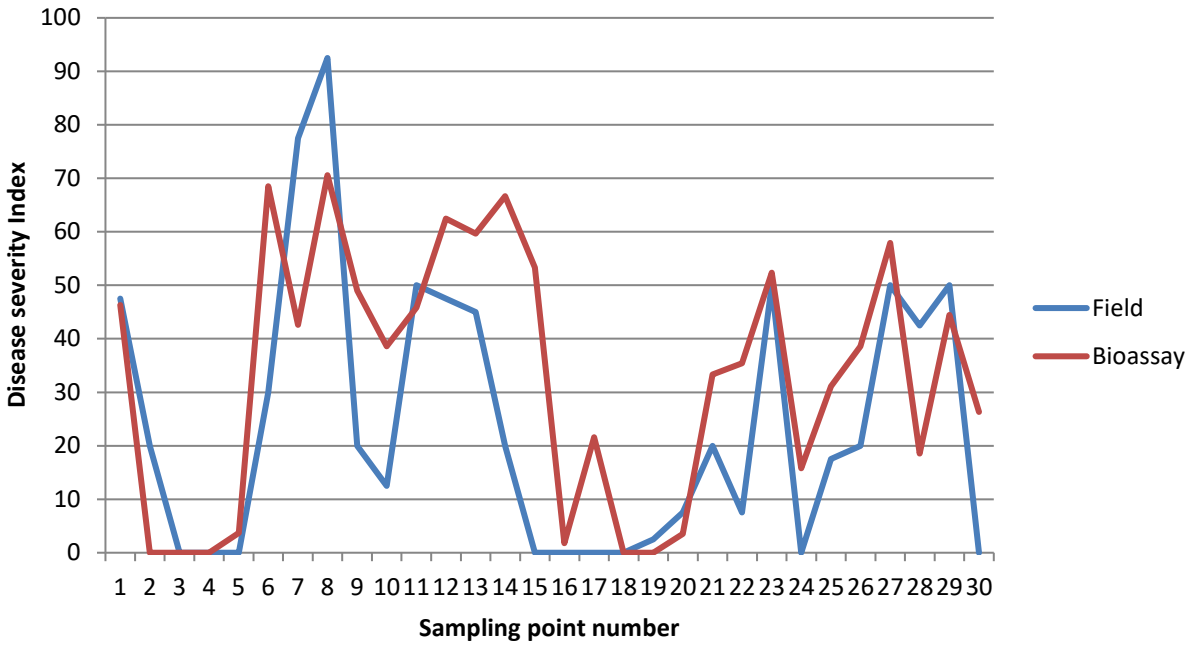


Figure 55. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Little Dilwyn field, 2017.

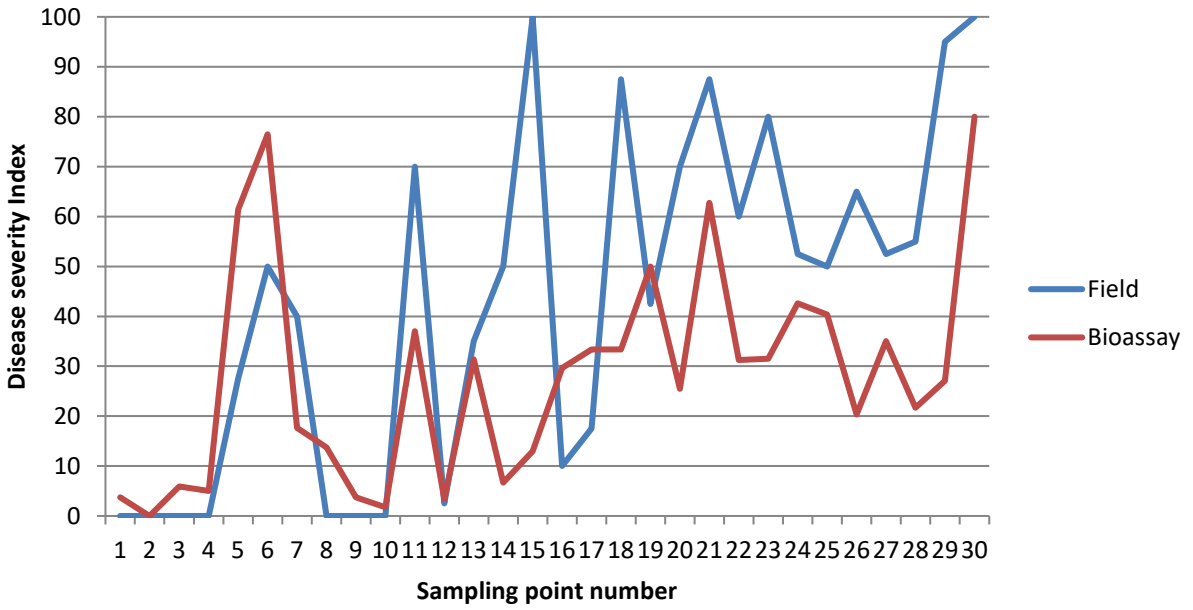


Figure 56. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Polly's Sutton field, 2017.

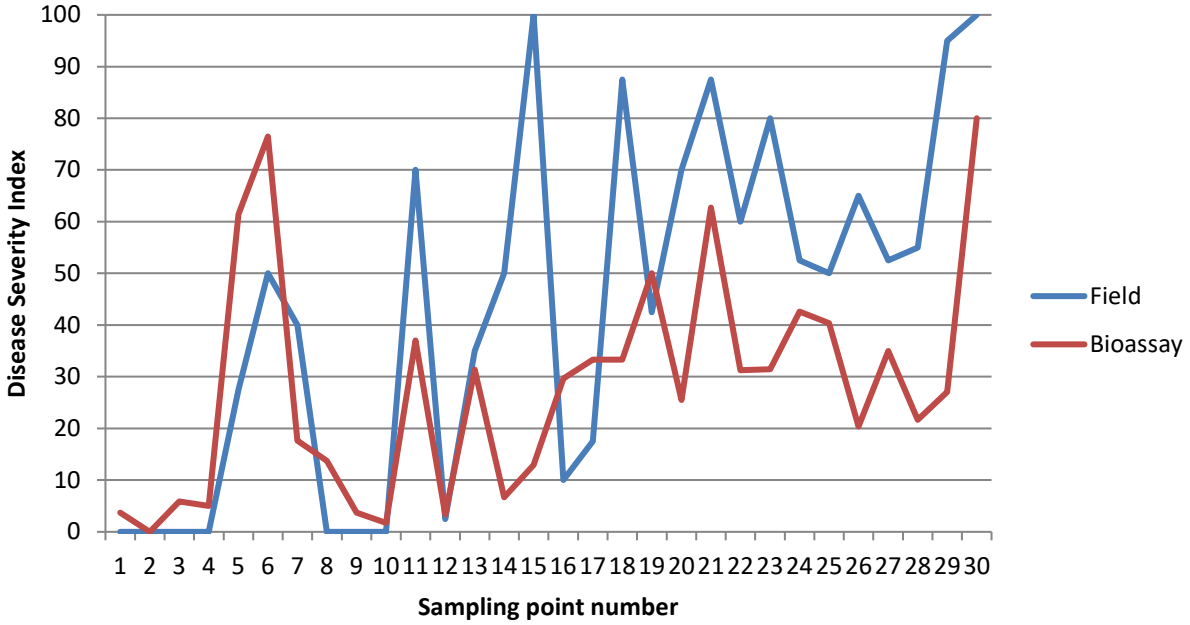


Figure 57. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Staffordshire field, 2017.

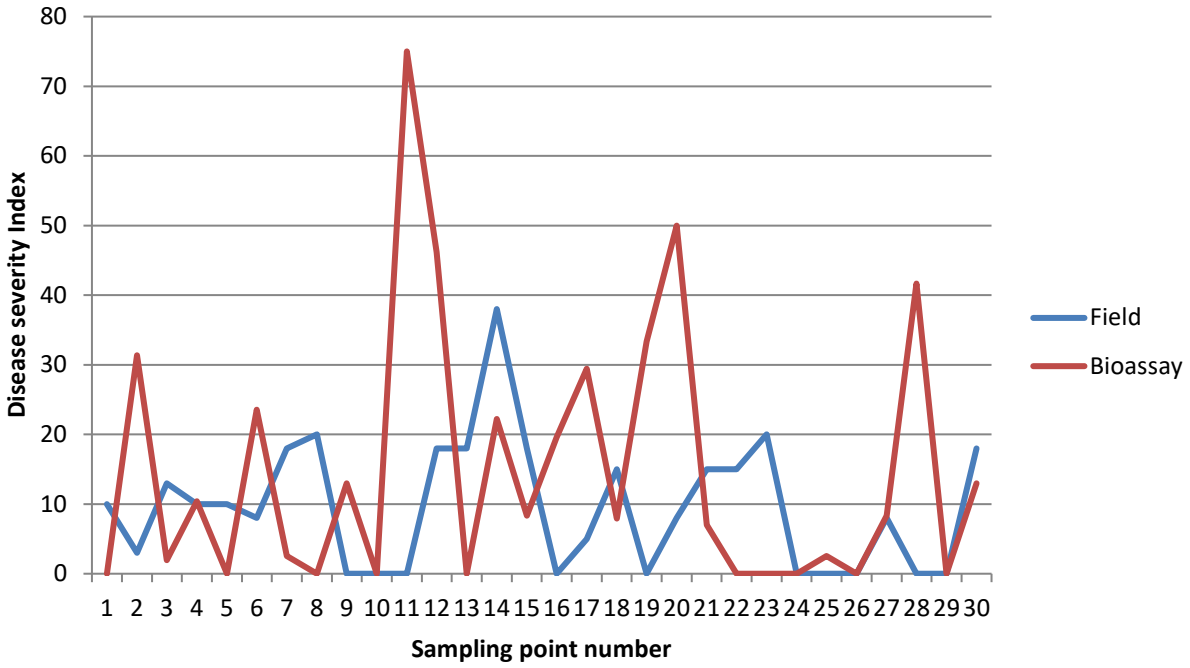


Figure 58. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Whinney Hill field, 2017.

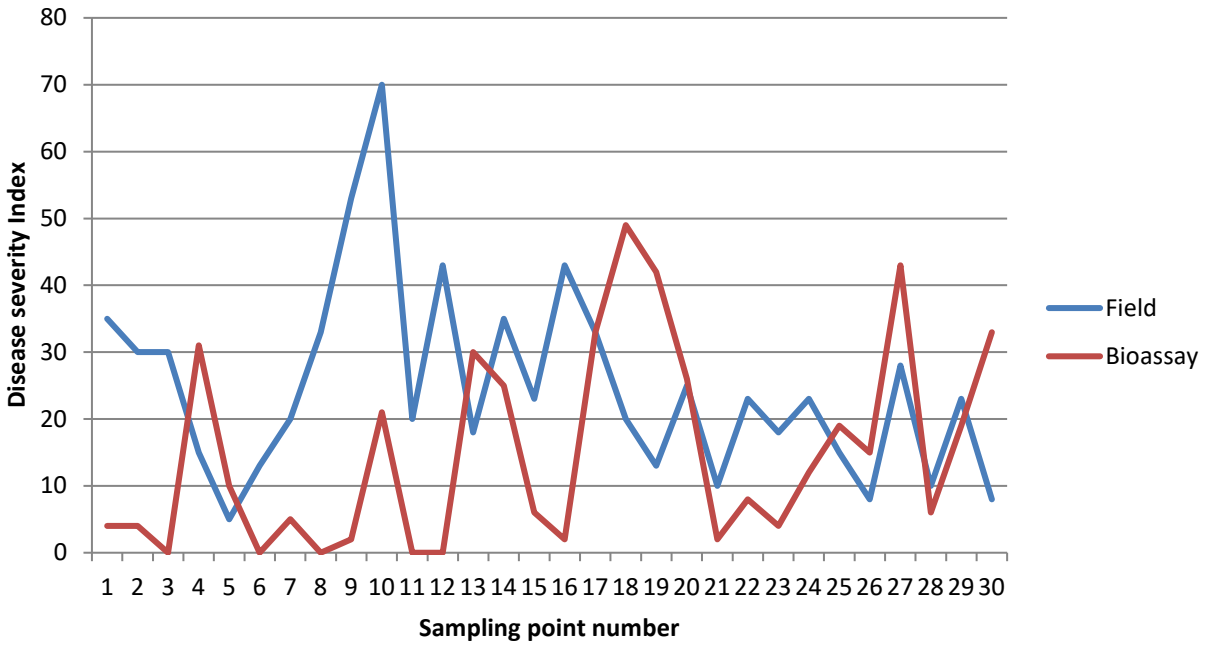


Figure 59. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Boysack field, 2017.

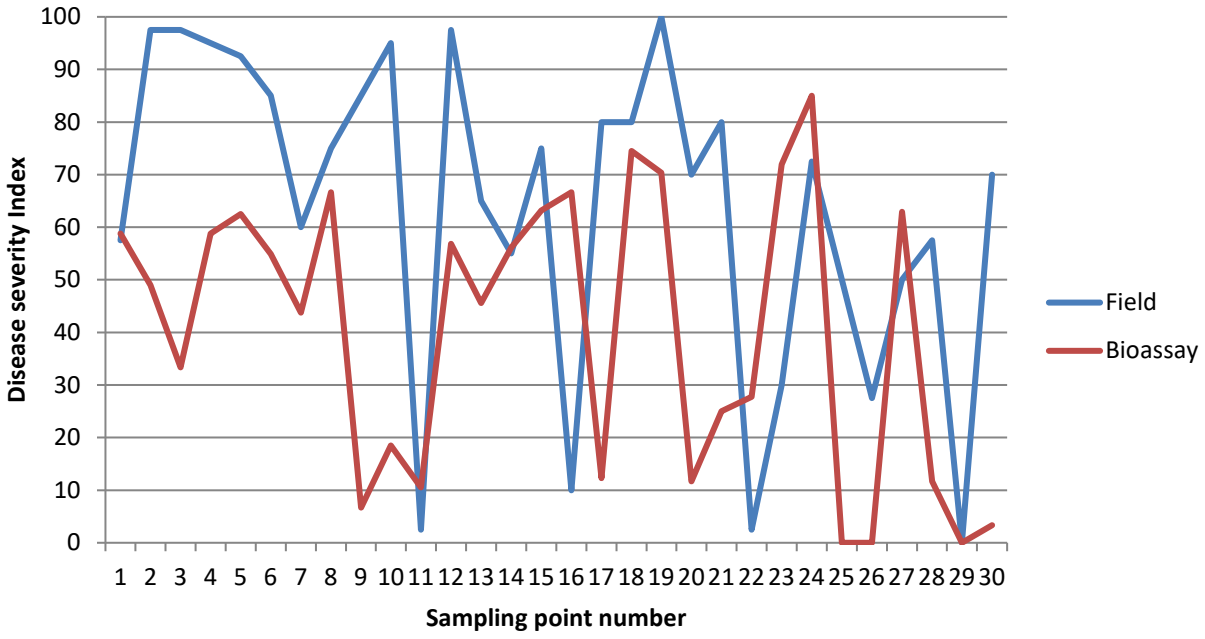


Figure 60. Disease severity index obtained from visual assessment in the field and from the bioassay. Samples were taken from the Pencombe field in 2017.

Appendix 7.3

September 2016

Student Bursary Report

10 week project

Defence elicitors for clubroot control in oilseed rape

Developing sustainable management methods for clubroot (*Plasmodiophora brassicae*) through an understanding of the prevalence of pathotypes present in the UK and through field mapping to establish the impact and spread of the disease in oilseed rape.

Ross Etherington, University of Birmingham

Under the supervision of Dr. Graham McGrann, SRUC

This is the report of a 10 week student bursary project which started in June 2016.

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1. Supervisor's report

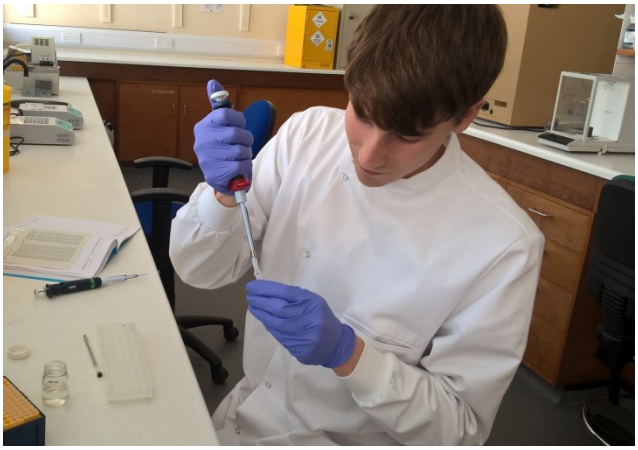
Clubroot is a serious threat to oilseed rape and vegetable brassica cultivation across the UK. The soil-borne nature of the disease, combined with limited sources of host plant resistance and evolving pathogen populations, means management of this disease is particular difficult. The AHDB funded project "Developing sustainable management methods for clubroot (*Plasmodiophora brassicae*) through an understanding of the prevalence of pathotypes present in the UK and through field mapping to establish the impact and spread of the disease in oilseed rape" investigates different reasons why clubroot control is not always successful. The project will use a soil mapping approach to quantify the distribution of the clubroot pathogen, *P. brassicae*, within infested fields. Soil samples from selected fields will be pathotyped using the European clubroot differential brassica lines to characterise pathogen variation within fields and across sites within the UK. A number of the sites for selected for pathotyping were previously pathotyped approximately ten years ago enabling an assessment of temporal changes to local pathogen populations. Insights in to in-field variation in pathogen distribution and resting spore concentrations, combined with information on local and regional pathotype variation from this project, will be used to devise sustainable disease management strategies to control clubroot. Control strategies, such as application of soil amendments to raise pH and calcium levels and the use of resistant oilseed rape varieties, can be targeted to regions of fields that have particularly high incidence of the disease. Those areas that are disease free can be left untreated, resulting in reduced costs for disease control. More selected use of the limited clubroot resistant varieties will protect their use in agriculture and lower the risk of pathogen adaptation resulting in resistance-breaking pathotypes.

Control options for clubroot are limited, such that novel management strategies are coveted by the industry. The work in this bursary builds on research from SRUC, which indicated that defence elicitors have the potential to lower clubroot in susceptible oilseed rape plants, and aimed to evaluate the effects of three defence elicitors applied as a seed treatment to control clubroot. Unfortunately, the clubroot disease infection assays did not work meaning that the time course analysis to assess how the different defence elicitors affect the build up of *P. brassicae* DNA in root tissue and how this equates to the development of clubroot galls over time. The reasons for the failure in the pathology experiment are currently unclear but lack of infection is an inherent problem when working with plant pathogens and is particularly frustrating when working with soil-borne pathogens where disease symptoms take weeks to develop.

Despite the problem surrounding the key experimental questions of the bursary, the project still yielded some important insights in to the potential effects of defence elicitors when applied as seed soak treatments on the early development of oilseed rape seedlings. Both SiTKO-SA and Inssimo appear to slow the early stages of oilseed germination after three days but this effect was not

observed after five days. However, SiTKO-SA appears to also reduce seedling emergence compared to control plants which could be problematic for oilseed growers, especially those in the more Northerly regions of the UK, where clubroot infestations are severe but establishment of the oilseed crop can be more difficult to achieve. Seedlings from SITKO-SA treated seeds also were shorter than controls, whereas those plants from Inssimo treated seeds were significantly taller. These are preliminary findings but they do indicate additional areas of study that will need to be taken in to account to facilitate the uptake of defence elicitors in clubroot control. For defence elicitors to be accepted as alternative control strategies in sustainable clubroot management programmes, industry must be convinced not only of their ability to control the disease but also that there are not adverse effects of the compounds that could affect yield potential.

2. Personal statement



This project has provided me with the opportunity to carry out my first piece of independent research during which, I have learned numerous pathology and molecular biology skills, including disease assessment, dilutions, DNA extractions, and running polymerase chain reaction based assays. These skills will prove invaluable during my final year project at university and for postgraduate study.

During the project I have also been able to work within a more applied discipline of plant biology than I have previously studied, with a clear link between my research and crops in the field. This has enhanced my insight into how scientific developments can be implemented into agricultural practises and I hope to carry out further research of this type in the future.

Due to the data produced from my research, for the first time I have been able to compare scientific literature with my own observations and appreciate how my own results would integrate with existing knowledge. This has given me a more complete understanding of how research from different projects and institutions can be combined in order to drive agricultural innovations.

3. Abstract

The hypertrophic growth of non-fibrous ‘clubroots’ caused by the protist *Plasmodiophora brassicae*, has a large impact on global yields of brassica crops. In the UK, 10-15% yield losses due to clubroot are common in the brassica crop oilseed rape (OSR). The disease is able to survive in soils for up to 20 years and is currently unable to be controlled by individual management strategies, as such, clubroot is widespread across the majority of the UK.

At present, clubroot is most effectively managed using soil amendments, such as raising pH, and through the growth of resistant OSR varieties. Combining these strategies, with lengthened crop rotations can effectively reduce clubroot severity but still fail to provide total clubroot control.

This study investigated the potential of defence elicitors to provide clubroot resistance in OSR. Additional control methods, such as defence elicitor treatments, could be incorporated into current integrated approaches. Development of additional methods of clubroot control can potentially reduce clubroot severity further as well as ease pressure on current management strategies, such as aiding the longevity of current resistant OSR cultivars.

The ability of defence elicitors to induce plant immune responses and enhance disease resistance has been demonstrated for numerous diseases across multiple crops. In the current investigation, the elicitors Innsimo, SiTKO-SA and Laminarin were applied to OSR as seed treatments – a practical method of administering elicitors on a large scale, before seeds were exposed to soils containing *P. brassicae*. The effect of each elicitor on plant development and disease resistance was assessed over eight weeks, with notable reductions in the developmental rate of seeds treated with SiTKO-SA observed. Germination assays showed a 17% reduction in the percentage of germinated SiTKO-SA-treated seeds after three days relative to untreated control seeds. Differences were also observed after two weeks of growth with 32.2% fewer SiTKO-SA seeds having developed into seedlings with an average height 13.2 mm less than that of the controls. Only minor levels of *P. brassicae* DNA were detected in roots of plants from each treatment (including controls) using quantitative polymerase chain reaction assays. Minimal levels of pathogen DNA prevented the reliable comparison of treatments in terms of clubroot resistance and further investigation is needed in order to assess their effectiveness.

4. Introduction

Clubroot is a disease affecting all forms of brassica crop, caused by the protist *Plasmodiophora brassicae.* The pathogen causes characteristic swelling of roots, forming non-fibrous clubs or galls by altering root production of growth hormones such as auxin and cytokinin (Hwang *et al*., 2012). The loss of fibrosity, combined with the diversion of water and nutrients towards galls rather than shoots and leaves can lead to deficiencies throughout the plant, thus increasing the likelihood of wilting, stunted growth and, in severe cases, death (Hwang *et al*., 2012).

In the common arable crop oilseed rape (OSR), clubroot is associated with the production of both fewer and lower yielding oilseeds (Hwang *et al*., 2012). This commonly leads to the loss of 10-15% of potential yields with much higher losses possible if the crop is severely infected (McGrann *et al*., 2016). In surveys carried out between 2007 and 2010, 52% of tested UK sites growing OSR were positive for *P. brassicae* (McGrann *et al*., 2016), a figure that is only likely to rise given the increased use of OSR in crop rotations and the current lack of effective management strategies.

Presently, the most commonly used methods of clubroot control include extended crop rotations, soil amendments and the use of resistant varieties of OSR. Whilst these strategies are able to reduce clubroot severity, they are not without problems.

4.1. Crop Rotation

Resting spores of *P. brassicae* can survive in soils for as long as 20 years (Hwang *et al*., 2012), far longer than the average crop rotation period. A 3.7 year half-life (Wallenhammar, 1996) makes 4-6 year rotations a viable method of limiting the prominence of the disease but market pressure hampers the implementation of such rotations. The problem is increased by the presence of cruciferous weeds, on which *P. brassicae* can survive, extending its half-life (Dixon, 2009a).

4.2. Soil Amendments

Clubroot severity can be partially controlled through the addition of calcium to soils and raising pH to a level above 6.0-6.5; the optimum pH for clubroot spore germination (Hwang *et al*., 2012). However, the mechanism by which these amendments provide protection remains unknown and their success is highly variable (McGrann *et al*., 2016).

4.3. Varietal Resistance

European clubroot resistant cultivars of OSR have been developed, which show a reduced susceptibility to *P. brassicae*. In most cases, the disease resistance is dependent on a single genetic locus originating from the resistant variety ‘Mendel’. The limited origin of this genetic resistance

makes it vulnerable to pathogen evolution, leading to isolates able to overcome the resistance gene. This is particularly a problem in areas where disease pressure is high and brassicas are grown in short rotations (Peng *et al*., 2014). The multiple forms or pathotypes in which *P. brassicae* can exist further exposes the vulnerability of resistance with most cultivars being resistant to specific pathotypes but susceptible to others (Hwang *et al*., 2012). This provides a source of genetic variability, which has the potential to permit the adaptation of the pathogen to overcome resistance. An additional issue is that the highest yielding OSR varieties are generally clubroot susceptible (AHDB, 2016), reducing the incentive for farmers to grow a resistant variety unless there is a history of severe clubroot infestation in their fields.

4.4. Fungicides

The application of fungicides is not currently considered a practical method of managing clubroot due to their ineffectiveness at high disease pressure and highly variable results when applied to less severe infestations (Peng *et al*., 2014). With little understood about fungicide-*P. brassicae* interaction mechanisms, combined with current legislation that restricts the use of many chemicals on crops, the development of an effective fungicide treatment for clubroot remains highly unlikely (Peng *et al*., 2014).

A synergistic approach combining multiple strategies is largely seen as the most practical and reliable method of managing clubroot. Integrated strategies benefit from both a greater accumulative effect and the reduced pressure on resistant cultivars.

4.5. Defence Elicitors

An underutilised option for clubroot control is defence elicitors. By inducing a plant's immune response, most commonly via the salicylic acid or jasmonate pathway, defence elicitors can reduce the impact of certain diseases (Walters *et al*., 2013). Elicitors can rarely provide complete control of a pathogen but may be a viable method of reducing disease pressure in order to prolong the effectiveness of resistant OSR varieties via, so called resistance stewardship. Previous applications of elicitors as a root drench have yielded promising results in clubroot control (McGrann *et al.*, unpublished data). However, a root drench is not a practical technique when applied to OSR crops in the field due to the huge quantity of chemical that would be required to drench soil across a broadacre field. Here we evaluate seed treatments of three different defence elicitors (Innsimo, SiTKO-SA and Laminarin), to assess their impact on plant development and their potential to provide eight weeks of disease protection across the most vulnerable stage of plant development (Walters *et al*., 2013).

5. Materials and methods

5.1. Seed Treatments

Seeds of the clubroot susceptible OSR variety Anastasia were soaked in separate solutions of three commercially available defence elicitors, at supplier recommended concentrations (Table 1). Control plants were soaked in water. Seeds were soaked for 24 hours at 4 °C. Prior to planting seeds were rinsed ten times in tap water, with a ten minute period of full submergence during the final wash.

Table 1 – Defence elicitor concentrations used for seed treatments

Elicitor	Concentration of soak solution
Innsimo	0.05 g L^{-1}
Sitko-SA	10 ml L^{-1}
Laminarin	5 ml L^{-1}
Water (control)	n/a

5.2. Seed Germination Assays

Treated seeds were germinated in darkness on damp filter paper and the germination percentage of 600 and 200 seeds per treatment was assessed after five and three days respectively. Data was analysed by modelling binomial proportions within a generalized linear model. Binomial proportions were calculated as the number of germinated seeds out of the total seeds in each experiment. The model assessed variation attributable to the different treatments examined and experimental replicates.

5.3. Preparation of Spore Suspension

Galls from clubroot infected OSR cultivar Fashion plants, harvested on 17/05/16 and stored at -20 °C, were homogenised and the resulting suspension filtered through two layers of wet muslin, before the removal of starch granules by centrifugation at 100 x g for 5 minutes. The concentration of spores was measured using a haemocytometer and diluted to $2x10^5$ spores ml^{-1}.

5.4. Planting/Harvesting

Four sets of 14 pots (9 x 9 x 8 cm) were filled with John Innes No. 3 compost and 50 ml of the $2x10^5$ spores ml^{-1} resting spore suspension poured onto the compost surface to provide a final concentration of $1x10^6$ spores per pot. A further ten pots were filled with compost but not inoculated with resting spores, to act as an uninoculated control. Seeds from each of the four treatments were planted across 14 pots, with four seeds sown and covered with vermiculite per pot. Four water-treated seeds were also planted in each of the 10 uninoculated pots.

After three weeks of growth in a glasshouse, plants were thinned down to two plants per pot and sampled at two week intervals for eight weeks. Each harvested plant was scored from zero to five for clubroot symptoms and the number of leaves and plant height was counted and measured respectively. Clubroot severity was scored on a 0-5 scale based on the level of galling present; 0 = no galling; 1 = small clubs present but most of fibrous root still healthy; 2 = galls visible around tap root and crown; 3 = moderately severe galling with healthy roots still visible; 4 = severe galling with few healthy fibrous roots present; 5 = severe galls with root system now rotten.

5.5. Extraction and Quantification of DNA

Roots were washed, removed from shoots, snap frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. DNA was extracted from the root tissue using a DNeasy Plant Mini Kit (QIAGEN). The amount of total DNA extracted was quantified using a Nanodrop-1000 spectrophotometer and diluted to a concentration of 10 ng μl^{-1}. Concentrations of *P. brassicae* DNA was measured via quantitative polymerase chair reaction (qPCR), carried out using a Stratagene MX3000P. A total volume of 20 µl was used containing 500 nM concentrations of both forward and reverse primers (Table 2), in addition to 10 µl SYBR® Green JumpStart™ Taq ReadyMix™ (Sigma Aldrich), 3 µl H_2O and 5 µl of DNA solution. Samples were initially heated to 95 °C for two minutes and then cycled 40 times at 95 °C for 10s, 62 °C for 20s and 72 °C for 25s. *P. brassicae* DNA was quantified via comparison to a standard curve of five-fold dilutions ranging from 50 ng to 5.12 fg produced using DNA obtained from clubroot spores (Fashion, harvested 17/05/16) using an Illustra Nucleon Phytopure DNA extraction kit (GE healthcare).

Table 2 – Primers used for qPCR

Primer	Sequence
PbITS3 (Forward)	5’-CGCTGCATCCCATATCCAA-3’
PbITS4 (Reverse)	5’-TCGGCTAGGATGGTTCGAAA-3’

The quality of extracted DNA was assessed through end point PCR using OSR specific primers. A total volume of 25 µl, made up of 12.5 µl Hotstar Master Mix (QIAGEN), 200 nM EF1α forward and reverse primers, 6.5 µl H_2O and 5 µl of DNA solution, was heated to 95 °C for 15 minutes and then cycled 40 times at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min. Gel electrophoresis of PCR products was then carried out on a 1.5% agarose gel and DNA visualised using GelRed Nucleic Acid Gel Stain (Biotium).

5.6. Two week emergence Assay

A further 56 seeds from each treatment were planted across 14 pots (as described in 5.4). The percentage of emerged seedlings and their heights was measured after two weeks of growth in pots in a glasshouse. Only plants with fully formed cotyledons and a minimum height of 20 mm were

classified as emerged. Differences in average height between treatments were compared using a student's t-test.

6. Results

6.1. Seed Germination

6.1.1. Five day

After incubation at room temperature for five days, no significant difference (P=0.73) was observed between the germination of elicitor-treated and water-treated control seeds (Figures 1A and 2).

6.1.2. Three day

Germination percentage after three days was highest in control seeds. Significant differences relative to the control seeds were identified from both the Innsimo (P<0.05) and SiTKO-SA (P<0.01) treatments (Figures 1B and 3).

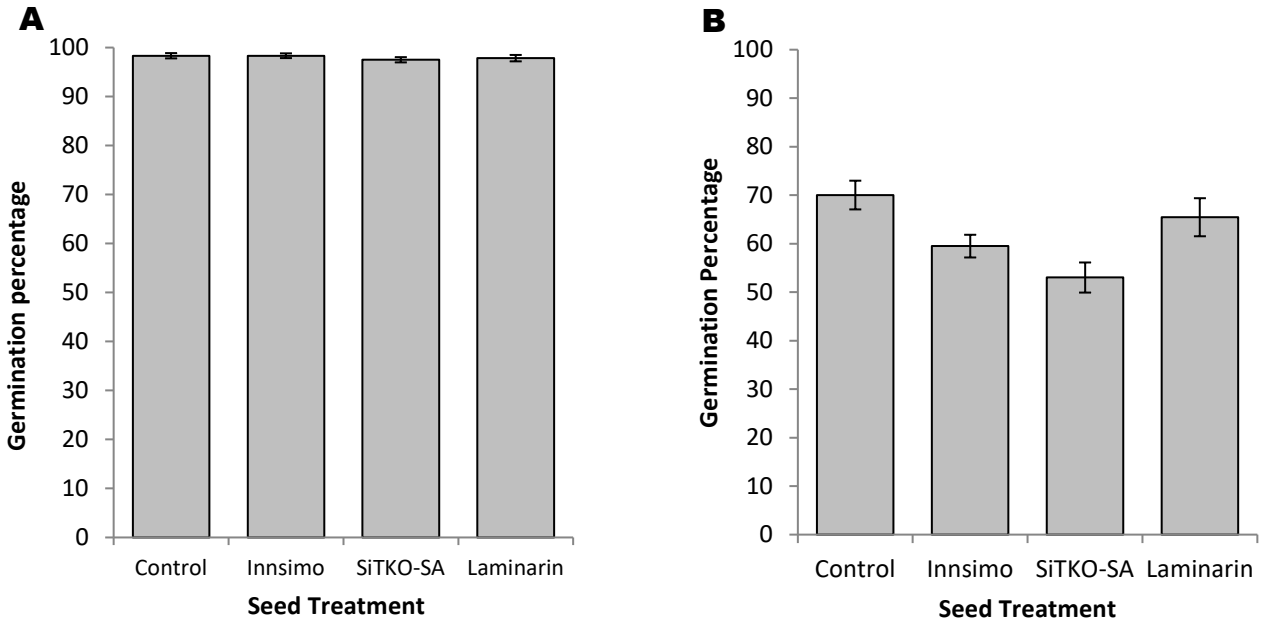


Figure 1 – Germination percentage (±SE) of seeds soaked in defence elicitors and in water (control) after five days (A) and after three days (B)

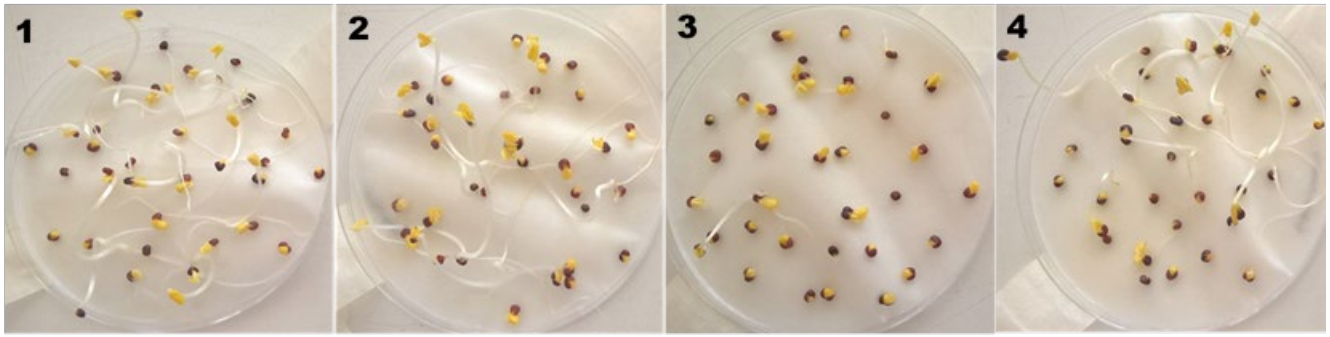


Figure 2 – Representative five day germination plates. 1=Control, 2=Innsimo, 3=SiTKO-SA, 4=Laminarin

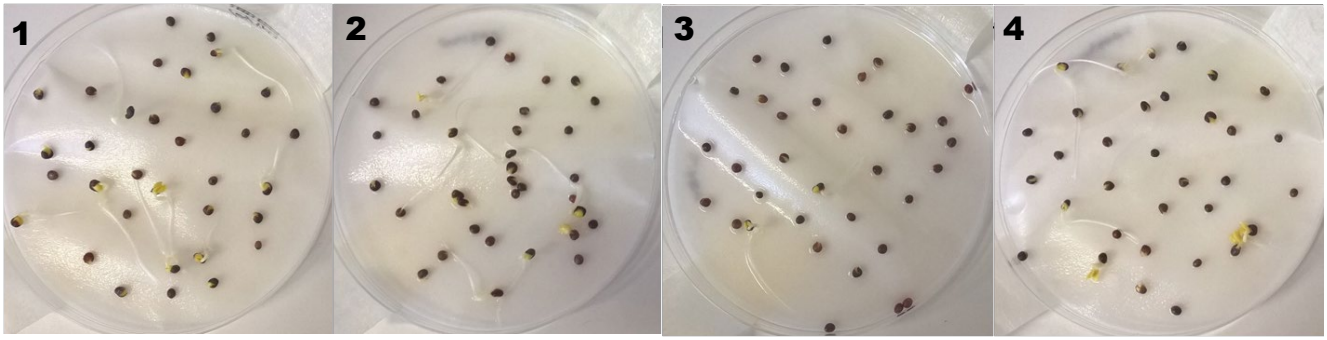


Figure 3 – Representative three day germination plates. 1=Control, 2=Innsimo, 3=SiTKO-SA, 4=Laminarin

6.2. Seedling Development

6.2.1. Two-week emergence

The proportion of SiTKO-SA treated seeds that had developed into seedlings after two weeks of growth was considerably lower than that of seeds soaked in water, Innsimo or Laminarin. The average height of those SiTKO-SA seedlings that had emerged was also significantly lower than seedlings previously exposed to both the Innsimo and Laminarin seed soaks ($P<0.05$). A significant difference in average height was also detected between control and Innsimo seedlings ($P<0.05$).

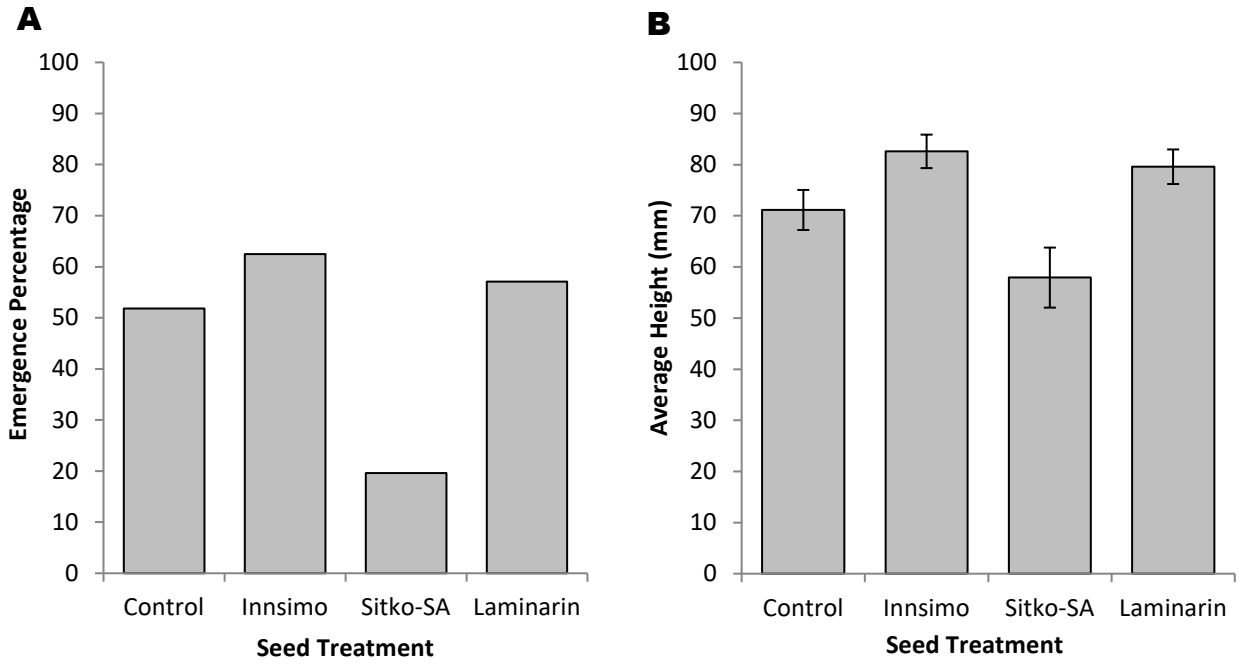


Figure 4 – (A) Percentage of treated seeds producing seedlings of greater than 20 mm in height after two weeks since planting. (B) Average height of emerged seedlings from each treatment.

6.2.2. Number of leaves

The number of leaves produced during the first eight weeks of growth showed limited variation between plants growing from treated seeds and untreated control seeds. Inoculation with *P. brassicae* spores also had little impact on the number of leaves, as a similar pattern of growth was observed for both inoculated and uninoculated plants. At the two week harvest, each set of seedlings

had, on average, produced of between 3.5 and 4 leaves. From four weeks onwards, the number of leaves plateaued, with an average of approximately 6 leaves per plant from each treatment (Figure 5).

A deviation from this trend was observed for uninoculated plants after four weeks of growth. This variation was generated because the sampled uninoculated plants were unable to be thinned to two plants per pot at the three week stage, due to an insufficient number of plants for the week four harvest. The presence of two anomalously small plants also led to the SiTKO-SA treatment producing a reduced leaf count at the six week stage. As such, both differences were considered unlikely to be biologically significant.

6.2.3. Plant height

Like, leaf number, all treatments produced a similar rate of seedling growth. Plants grew to approximately 75 mm tall after two weeks and to 220 mm tall after four weeks. Growth then slowed, with plants only increasing by approximately 40 mm in height over the next four weeks. Only minor variation between treatments was observed, potentially attributable to the problems described in section 6.2.1 (Figure 6).

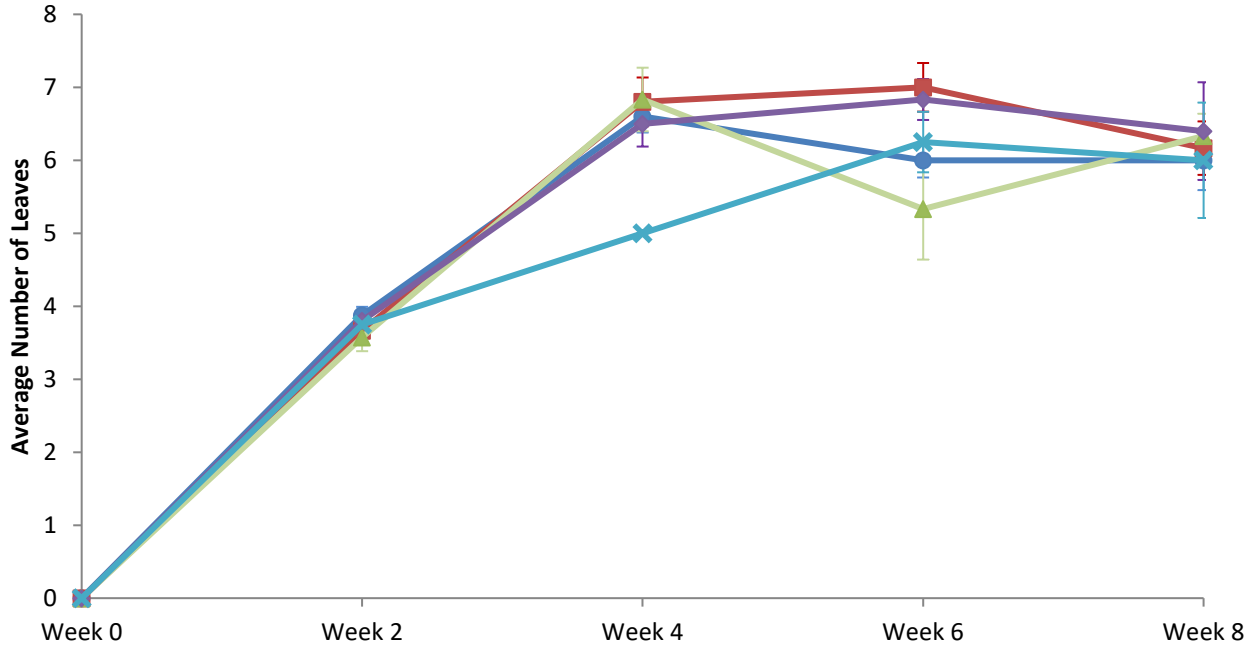


Figure 5 – Average number of leaves on plants harvested over an eight week period (±SE). Dark blue circles=control, Red squares=Innsimo, Green triangle=STKO-SA, Purple diamond=Laminarin, Light blue cross=Uninoculated

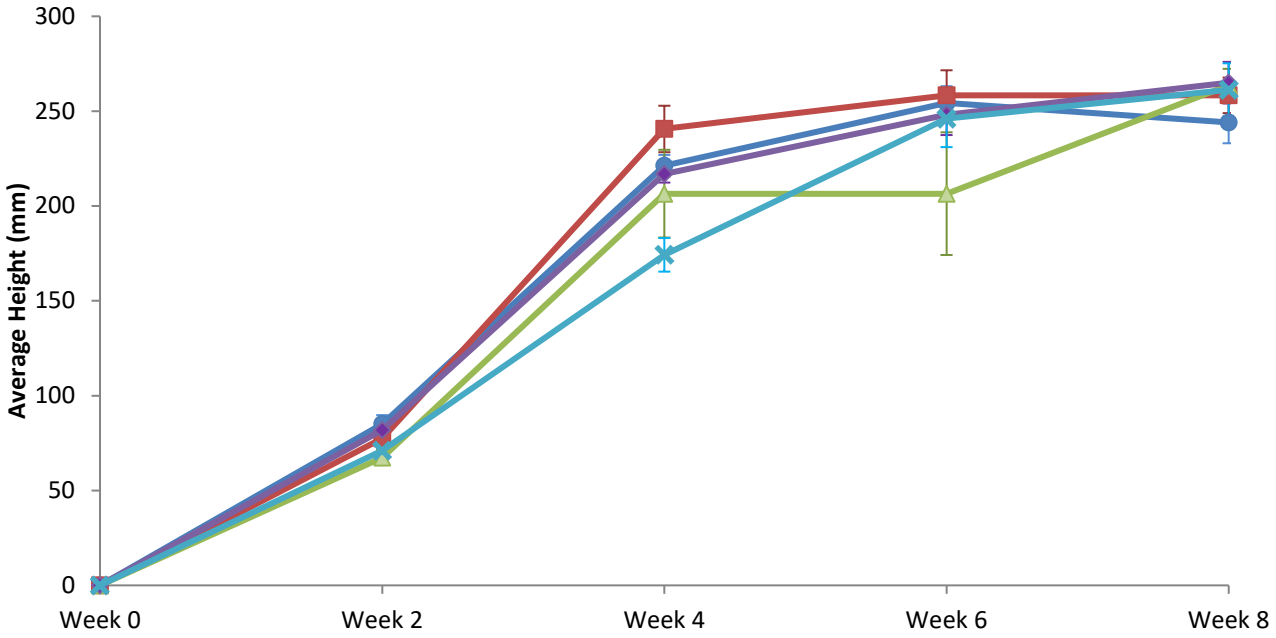


Figure 6 – Average height of plants harvested over an eight week period (±SE). Dark blue circles=control, Red squares=Innsimo, Green triangle=STKO-SA, Purple diamond=Laminarin, Light blue cross=Uninoculated

6.3. Clubroot control

The ability of the defence elicitors to manage clubroot was unable to be effectively analysed due to minimal disease incidence. No galls were formed on the roots of inoculated samples and all observed patterns of growth appeared not to be limited by the presence of disease. *P. brassicae* DNA was detected in some samples via qPCR but only at trace levels. The percentage of all harvested roots containing a measurable level of *P. brassicae* DNA trace was highest after two weeks, decreased to a minimum at week 6 and then increased slightly after eight weeks (Table 3).

Table 3 – Percentage of samples testing positive for *P. brassicae* DNA per harvest

Harvest	Percentage of samples with *P. brassicae* DNA detected
Week 2	62.5
Week 4	20.5
Week 6	6.3
Week 8	8.7

The quality of extracted DNA was analysed using end point PCR with the OSR specific forward and reverse EF1α primers. Of the nine random samples selected, each produced an EF1α DNA band (Figure 5).

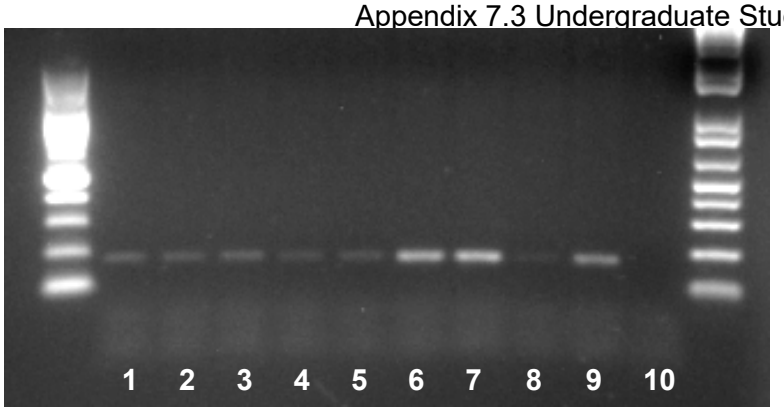


Figure 7 – OSR EF1α DNA bands from end point PCR of nine random samples.
1=Week 2 Control, 2=Week 2 SiTKO-SA, 3=Week 2Laminarin, 4=Week 4 Control, 5=Week 4 Innsimo, 6=Week 4 SiTKO-SA, 7=Week 6 Innsimo, 8=Week 6 SiTKO-SA, 9=Week 6 Laminarin, 10=Blank.

7. Discussion

7.1. Germination and development

Previous investigations have shown the effectiveness of salicylic acid at controlling disease but have also noted a corresponding reduction in the growth of roots and shoots (Lovelock *et al*., 2013). To be a valid clubroot management strategy, defence elicitors must have minimal effects on the germination and development of OSR so as to avoid a significant yield penalty.

Germination assays within the current study show that, when applied as a seed treatment, defence elicitors permit successful seed germination, with a high germination percentage observed for all treatments (Figure 1A). Whilst germination percentage remained high for each treatment after five days, variation was noted in the rate of emergence and subsequent development of the treated seeds. The three day germination assay (Figure 1B) and two week emergence and height data (Figure 4) used to investigate this each showed a reduced level of growth from seeds pre-treated with SiTKO-SA. No major effect of the SiTKO-SA treatment was noted from the leaf counts and height measurements of harvested plants across the eight weeks (Figures 5 and 6) but pots with larger plants were specifically selected at week two, as plants were required to be a minimum size in order to be harvested. From week four onwards, it was likely that growth was restricted by nutrient availability due to the limited pot size, therefore leading to the growth plateau observed, regardless of treatment. Further tests are required to evaluate the relevance of these findings with respect to the use of Inssimo as a plant protection product in OSR.

The three day germination assay (Figure1B) and two week plant height measurements (Figure 4B) also highlighted significant differences between the control seeds and Innsimo treated seeds. However, the differences between the two treatments were observed in opposite directions, with control seeds germinating earlier but Innsimo seedlings growing taller after two weeks. It is unreasonable to draw significant conclusions about the impact of Innsimo on plant development from this limited and contradictory data. The opposing variations highlight limitations with the investigation, such as the relatively small sample sizes, and an appreciable level of background variation must be considered when interpreting the presented results.

SiTKO-SA treated seeds demonstrated a slower rate of development compared to the control seeds. Unlike differences between control and Innsimo treatments, the effect of SiTKO-SA was consistent and always in the same direction. Differences were also more strongly statistically significant. It can therefore be more reliably concluded that a SiTKO-SA seed soak treatment leads to a slower rate of early development in OSR. This result may be specific to the unique conditions that plants and seeds were exposed to in this experiment. However, obtaining similar results in subsequent trials may indicate that slowed plant development is a possible negative consequence of SiTKO-SA as a disease management strategy in OSR that could hinder yield potentials.

SiTKO-SA and Innsimo are based on the same active ingredient: salicylic acid (SA), with SiTKO-SA containing a 4% concentration and Innsimo containing a 50% concentration of acibenzolar-S-methyl (ASM), a synthetic analogue of SA. The obtained results may indicate that SA has a greater inhibitory effect on plant development than its synthetic equivalent. This in turn could correlate with a stronger immune response to SA, although a much more in depth investigation would be required to validate these initial observations and hypotheses. Alternatively, another component of SiTKO-SA may have caused the reduced developmental rate, however, this is unlikely, as the only other active ingredient of SiTKO-SA, silica, has never been previously recognised as having any inhibitory effect on growth.

No negative effects on the germination and development of Laminarin treated seeds were observed. This minimal effect of Laminarin makes it ideal for use as a defence elicitor, provided that it is also able to effectively induce disease resistance to clubroot. Unfortunately, due to failed disease uptake, the current investigation was unable to analyse this aspect of the tested elicitors.

7.2. Clubroot Control

The effectiveness of defence elicitors at inducing resistance towards multiple diseases is well documented (Walters *et al*., 2013). Their efficacy, however is not replicated, in every disease of all crop species, ASM for example has effectively aided resistance to numerous pathogens but no effect has been observed against others (Graham and Leite, 2004; Huth and Balke, 2002; Zhang *et al*.,

2001). The intention of this investigation was to assess the ability of three elicitors to protect OSR from the clubroot pathogen *P. brassicae* when applied as a seed treatment. Unfortunately, due to poor uptake of the disease into the OSR test plants, assessment of this nature was unable to be performed. No visible clubroot symptoms were formed and the concentrations of *P. brassicae* DNA detected using qPCR were too small to make meaningful comparisons between the treatments. Because the failure of the investigation resulted from problems with pathogen inoculation, not the ineffectiveness of elicitors, the potential remains that elicitor seed soaks will be able to induce clubroot resistance, but further studies will be required to provide evidence for this.

A higher proportion of root samples with detectable levels of *P. brassicae* DNA were present in earlier harvests (Table 3). It is likely that this was due to root growth, and hence an increase in OSR DNA, occurring more quickly than *P. brassicae* DNA accumulation leading to the dilution of pathogen DNA. Pathogen DNA build up may have been slowed by adverse glasshouse conditions for disease development. The presence of *P*. *brassicae* DNA at low levels in sampled roots suggested that the primary infection of the pathogen had taken place. Following the primary infection and formation of plasmodia within root hairs, secondary zoospores are produced which are able to penetrate the root epidermis and infect the cortex (Hwang *et al*., 2012). This secondary infection requires damp soil in order for the zoospores to be able to travel through water (Dixon, 2009b). It is possible that hot weather conditions may have temporarily led to the excessive drying of soil, preventing zoospore movement and therefore the secondary infection. The development of only a primary infection would also correlate with the dilution of *P.brassicae* DNA observed from week two to eight, with roots continuing to grow, whilst the secondary infection failed to take place.

An alternative, although unlikely, possibility for the minimal disease uptake may be a previously unrecognised clubroot resistant property of Anastasia to the isolate used. In a subsequent trial, in the same glasshouse, severe galling developed on Anastasia plants just four weeks after inoculation with a different field isolate. In future investigations into clubroot disease resistance, improved results are likely to be obtained by inoculating plants using a more aggressive field isolate in order to ensure the development of the disease.

8. References

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