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Management of clubroot (*Plasmodiophora brassicae*) in winter oilseed rape

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1. ABSTRACT

Clubroot is an increasing problem in oilseed rape crops throughout the UK and has been exacerbated by close rotations, as the disease can persist for upwards of 15 years in soils. The galls on roots formed by the clubroot pathogen, *Plasmodiophora brassicae*, affect normal root function and reduce the uptake of water and nutrients such that even low levels can reduce yield. Root galls will commonly breakdown with secondary rots such that root function is seriously impaired.

Yield losses to clubroot in this project were 0.3 t/ha for every 10% clubroot severity. Losses in affected crops can therefore equate to over 50% of potential yield in the most severely infected crops. Survey work in this project, for the years 2007-2009 showed that the disease was present in all areas of the UK where oilseed rape was grown. Of the sites surveyed, 52% tested positive. The sites sampled were not randomly selected and growers and agronomists who participated tended to select fields of concern. These positive test results were often at sub clinical levels in the crop.

An aim of the project was to investigate the use of varietal resistance and /or soil amendments, Calcium carbonate (LimeX70), Calcium cyanamide (Perlka) and boron (Solubor) to manage clubroot in affected fields. Soil amendments gave variable control but showed some potential as part of a clubroot management strategy. Varietal resistance remained the more effective management tool for growers, but varietal resistance is under pressure at sites where it has been heavily used in back rotations and varietal control was not effective at sites in Aberdeenshire. The varieties Mendel and Cracker, gave 50 – 95% disease control at three sites in the West Midlands. Control with soil amendments ranged from 0 – 90% but analysed over the trial series control meaned at 25% for the best solo treatment (Calcium carbonate at 8 t/ha). The Calcium cyanamide product is no longer supported for use on oilseed rape crops in the autumn in the UK. Soil testing for clubroot and soil pH, and lengthening rotations are important to the long term management of clubroot on farms, as varietal resistance and soil amendments give inconsistent results.

2. SUMMARY

Clubroot is an increasing problem in oilseed rape crops throughout the UK and has been exacerbated by close rotations. The disease can persist in soils for upwards of 15 years and has a half-life of 3.7 years so that in relatively short three or four year rotations it builds in severity in affected fields. The galls on roots formed by the clubroot pathogen affect normal root function and reduce the uptake of water and nutrients such that even low levels can reduce yield. Root galls will commonly breakdown with secondary rots such that root function is seriously impaired and yield in affected crops can be reduced to less than half of potential yield. A series of warm and wet autumns prior to the start of this project also exacerbated symptoms in oilseed rape crops and it was a common advisory problem in diagnostic clinics, but the extent of the problem was unknown.

The disease affects a wide host range and is very problematic in brassica vegetable production. In the vegetable scenario land is commonly rented for production and clubroot is typically managed by soil testing prior to cropping and then rejecting heavily infested fields. Clean land is a diminishing resource and for oilseed rape cropping on affected farms, avoiding infected fields is seldom an option. Disease severity is linked to soil acidity and available calcium content and work on vegetables has shown that soil amendments which can raise these parameters can significantly reduce clubroot infection and maintain yields. Clubroot has thick-walled resting spores which are very resistant to both environmental stress and to soil treatments, hence their ability to persist in soil for very long periods. In order to infect, these resting spores germinate into motile zoospores that can swim towards, and infect the roots of host plants. It is at this stage that they are most vulnerable to treatments or to hostile soil conditions and previous work on vegetable crops has demonstrated that if a spike in pH and calcium can coincide with early infection, soil amendment treatments are likely to be most effective. This is easier to achieve with transplanted brassica crops where the root exudates stimulate the clubroot spores to germinate and soil treatment is closely timed to planting. Treatments with greatest efficacy in vegetable crops in previous work were very fine forms of lime where the pH and calcium availability rise in soils was rapid. Limex70 (a Calcium carbonate by-product of the sugar beet industry) and Perlka (a calcium cyanamide product) are widely used on vegetable crops in this context but their efficacy on oilseed rape was unknown.

The current project aimed to investigate management of clubroot in oilseed rape, with five specific sub aims. The first was to determine through survey work the extent of the problem in UK crops. The second was to test varieties for disease resistance or tolerance and determine the potential of varietal resistance as a management tool. The third objective was to investigate the efficacy of soil amendments in field trials. The fourth objective was to determine if future climate change scenarios would increase or decrease clubroot severity and the final objective was to develop a quantitative real-time PCR diagnostic assay for the disease.

Survey work in this project, for the years 2007 – 2009, revealed that 52% of sampled oilseed rape fields were infected with clubroot when tested in bioassays of the soil. Scottish sites sampled had a 50% infection rate and English samples 54%. Samples size was 96 sites. Sites were not randomly selected and the agronomists and growers who participated tended to select fields of concern (because of intensive rotation or because they had seen patches of poor growth). The actual incidence of infection therefore is probably somewhat lower than this but nevertheless these figures are representative of infection levels on intensive holdings. These positive test results were often at very low levels of severity in the bioassay test and were therefore still at sub clinical levels in oilseed rape and not yet problematic or observable in the affected fields surveyed. This indicates that the problem will build in severity in future crops, and increase in importance to the industry. Where fields were sampled and assessed in subsequent years within this project the decline in clubroot noted between seasons was minimal, which is indicative of its longevity within the soil. The declines noted were in line with those observed in previous literature with a half-life of around 3.5 - 4 years.

Maps of the distribution of affected fields in the UK were produced which showed 'hot spots' of infection relating to areas of intensive brassica or oilseed rape production. The disease was noted in all areas tested, but was more prevalent in relation to the acreage of crop grown in the north east of Scotland. This is indicative of the short rotations of oilseed rape in this area and the lack of alternative reliable break crops for cereals at this latitude. Soils sampled in the course of the survey were also tested for pH and extractable calcium content but there was no correlation between these parameters and disease severity in the survey. Disease severity was more influenced by previous field history, and high levels were noted at Aberdeen sites with rotations of one in two, with winter barley as the intervening crop.

The progress of a clubroot epidemic within a season is dependent on weather and in particular is favoured by wet and warm autumn conditions. Infection ceases below soil temperatures of 8^{o}C. Using climate change modelling and weather predictions for the 2020 and 2050 timeframes (using data from the A1B Global Climate Model), maps of future clubroot risk were produced using the CLIMEX^{TM} computer simulation programme. The modelling indicated that the pathogen can survive in a wide range of conditions and will be reasonably resilient to drought. It will benefit from predicted higher temperatures which will extend its growing season and allow for more generations in some regions so that, by 2050, larger areas of the UK than currently will become suitable for clubroot. This picture will worsen if crops are irrigated.

Using a bioassay, varieties were screened for tolerance to clubroot in the hope that this would offer a sustainable strategy for growers. The bioassay included Mendel which carries a known resistance to clubroot. This was the only variety where a significant reduction in clubroot was

noted. What was of concern, however, was that a high level of infection was still noted in the Mendel (an index of 26.9% compared to a mean of 74.1 for the susceptible varieties). The soil used in the bioassay was taken from a site in Fife with a back history of using Mendel. Little is known or understood about clubroot populations but variable host pathogenicity is known. It has been observed that the Mendel resistance is eroded at sites where it is commonly used and it is likely that strains of clubroot that can overcome this resistance mechanism build up in this scenario. Susceptible volunteers in the field can be one explanation of clubroot infection in fields of Mendel, however the bioassay results, in controlled conditions and with certified seed, confirms that strains of clubroot exist that can overcome this host resistance.

The variety Cracker was introduced to the market midway through this project and was evaluated in the field trials described below. Although Cracker has better foliar disease ratings and improved agronomic characterises and yield, it carries the same resistance mechanism as Mendel and is not therefore an alternative strategy in managing clubroot at sites where Mendel has previously been used.

Clubroot is favoured by acid soils and liming has been a common strategy in reducing disease severity. Previous work on vegetable brassicas demonstrated the potential of different sources of lime and indicated that finely ground and more available types were more effective. Products that raise soil calcium are also successfully applied to vegetable brassica crops to reduce clubroot infection, for example Calcium cyanamide. Boron is another soil amendment used in the vegetable scenario.

An aim of the project was to investigate the use of soil amendments and varietal resistance to manage the problem. Six field trials were carried out in the three seasons of this project, 2007-2008, 2008-2009 and 2009-2010. Two sites were established per season, one in Aberdeenshire and one in the West Midlands. The clubroot epidemics observed at the sites varied hugely, even between two sites in the same season. In some trials clubroot was severe from early autumn, throughout the whole season, at others it was low in the autumn but increased to moderate levels in the following spring and at some it was low throughout the season. Soil amendments were therefore evaluated in a wide range of disease scenarios. Calcium cyanamide (Perlka), Calcium carbonate (LimeX70) and boron (Solubor) were evaluated. Treatments were adjusted over the course of the trial series such that only the most effective treatments were continued, and in the later years attention was given to combined treatments. The susceptible variety Kommando was used throughout, as was the resistant variety Mendel. The variety Cracker was trialled in the final season only, as a coded variety prior to its launch on the UK market.

Results showed that both soil amendments and varietal resistance gave variable control but that varietal resistance remained the more effective management tool for growers at sites where it had not already been deployed.

In six trials over three seasons, varietal resistance as found in Mendel and Cracker gave 65- 99% disease control at three sites in the West Midlands (Warwickshire, Shropshire and Hereford). Varietal control was not evident at the three Aberdeen sites where Mendel had been commonly used prior to the trial series. Yield benefits varied. Mendel yielded significantly better than Kommando at the 2007-2008 Shropshire trial site where disease severity was high but did not improve yield the following year at a Hereford site where disease severity was lower. This is indicative of the lower yield potential of Mendel, which is now somewhat outclassed agronomically. At a Warwickshire site in the final year of trials the varieties Mendel and Cracker did give significant yield improvements in a similar disease epidemic. Mean yields averaged over all treatments were 4.12 t/ha for Kommando, 4.52 t/ha for Mendel and 4.53 t/ha for Cracker.

Clubroot control was also highly variable with soil amendments. Analysis over the whole trial series demonstrated potential disease control of around 25% with Calcium carbonate at 8 t/ha, but control ranged from 0% control to over 95% control at different sites. Control was not noted with solo field amendments at the three Aberdeen sites, although a combined Calcium carbonate, Calcium cyanamide and boron treatment did give control in the latter year of trialling, but did not raise yield significantly. The calcium cyanamide product trialled did reduce clubroot severity (but did not significantly increase yield) at the Warwickshire site in 2009/2010 and at the preceding season's Herefordshire site, but the rate used (250 kg/ha) equates to an additional 50 kg of nitrogen applied and this exceeds the nitrogen limits that can legally be applied to oilseed rape crops in the autumn in the UK, so the product is no longer promoted for use on oilseed rape. This might be reviewed in 2013, and banded applications are being investigated by the manufacturer.

Yield benefits in response to soil amendment treatments were noted in two trials out of the six. Calcium carbonate at 8 t/ha raised yield by over a tonne per ha in the final year of trialling at a site in Warwickshire where disease intensity was low in the autumn and moderate in the spring. Similar treatments performed poorly at sites where disease intensity was either very low, so that differences were not significant, or very high where treatments were overwhelmed. Note that phytophthora root rot also contributed to plant losses at some sites, notably Shropshire in 2008. Analysis of the whole trial series did show significant yield benefits with predicted mean increases of 5% for Calcium carbonate and up to 20% for combined three way treatments with calcium carbonate+ calcium cyanamide + boron.

There were significant correlations between soil calcium content and diseases severity and also between soil pH and disease severity. There was no evidence in the data of any additive effect between the two.

A new, quantitative molecular test was developed. This was highly sensitive to clubroot infection in plants where it correlated well with disease severity and was more sensitive than existing tests. Interference issues with different soil types could not be resolved however, preventing its use for routine soil testing. This is thought to relate to the presence of inhibitors to the DNA amplification process in different soils types and soil results were inconsistent and related poorly to bioassay or observed field infections. A bioassay, or a shortened bioassay quantified by PCR testing on the plants therefore remains more accurate for growers who wish to test soils.

Of the management strategies available to growers, both varietal control and soil amendments were variable in their success and this demonstrates that soil testing for clubroot and lengthening rotations are important to the long term clubroot management strategy on farms. This should be combined with routine testing for soil pH as literature shows acidity will increase disease build up. Many of the fields tested in the course of this project were positive for clubroot but at levels not yet problematic to the crop. The disease severity in such fields is likely to become worse in subsequent years, making this issue more problematic. In addition, climate change modelling indicated that the pathogen can survive in a wide range of conditions and will be reasonably resilient to drought. It will also benefit from predicted higher temperatures and maps produced for the project show that by 2050 larger areas of the UK than currently will become suitable for clubroot. This picture will worsen if crops are irrigated.

3. TECHNICAL DETAIL

3.1. General introduction

Clubroot, caused by *Plasmodiophora brassicae,* is the most damaging disease of brassica crops globally and accounts for £30 million losses in the UK *per annum* (Donald *et al*., 2006). It persists in soils for upwards of 15 years, with a half-life of 3.7 years (Wallenhammer, 1996), and is therefore a major issue in short rotations. The introduction of oilseed rape into rotations in the UK in the mid-1970s increased the risk of disease spread and build up. Oilseed rape is particularly important in arable rotations in the north of the UK as other break crop choices become increasingly limited. Rape is now commonly grown one year in three in arable rotations in Scotland and England and one year in two is not uncommon. This frequency in the rotation increases the risk of the disease multiplying and spreading on farms. In the last few seasons, problems with clubroot infection in rape and yield losses in commercial crops have been widely evident. Mendel, a variety with resistance, is widely grown on soils known to be contaminated but this variety requires higher levels of agronomic inputs such as agrochemicals and does not have the same yield potential as newer varieties (see HGCA Recommended List). However, the variety Cracker, recently introduced to the market, carries the same 'Mendel' resistance but has better agronomic and yield characteristics than Mendel (see HGCA Recommended List). The overuse of a single management strategy exerts a strong selection pressure and the race specific nature of the 'Mendel' resistance means it may not be durable (Werner *et al*., 2008). This resistance mechanism is under further pressure as it is present in other crops such as swedes and stubble turnips, which increases the selection pressure where these are also grown in the rotation. The symptoms of clubroot are shown in Figure 1.

Results from a Defra-funded SAC/ADAS project on horticulture brassicas (Harling, 2006) demonstrated that lime products (e.g. sugar beet factory lime as LimeX, and calcium cyanamide as Perlka) were the most effective available control agents against clubroot. In vegetable cropping they are applied close to planting in a fine powder form and are moreover relatively inexpensive and environmentally benign. Other nutrients such as boron may also have an effect on clubroot (Donald and Porter, 2009). Some fungicides have shown some control of clubroot (Stewart, 2007), but in the current legislative climate it is unlikely that any soil applied fungicide would gain approval for use, particularly on the scale of planting of a crop like oilseed rape.

Figure 1. Clubroot infected roots of clubroot susceptible oilseed rape (left) and healthy roots from the resistant variety Mendel in same field (right)

Clubroot survives in the soil as thick walled resting spores which can withstand long periods in the absence of any host. In the presence of a host, and with available water to move in, the spores germinate and motile zoospores swim towards the host roots. This is thought to be stimulated by root exudates. During the primary infection phase the zoospores infect root hairs where they can multiply and go on to spread and form secondary infections in the roots (Webster, 1986). Clubroot can infect and survive on cruciferous weeds in arable rotations.

High soil pH and calcium ion content are known to reduce clubroot severity in host crops but the precise mechanism for this control is not fully understood. A direct effect from calcium and pH on resting spores has been shown in previous research (Donald and Porter, 2009) but only at extremes of low inoculum and high values for these two parameters. The main effects are seen early in the infection process when calcium may work by enhanced uptake by the host which strengthens cells walls, but also by limiting the multiplication of clubroot in root hairs in the early stages of infection. Similarly, high pH reduces the number of primary infections in root hairs by clubroot, and the subsequent infection process. There is also limited evidence from other pathogens that calcium can limit zoospore mobility. Timing of soil amendment application is critical and the immediate three or four days following transplanting of vegetable brassicas has been identified as the most important (Webster, 1986). The control offered by raised pH and calcium, although it can be significant, is not complete. Even distribution in soil is also a key factor and this can be hard to achieve, which allows infection in pockets of lower pH and available calcium.

Boron has also been used to reduce clubroot infections in vegetable brassica crops. The mode of action is unclear but it is thought to act by reducing subsequent disease development in plants, rather than by reducing primary infection rates (Webster, 1986)

There is some limited movement of the resting spores through the soil profile and spread following field flooding is also commonly noted. Spread can occur via contaminated soil transferred on machinery wheels and infection is often first noted around field gateways. At first appearance in crops it is often patchy and linked to gateways and wet hollows in a field but from there it will spread in distribution in subsequent crops. In vegetable cropping, land is often rented and field testing prior to field selection allows infected sites to be rejected. Clean land is a diminishing resource and infected farms rejecting fields for oilseed rape gives additional pressures in other crops and fields on the holding, as well as having financial implications. Growers will usually opt to drill a clubroot resistant oilseed rape variety like Mendel, but the specific host resistance present (a single major gene resistance) in this variety does not control all strains of clubroot and significant disease can develop where it has been used several times in a rotation (Oxley, 2007)

A number of PCR assays have been designed in the past for the specific detection of clubroot from soil. However, none of the published methods have used quantitative real-time PCR but rather end point PCR, the use of real-time PCR provides much greater information about the critical infection levels in different soil types and when these results are used alongside the existing bioassay test data, this could provide valuable information on the critical levels of *P. brassicae* inoculum required.

3.1.1. Aims

The aim of the work was to develop management strategies for clubroot with the following specific sub aims.

1. To survey soils and determine the incidence and distribution of infection in oilseed rape crops
2. To test varieties for tolerance or resistance in a bioassay
3. To examine the efficacy of soil amendments in field trials
4. To predict future severity of disease index predicted by climate change scenarios
5. To develop a quantitative molecular assay to detect and measure clubroot in plants and soils

3.2. Materials and methods

3.2.1. Survey of oilseed rape fields

Aim: To assess incidence and distribution of clubroot infection

In 2008, 2009 and 2010 a survey of commercial oilseed rape fields in Scotland and England was undertaken. A total of 96 samples were collected, 44 from England and sampled by ADAS and local agronomists and 52 from Scotland and sampled by SAC. The sample was not random and the growers and agronomists who took part selected the fields that they wanted to sample, and some contributed multiple samples from fields in close proximity. Fifty cores were taken from each sampling area to give a soil sample of approximately 2 kg using a narrow bladed fern trowel or auger and the cores were taken to a depth of 15-20 cm. The cores were collected at regular intervals in a “W” or multi “W” pattern. Large stones and plant material were removed from the sample before being bagging in a large heavy gauge polythene bag. The soils were mixed by hand and plastic seed trays with drainage holes (20 x 14.5 x 5.5 cm) were filled with the test sample soil for the bioassay tests. Each of these trays was then put into a larger tray with no drainage holes (33.5 x 21 x 5.5 cm). Positive and negative control samples were set up with every batch of tests. The positive control used was soil sampled from a heavily infected site in Fife and the negative control was filled with John Innes No. 2 potting compost, pH measured at 5.5. Twenty seedlings of untreated Chinese cabbage variety SB1 Kilo were planted per tray, shown in Figure 2.. The trays were then put on raised benches in a glasshouse and watered daily by bottom watering. The glasshouse air temperature was 18^{o}C. After six weeks, roots were assessed and scored for clubroot infection on a four point scale, where 0 = uninfected, 1 = slight clubbing, 2 = moderately clubbed and 3 = severely clubbed. A percentage severity index was then calculated by weighting the incidence of plants in the three positive categories by a factor of one, two or three respectively using the following calculation:- Index = ((1*slight) + (2* moderate)+ (3* severe)) * (100/3*number of plants assessed).

Figure 2. Chinese cabbage seedlings in a bioassay test showing an infected tray (right) and uninfected (left).

Test soils were also tested for:-

- pH
- Phosphorus
- Potassium
- Magnesium
- Calcium

3.2.2. Bioassay variety tests

Aim: To test available varieties for tolerance or resistance to clubroot infection

A bioassay of 31 varieties going through the NL or RL testing system at the time, plus a Chinese cabbage control, were used to test for varietal resistance to clubroot (Table 1). Plastic seed trays with drainage holes (20 x 14.5 x 5.5 cm) were filled with John Innes No. 2 potting compost, pH 5.5. Each of these trays was then put into a larger tray with no drainage holes (33.5 x 21 x 5.5 cm). To inoculate the compost, a 50 ml clubroot resting spore suspension containing 10^5 spores/ ml was poured over the top of the soil to give a final concentration of 10^4 spores/g soil. The spore suspension was prepared by planting twenty seeds of untreated Chinese cabbage var. SB1 KILO into soil selected from an infected site in Fife into trays as described above. The trays were then put on raised benches in the glasshouse and watered daily by bottom watering. The glasshouse air temperature was 18^{o}C. After 6 weeks, the resultant galls were washed free of soil, homogenised, filtered through 8 layers of muslin using 20 – 25 ml tap water and centrifuged at 100 x g. The pellet from this step was discarded and the supernatant spun at 6000 x g for 15 minutes to pellet the clubroot spores. The spores were then re-suspended in 25 ml de-ionised water and a haemocytometer used to count the spores in 25 ml solution.

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

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

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

3.2.3. Field trials

Aim: To test the efficacy of soil amendments and varietal resistance in field experiments.

Field trials were established in the autumns of 2007, 2008 and 2009. There were two sites per season, one in Aberdeenshire and one in Shropshire (2007/2008), Herefordshire (2008/2009) and Warwickshire (2009/2010). Sites with a history of severe clubroot were selected, and infection confirmed with a pre-establishment bioassay.

Trial design was as four fully randomised blocks. Treatment products were selected as having shown efficacy in previous work on transplanted vegetable brassicas (Harling, 2006). Plot size was at least 30 m^2. Treatments were adapted between years in response to early results and specific

treatments are detailed in the tables below (Tables 2, 3 & 4). The soil amendments evaluated were a precipitated calcium carbonate product (LimeX70) and a calcium cyanamid product (Perlka). The former was applied to plots before cultivation and incorporated. The latter was applied after soil preparation for drilling and then incorporated in two of the treatments and not incorporated for a third treatment. Boron was also evaluated as a soil amendment. The boron was applied as Solubor (20.8% boron) using a knapsack sprayer to apply as a soil drench. Varieties selected were Kommando, which carries no known resistance to clubroot, and has good phoma and light leaf spot resistance, and Mendel and Cracker which are clubroot resistant. Mendel is weak for light leaf spot and phoma resistance, but was the only variety available in the first year of trialling. Cracker has better foliar resistance and better yield potential but the same clubroot resistance as Mendel. It was kindly made available to the project by LS Plant Breeding as a coded variety in year three.

Trial season 1 – 2008/2009

The soil amendments evaluated in this first trial season were Calcium carbonate (as LimeX70) and Calcium cyanamine (as Perlka). The former was applied to plots before cultivation and incorporated. The latter was applied after soil preparation for drilling and then incorporated in two of the treatments and not incorporated for a third treatment. Boron was also evaluated as a soil amendment. The boron was applied as Solubor (20.8% boron) using a knapsack sprayer to apply as a soil drench. There were two control treatments, one untreated and a further control to balance the 50 kg/ha of nitrogen in the calcium cyanamide product. Other inputs were as per local practice. The varieties tested were Kommando (susceptible) and Mendel (resistant). Plot size was 30 m^2.

Table 2. Soil amendments tested in field trials 2007/2008 season.

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

Total – 8 treatments x 2 varieties x 4 replicates = 64 plots

Assessments:

Before treatments applied

- Each block of treatments was sampled and tested separately for clubroot, extractable phosphorus, potassium, magnesium and calcium and soil pH in CaCl (4 samples per trial). An additional 100g of soil was tested for clubroot using PCR probes developed in section 5 of the methods.

Establishment

- Establishment was assessed on 1 - 9 scale where one was very poor emergence and nine complete emergence. Vigour was also scored if differences were evident, also on a 1-9 scale where one was poor vigour and nine was excellent vigour.

In season assessments

- Clubroot development was monitored over the season to select appropriate dates for full assessments. This was done by digging up some plants from control plots every couple of weeks to check on disease progress.
- In early autumn (October) 2 kg of soil was sampled from each treatment area. Samples were bulked across varieties and replicates and tested for clubroot and soil nutrients (8 samples per trial). An additional 100g of soil was tested for clubroot using PCR probes developed in section 5 of the methods.
- In early Autumn (October) roots were assessed for clubroot using the standard assessments on 0-3 scale described in the bioassay section. Foliar infection (light leaf spot and phoma) were assessed at the same time as the percentage surface area of plant affected.
- In early February or March roots were again assessed for clubroot and foliar infection. Vigour was also scored on a 1-9 scale at this time.
- Just before harvest, roots were assessed for clubroot as described. Plant survival was also assessed.
- At harvest yield was assessed and adjusted to 91% dry matter.

Soil analysis

Soil analysis was carried out at SAC to determine phosphorus levels using the Modified Morgans method. The original Morgans method (Morgan, 1937) was adapted specifically for acidic Scottish Soils by SAC in the mid 1980's hence the name "Modified Morgans". As the method is commercially sensitive, there has been no publication of it outside SAC. The most appropriate method to use should depend on the actual pH of the soil. On the whole English soils are alkaline and bicarbonate (Sodium Hydrogen carbonate) would be used as the extraction solution. However if bicarbonate is used on an acidic (Scottish type soil) there is a neutralisation effect or acid / base

reaction and so minimal P extraction is obtained. The same theory also applies in reverse, the Modified Morgans reagent is slightly acidic and when used on alkaline soils minimal extraction occurs and so the P value is lower than by using bicarbonate. As there is no real "fix factor" to convert one to the other the Modified Morgans method was used for consistency. From literature it was not anticipated the phosphorus levels would be important in clubroot development and were only carried out as it is part of the routine soil analysis package carried out on soils at SAC.

Trial season 2 - 2008/2009

The same protocol as year one was broadly followed but the lowest rate of LimeX70 (2 t/ha) was dropped and replaced with a combined treatment of LimeX70 and Perlka. The former was applied to plots before cultivation and incorporated. Perlka was applied after soil preparation for drilling to treatments 2, 5 and 6 in table 3, below, and shallow incorporated for treatments 2 and 5. It was not incorporated for treatment 6, where it was left on the soil surface for this treatment. As before there were two control treatments, one untreated and a further control to balance the nitrogen in the Perlka calcium cyanamide product. Other inputs were as per local practice. The varieties tested were Kommando and Mendel. Plot size was 30 m^2.

Table 3. Soil amendments tested in field trials 2008/2009 season.

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

Total – 8 treatments x 2 varieties x 4 replicates = 64 plots

Assessments:

Before treatments applied

- As per trial season 1.

Establishment

- As per trial season 1.

In season assessments

- As per trial season 1.

- At harvest yield was assessed and adjusted to 91% dry matter. Soil was sampled from each replicate treatment and then bulked across replicates and then tested for clubroot using bioassay and PCR, pH and soil nutrients (16 samples per trial).

Trial season 3 – 2009/2010

The protocols from trial seasons 1 and 2 were adapted and only the most effective treatments selected. A second clubroot resistant variety, Cracker, was introduced and tested alongside Mendel and Kommando. Two sites were established, one in Aberdeenshire and one in Warwickshire. The Aberdeenshire site had high levels of oilseed rape volunteers such that the varietal component to the trial had to be abandoned and the Kommado treatments only were assessed and analysed at this site. Soil amendment treatments were adjusted from the previous seasons' protocols as follows: there was only one control treatment and the second control balancing the nitrogen was dropped to accommodate the extra varietal element. The soil incorporated Perlka treatments were also dropped, leaving treatment 4 where it was not incorporated, as there had been no demonstrable benefit from incorporating Perlka into the soil prior to drilling in the preceding four trials. Plot size was 30 m^2. Treatments were applied as described for the previous trial season.

Table 4. Soil amendments tested in field trials 2009/2010 season.

Treatment	Product and rate
1.	Untreated control
2.	Calcium carbonate 4 t/ha
3.	Calcium carbonate 8 t/ha.
4.	Calcium cyanamide 250 kg/ha
5.	Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha
6.	Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha

Total – 6 treatments x 3 varieties x 4 replicates = 72 plots

Assessments:

Before treatments applied

- As per trial season 1.

Establishment

- As per trial season 1.

In season assessments

- As per trial season1.
- At harvest yield was assessed and adjusted to 91% dry matter. Soil was sampled from each replicate treatment and then bulked across replicates and then tested for clubroot, pH and soil nutrients (18 samples per trial).

Statistical analysis of field trial data

Analysis of variance was conducted using GenStat 13th edition. Split-plot analyses were used for the individual trials, since soil treatments were applied to whole plots within the four replicates, and varieties were analysed as sub-plots: treatment and variety effects and their interactions were included.

For the combined analyses over trials, there were potentially trial-by-variety, trial-by-treatment, treatment-by-variety, and trial-by-variety-by-treatment interactions. Because the data were incomplete, only trial-by-variety interactions were included in the analysis. These analyses used REML, and allowed residual variances to differ between trials.

3.2.4. Climate change impact on disease severity

Aim: To predict the likely severity of clubroot under standard climate change predictions.

CLIMEX methodology

The potential distribution of clubroot in the UK under current and projected climates were estimated using CLIMEX™, a computer simulation program sold by Hearne Scientific Software that is widely used to estimate potential distribution and abundance of pest species based on climatic conditions (Sutherst & Maywald, 1985; Sutherst *et al.,* 2007). Turkington *et al.* (2004) developed a CLIMEX species-specific model for clubroot based on temperature and moisture parameters from previously published information and estimated from disease occurrence in Canadian epidemic areas.

The parameters used by Turkington *et al.* (2004) have been modified to take into account recent publications on the development of clubroot at different temperatures (Sharma *et al*., 2011; Gossen *et al*., 2012), and personal communication from Kelly Turkington at Agriculture & Agri-Food Canada, to develop an updated clubroot model for use in CLIMEX.

The following temperature parameters were used in the CLIMEX model:

Limiting low temperature (DV0)	8°C
Lower optimal temperature (DV1)	20°C
Upper optimal temperature (DV2)	26°C

Limiting high temperature (DV3) 32°C

Another parameter (PDD) is the number of degree days above the lower temperature threshold for population growth (DV0) that is required to complete an entire generation of clubroot. A value of 300 for PDD was recommended by Kelly Turkington based on his modelling using CLIMEX.

Discussion with Kelly Turkington and his colleagues suggests that winter stress (cold, heat, wet stress) is not likely to play a large role in terms of clubroot survival. Given the world wide occurrence of the clubroot pathogen in climates that range from cold (e.g. Canada and Sweden) to warm (e.g. some locations in Australia) resting spores can likely survive a range of cool to hot and wet to dry conditions. Consequently, these stress parameters within the CLIMEX model were not used.

The soil moisture parameters used within the clubroot CLIMEX model are based on the observation by Monteith (1924), and subsequently adopted by Turkington *et al.* (2004), that resting spore germination can occur as low as 45% of soil water-holding capacity.

The following soil moisture parameters were used in the CLIMEX model:

Limiting soil moisture (SM0)	0.4
Lower optimal soil moisture (SM1)	0.6
Upper optimal soil moisture (SM2)	1.5
Limiting soil moisture (SM3)	5.0

(SM = 0 indicates no soil moisture; SM = 0.5 indicates soil moisture content is 50% of capacity; SM = 1 indicates that the soil moisture content is 100% of capacity; SM > 1 indicates a water content greater than the soil holding capacity of (Smax) i.e. run-off)

The CLIMEX software includes a climate-matching function that can be used to directly compare the temperature, rainfall, rainfall pattern and relative humidity of a given location with any number of other locations, usually a location where that species is endemic and a perennial problem - i.e. a 'hot spot'. Within the UK, the location of Bacup in Lancashire (Latitude 53.71; Longitude – 2.20) was chosen to represent an area in the UK where clubroot is particularly problematic, and represents a ‘hot spot’ for this disease.

The historical meteorological data used in CLIMEX is sourced from the WorldClim and the Climate Research Unit (CL1.0 and CL2.0) datasets. Full details of how this data has been reformatted, adjusted and recombined to generate all of the required variables are detailed in Kriticos *et al*

(2012). This data (10' resolution (approximately 100 km^2 grids)) was downloaded from the CLiMond (www.climond.org) website pre-formatted for use in CLIMEX.

For future climate change scenarios, data from the A1B Global Climate Model was used to create estimates of the future climate for a range of future dates. Full details can be found in Kriticos *et al.* (2012). This data (10' resolution) was downloaded from the CLiMond (www.climond.org) website pre-formatted for use in CLIMEX. . The A1B model is defined by the Inter Governmental panel on Climate Change (IPCC) as describing a future world of very rapid economic growth, global population that peaks in mid-century and declines thereafter, and the rapid introduction of new and more efficient technologies. Major underlying themes are convergence among regions, capacity building and increased cultural and social interactions, with a substantial reduction in regional differences in per capita income. The A1 scenario family develops into three groups that describe alternative directions of technological change in the energy system. The three A1 groups are distinguished by their technological emphasis: fossil-intensive (A1FI), non-fossil energy sources (A1T) or a balance across all sources (A1B) (where balanced is defined as not relying too heavily on one particular energy source, on the assumption that similar improvement rates apply to all energy supply and end use technologies).

Climate matching

The current climate for Bacup in Lancashire was matched with the current, 2030 and 2050 projected climates (A1B Global Climate Model) using the CLIMEX 'Match Climates' functionMaps showing the match of soil moisture between the dates of 1st October and 4th March to Bacup were prepared.

3.2.5. Development of quantitative a molecular test for clubroot

Aim: To develop a real-time PCR detection of *Plasmodiophora brassicae* from soil and plant material

Resting spore extraction and purification from frozen clubroot gall tissue.

The following procedure was adapted from the methods in Möller and Harling (1996). Frozen root galls were thawed and placed into a 2 % sodium hyperchlorite solution for 15 minutes to remove any contaminating endophytic bacteria. Tissue from individual galls was rinsed with distilled water and placed into ball mill canisters containing two ball-bearings for homogenisation (Retsch MM200 ball mill; programmed for a vibration frequency of 30 reps/second for 30 seconds, which was suitable to completely homogenise up to 2 ml material). Five mls of ice cold homogenisation buffer (0.25 mol l^{-1} sucrose, 20 mmol l^{-1} MOPS at pH 7.5, 2.5 mmol l^{-1} EDTA, 0.2 % BSA) was added to the material and the resulting solution was filtered through sterile Whatmann No. 1 filter paper into disposable sterile Bűchner funnels, rinsed through with an additional 5 mls of buffer. The resulting

filtrate was split into four 2 ml Eppendorf tubes. Large starch grains were removed from the filtrate by centrifugation at 100 g for 5 minutes. The supernatant was retained and centrifuged at 1700 g for 5 minutes to pellet out the resting spores. Any residual small starch grains were removed by centrifugation at 13,000 g for 15 seconds with repeated re-suspension of the spore pellet in Triethanolamine (TEA, 40 mmol l^{-1} Tris-HCl at pH 7.6, 20 mmol l^{-1} sodium acetate, 2 mmol l^{-1} EDTA). Contaminating host proteins were removed with a 1 hour incubation (37^{o}C) with Proteinase K (0.01 mol l^{-1} Tris-HCl at pH 7.8, 5 mmol l^{-1} EDTA, 0.125% SDS, 20 mmol l^{-1} DTT, 0.05 mg ml^{-1} proteinase K, followed by 5 minutes at 85^{o}C to destroy enzyme activity. Following proteinase treatment, spores were washed three times in TE (10 mmol l^{-1} Tris HCl at pH 7.8, 0.1 mmol l^{-1} EDTA solution before being stored in 50 µl of sterile distilled water (SDW) and at 4^{o}C until further use.

DNA extraction from resting spores

The re-suspended spores were transferred to sterile 2 ml tubes (Eppendorf tubes) containing Ballotini beads and 440 µl of TENS extraction buffer (0.1% (v/v) β-mercaptoethanol, 250 mM NaCl, 200 mM Tris-HCl, 25mM EDTA, 2% (w/v) polyvinylpyrrolidone (PVP), 5mM Phenanthroline monohydrate). Samples were then agitated in a Fastprep FP120 Cell disrupter for three 40 second repetitions at a speed setting of 6.0 with the samples being cooled on ice in between repetitions to prevent overheating. 400 µl of 2 % sodium dodecyl sulphate (SDS) was added, the tubes were vortexed briefly and then incubated at 65^{o}C for 30 minutes. 800 µl of phenol:chloroform:isoamyl mix (25:24:1) was added to each tube before centrifugation for 10 minutes at 13,000 rpm. The resultant aqueous phase was transferred to fresh 1.5 ml tubes along with 40 µl 7.5 M ammonium acetate and 600 µl isopropanol and the samples were stored at -20^{o}C overnight. The samples were centrifuged for 20 minutes at 13,000 rpm with the supernatant then being discarded and the DNA pellet being washed in 600 µl 70 % ethanol. The ethanolic supernatant was poured off and the DNA pellet was dried in a centrifugal evaporator. The DNA was re-suspended in 50 µl SDW and quantitative measurements were taken using a Nanodrop spectrophotometer. The DNA was diluted to 10 ng $µl^{-1}$pior to storage at -20^{o}C.

DNA extraction from soil

Soil samples were oven dried and sieved through a 2 mm mesh, weighed (60 g for each sample) and placed in a milling cup along with 120 ml of sterile CTAB-EDTA buffer (20 g CTAB (Hexadecyltrimethyl-ammonium bromide), 100 ml of 1 M Tris-HCl (pH 8.0), 40 ml of 0.5 M EDTA, 280 ml of 5 mM NaCl, 30 g of PVP-40, 1.0 g of Dithiothreitol, 580 ml deionised water). To each of these cups 15 ball bearings were then added. The samples were then milled at 300 revolutions per minute (rpm) in the Retsch planetary ball mill. Four aliquots were taken from each sample and transferred to a 2 ml Eppendorf tube. The samples were then centrifuged at 14,000 rpm for 15 minutes and the resulting supernatant transferred to a clean 2 ml Eppendorf tube. Nine Hundred µl

of chloroform was added and mixed thoroughly before centrifugation at 14,000 rpm for 10 minutes. The resulting supernatant was then added to 2 ml Eppendorf tube containing 90 µl 3M sodium acetate and 900 µl isopropanol, to precipitate the DNA. After incubating on ice for 1 hour the samples were centrifuged at 14,000 rpm for 20 minutes. The supernatant was removed and 500 µl 70 % ethanol (ice cold) was added before centrifuging again at 14,000 rpm for 15 minutes. The ethanol was removed and the DNA pellet was dried in the 37 ^{o}C incubator, before adding 150 µl of DNA free water to re-suspend the DNA. The samples were then further processed to help to remove some of the humic acids which are present in soil samples by putting the sample through a spin column containing polyvinylpyrrolidone (PVP-40). Each of the Bio-spin columns (BioRad, CA. USA) were loaded with 15 mm of PVP, each column was primed before use by loading with 150 µl of DNA free water and centrifuging at 5,000 rpm for 5 minutes. The columns were then loaded with the DNA solutions and centrifuged as before. The collecting tube then contains the purified soil DNA sample which was then quantified using a Nanodrop spectrophotometer ND-1000 before diluting the samples to 10 ng/µl. The samples were then stored at -20^{o}C.

DNA extractions directly from roots

Plant material was placed in a pestle and mortar and ground to a powder in liquid nitrogen. To this powder, DNA extraction buffer (0.1 % (v/v) β-mercapthoethanol, 250 mM NaCl, 200 mM Tris-HCl, 25 mM EDTA, 2 % (w/v) SDS, 2 % (w/v) polyvinylpyrrolidone and 5 mM 1, 10-phenanthroline monohydrate; pH 8.0) was added. Enough extraction buffer was added to produce a pouring consistency, the amount used varied according to the sample. An aliquot of 800 µl of sample/extraction buffer was transferred to a 1 ml tube and stored at –20^{o}C until all the samples were ready to be further processed. Samples were then incubated for 30 minutes at 70^{o}C in a water bath, then 800 µl of ice-cold ammonium acetate (7.5 M) were added and the suspension kept on ice for 30 mins. The suspension was then centrifuged for 10 minutes at 14,000 rpm and the supernatant transferred to a fresh 2 ml tube (Eppendorf tubes) containing 600 µl of ice-cold isopropanol. The tubes were inverted several times to mix and stored at room temperature for 15 minutes. The tubes were again centrifuged at 14,000 rpm for 10 minutes and the supernatant removed, leaving a DNA pellet. The pellet was washed by adding 600 µl of 70 % ice-cold ethanol, centrifuging at 14,000 rpm for 10 minutes and then removing the ethanol. The pellet was allowed to dry in a fume cupboard for 10 minutes and re-suspended in 100 µl of sterile distilled water and stored at -20^{o}C until further use.

Amplification of the ITS region from clubroot isolates

This was carried out using a range of resting spores from 15 different locations throughout England and Scotland using soils received for commercial testing at SAC.

Sequencing PCRs were carried out to check for the uniformity of the ITS sequence. This was performed in an GeneAmp 9700 PCR System (Applied Biosystems, Foster City, CA. USA) with each PCR mixture (final volume, 50 µl) containing 5 µl of 10 X Buffer, 10 µg BSA, 0.5 µM of each primer (1406F 5'-tgyacacaccgcccgt-3', 3126T 5'-atatgcttaagttcagcgggt-3'), 200 µM of dNTP's, 4 U of GO-Taq DNA polymerase (Promega, Madison, WI, USA.), and 50 ng of DNA. The conditions for the PCR reactions were: 94°C for 3.5 minutes, followed by 40 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 1.5 minute PCR was terminated at 72°C for 8.5 minutes. PCR products were then separated on gel red stained 1.5 % (w/v) agarose gels run in 0.5 x Tris-borate-EDTA buffer and exposed to UV light to visualize DNA fragments. Following PCR the excess primers were removed with the High pure PCR product purification kit (Roche diagonostics, Germany) and the PCR products directly ligated into pGEM-T easy vector (Promega) according the manufactures protocol. Plasmids were transformed in *Escherichia coli* JM109 cells (Promega) according to a standard protocol (Sambrook *et al.,* 1989), and plasmid DNA extracted using the Wizard PCR clean system (Promega), finally the nucleotide sequence was determined in a dideoxy chain termination method reaction (Sanger *et al.,* 1977). This procedure is required to check that the target sequence for any detection methodology does not differ in some individual strains of the pathogen.

These results are not shown as the sequence was uniform between all the isolates tested and in this section of the ITS fragment, no variation was seen. The data have not been put in the EBI or other databases as yet. This result was not unexpected as ITS sequences are not that variable in certain sections in this pathogen, which is why ITS has been used as a target for other groups (Wallenhammer, 2012).

Primer and probe design

A set of Taqman probe and primers were designed using the ribosomal repeat and internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) GenBank accession number AB094979 and the amplified sequences for a range of other strains from across the UK. All strains that were sequenced had the same sequence as accession number AB094979.

This region of the DNA sequence is widely used for specific detection of fungal species, and has been widely used in previous detection methods for *P. brassicae* (Moller and Harling, 1996). However, all of these methods do not provide quantitative information on the levels of infection, merely if the pathogen is present in the soil or not. The TaqMan probe and primers set were designed by aligning similar sequence data together so as to design highly specific primers and probes that only amplify the target *P. brassicae* sequence. The chosen probes and primers were then checked using primer express 1.5 software (PE, Applied Biosystems). The specificity of all primers and probes used was also checked using the National Center for Biotechnology

Information BLAST server and confirmed by PCR testing against a panel of fungi, bacteria and plant material. The absence of signals and PCR products in real-time PCR or gel electrophoresis respectively, confirmed the specificity of the assays.

Real-time PCR assays

The amount of clubroot was quantified in DNA extracted from soil samples. Each reaction was performed consisting of 50 ng of total DNA containing 12.5 µl of PCR master-mix (60 U/ml Platinum® *Taq* DNA polymerase, 40 mM Tris-HCl, 100 mM KCl, 6 mM $MgCl_2$, 400 µM dGTP, 400 µM dATP, 400 µM dCTP, 800 µM dUTP, 40 U/ml UDG and stabilizers (Invitrogen Ltd, Paisley, UK), 600 nM of clubroot forward primer (5'- cccatatccaacccca tgt – 3'), 200 nM of clubroot reverse primer (5' - cgaaacacaactaaagttcca- 3'), 200 nM fluorogenic probe (5'-ctgcgtgtttcattttcgaac -3'), 0.375 µl of 50 x ROX reference dye (Invitrogen Ltd) and adjusted to a final volume of 25 µl using sterile distilled water. The reactions were carried out in duplicate in capped Thermo-Fast® 96-well non-skirted reaction plates (ABgene). Amplification and detection was performed in a Stratagene MX 3000P real-time PCR machine (Stratagene, La Jolla, California, USA) under the following conditions:1 cycle at 50^{o}C for 2 minutes and then 1 cycle at 94^{o}C for 2 minutes, followed by 50 cycles of 95^{o}C for 15 seconds and 60^{o}C for 1 minute. The increase in sample fluorescence was measured throughout the reaction. For each sample, the threshold cycle (Ct; cycle at which increase of fluorescence exceeded the background) was determined.

Standard curves were generated for each real-time PCR experiment by plotting known amounts of *P. brassicae* DNA against C_t values. This was done by spiking cabbage leaf DNA samples (50 ng of total DNA) with 10 different amounts of genomic *P. brassicae* DNA obtained from resting spores (Fivefold dilution series from 80 ng to 0.04 pg). A minimum standard for standard curves was set at an R^2 value of >0.95 Two negative controls, Chinese cabbage DNA (50 ng in total) and sterile distilled water were used in each real-time PCR run. Samples detected earlier than controls were regarded as positive. The data obtained against the standard allowed for the determination of the initial amount in the original samples to be determined this data is presented in the results section.

3.3. Results

3.3.1. Survey results (bioassay method)

The survey results showed that in 2008 57% of soils tested positive for clubroot and in 2009 40% were positive, with a total mean of 52%. Infection levels across English and Scottish samples were similar – 50% (26/52) in the Scottish samples and 55% (24/44) in the English samples. The mean clubroot infection index after bioassay testing was 26.6% range 1.7 to 100%). The mean pH of soils was 6.5 (range 5.7 – 8.2). The mean extractable calcium level was 2564 mg/l (range 894 – 21800). There was no significant correlation between these parameters and the disease index for infected soils. A summary of the extractable phosphorous, potassium, magnesium and calcium levels in soils that tested positive is shown in Table 5.

Table 5. Survey results for positive sites (determined by bioassay) in 2008 and 2009 showing clubroot disease index and extractable phosphorus, potassium, magnesium and calcium levels.

Variable	Disease	pH	P mg /l	K mg/l	Mg mg/l	Ca mg/l
Number	50	40	40	40	40	40
N missing	0	10	10	10	10	10
Mean	26.6	6.5	14.8	187.0	135.0	2564.0
SE mean	3.84	0.09	2.15	11.4	12.20	510.00
St. Dev.	27.20	0.54	13.60	72.10	77.30	3225.00
Minimum	1.67	5.70	2.20	61.50	34.80	894.00
Lower Quartile	5.00	6.00	7.03	132.00	81.80	1520.00
Median	17.9	6.4	11.2	188.0	124.0	1915.0
Upper quartile	42.8	6.7	18.8	228.0	171.0	2695.0
Maximum	100.0	8.2	76.6	393.0	422.0	21800.0

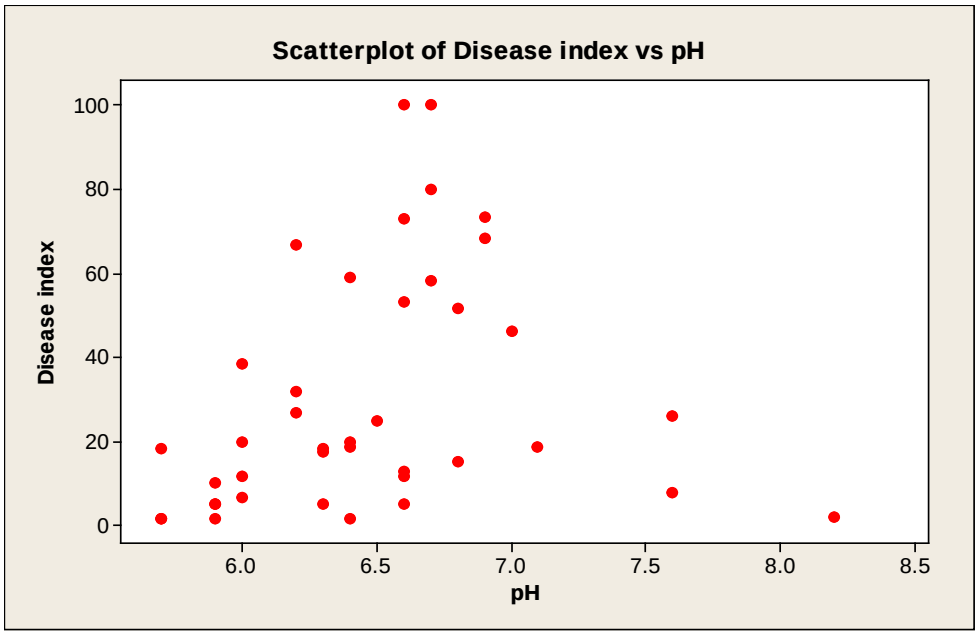

Figure 3. Clubroot index determined by bioassay from survey sites in 2008 and 2009 against soil pH.

There was no correlation between disease severity and soil pH for the positive survey sites shown in figure 3 (r=0.223, *P*=0.168).

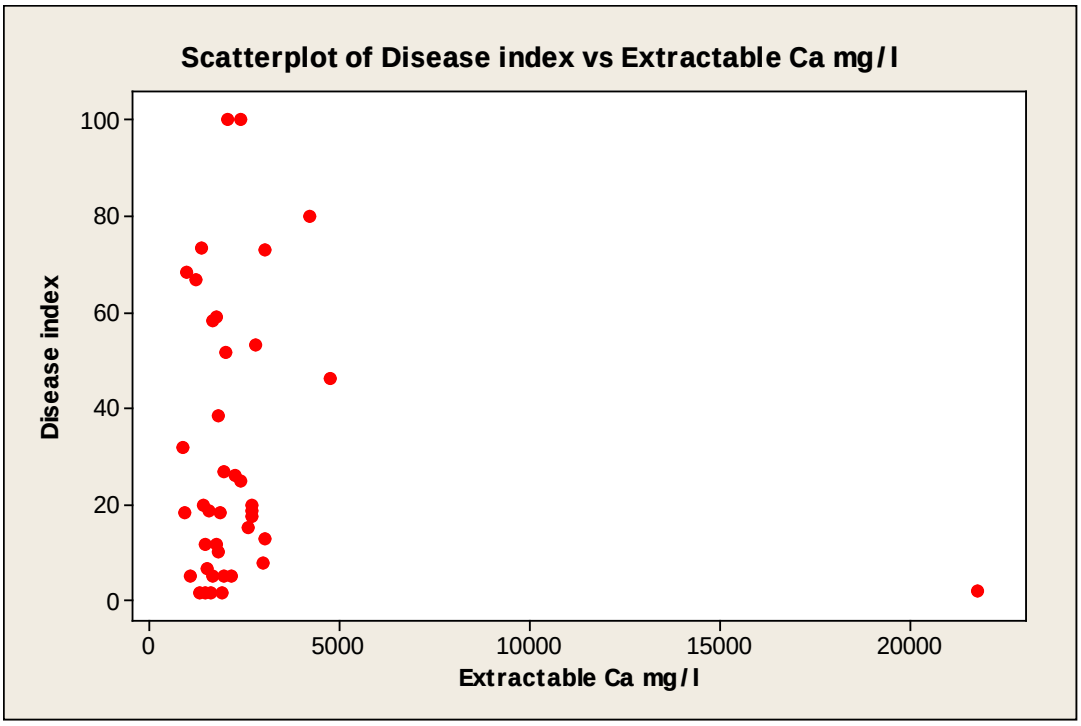

Figure 4. Clubroot index determined by bioassay in postitive survey soils in 2008 and 2009 against extractable calcium values (mg/l).

There was no significant correlation between clubroot index and extractable calcium values, shown in figure 4, even with the outlier value removed (r= -0.1303, P= 0.525 (with outlier) and r=0.209, *P*=0.202 (without outlier)).

Many samples had low clubroot indices in the bioassay, detailed below in figure 5. Decline over the two years of the survey was minimal (figure 6).

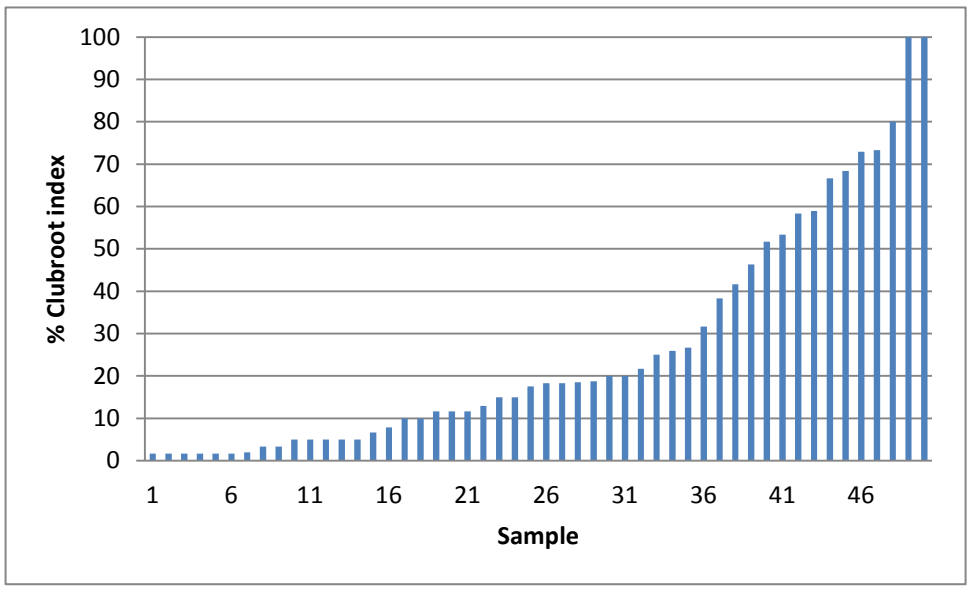

Figure 5. Postive clubroot soil samples sorted by plant bioassay clubroot severity index collected in 2008 and 2008 surveys. Many sites had low infection severities that were subclinical in the field.

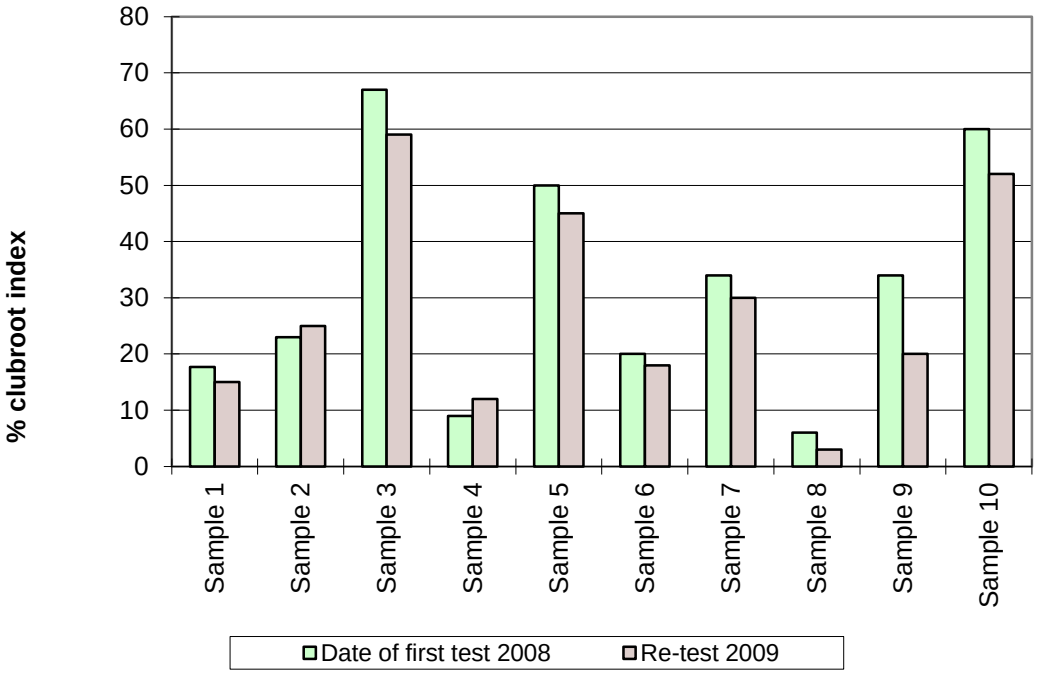

Figure 6. Bioassay results for soil samples retained and tested in consecutive years (2008 and 2009).

Results from survey sites were plotted over maps showing the proportion of land in oilseed rape production in 2009. The negative samples are plotted in figure 7.

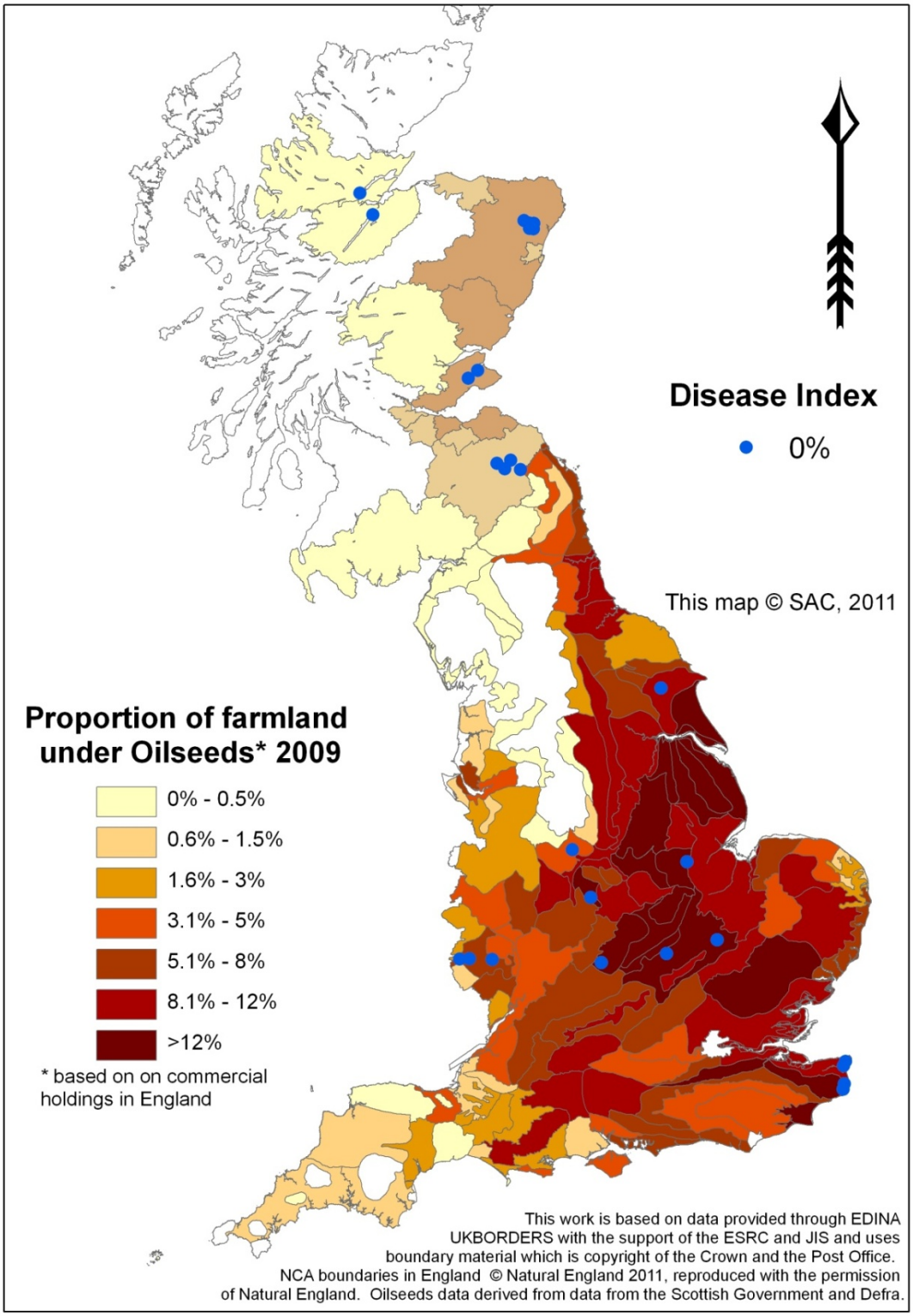

Figure 7. Negative clubroot 2008 and 2009 survey samples, determined by bioassay, plotted over the proportion of land in oilseed rape production.

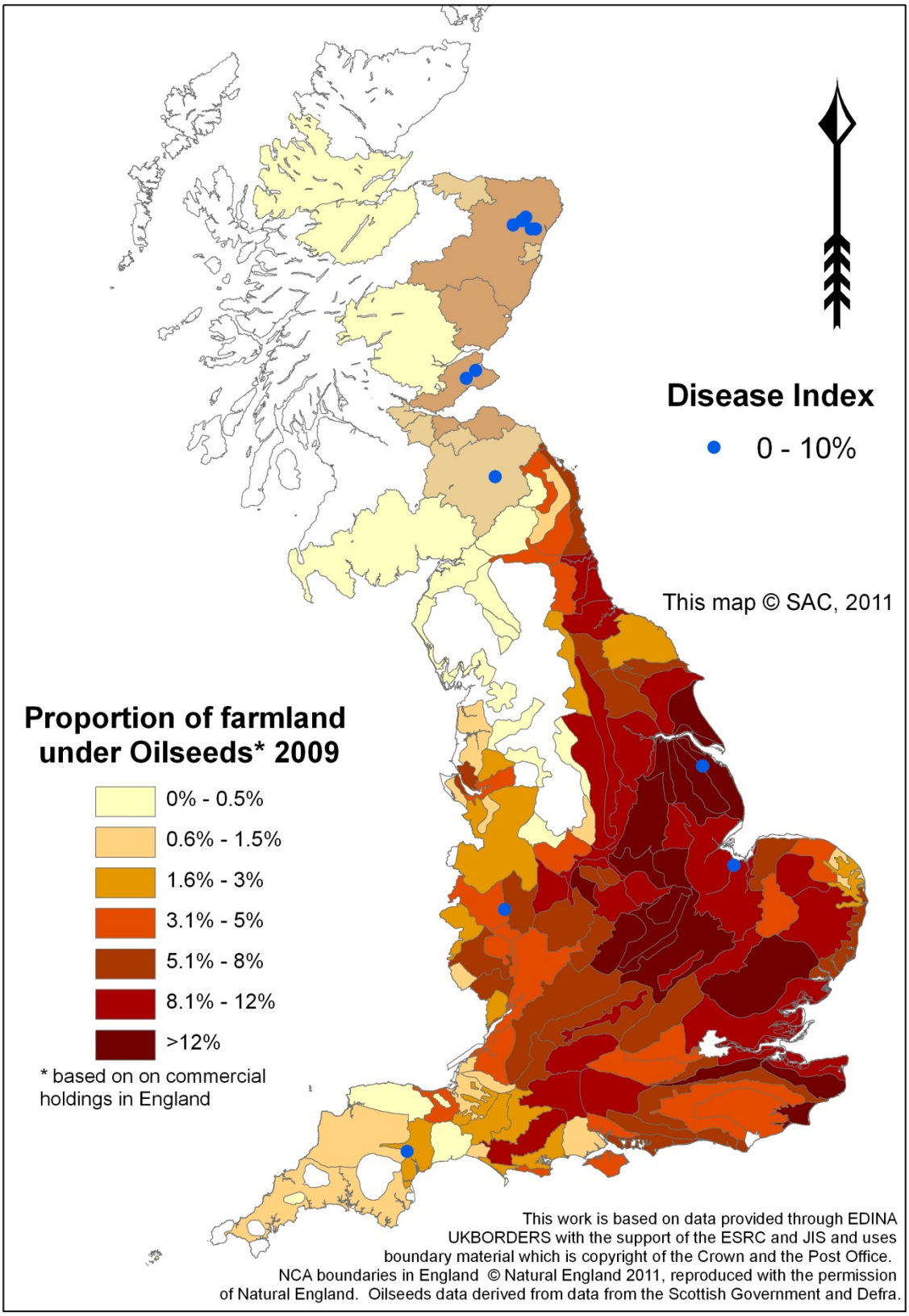

Figure 8. Clubroot 2008 and 2009 survey samples with a disease index <10%, determined by bioassay, plotted over the proportion of land in oilseed rape production.

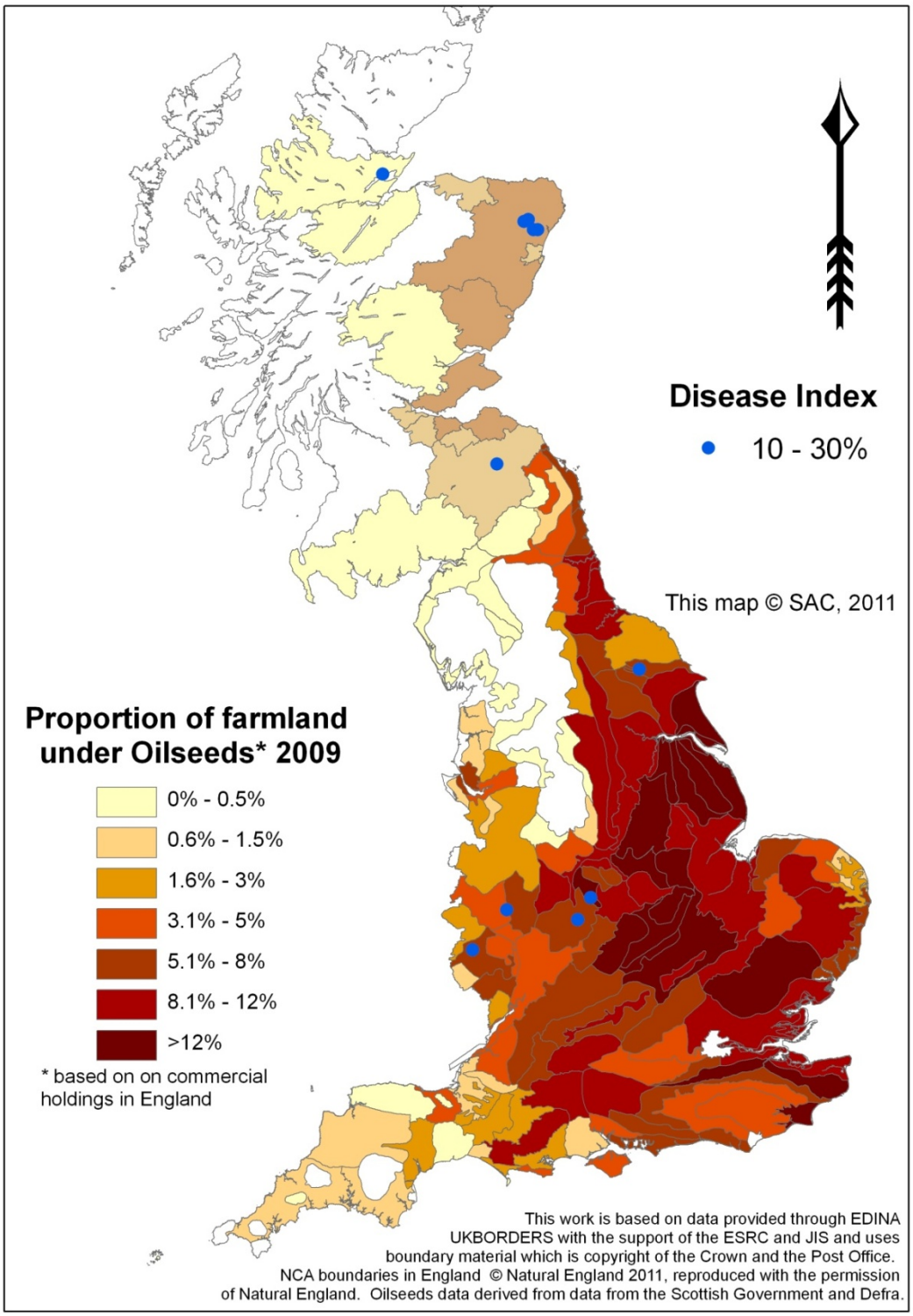

Figure 9. Clubroot 2008 and 2009 survey samples with disease indices of 10 - 30%, determined by bioassay, plotted over the proportion of land in oilseed rape production.

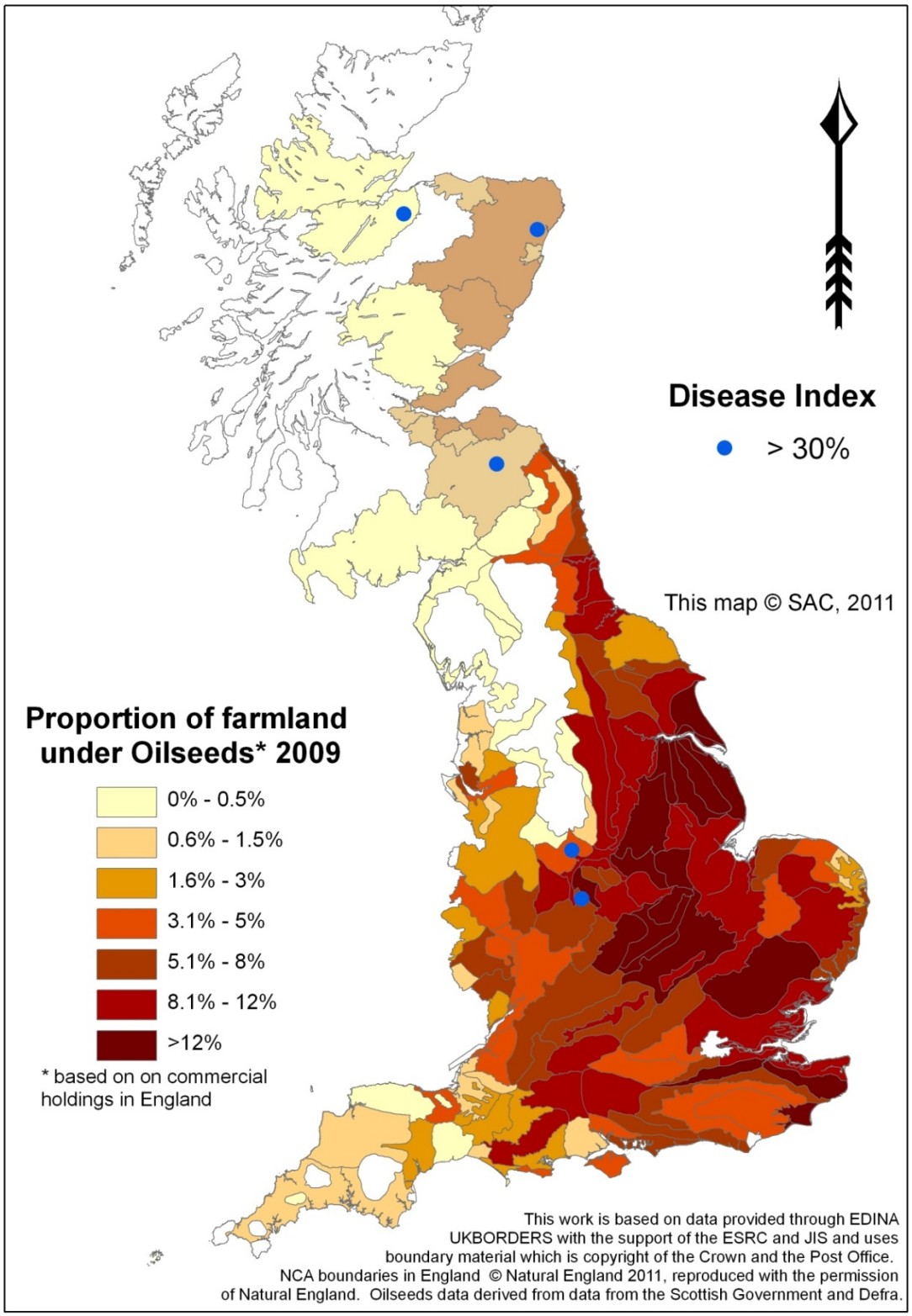

Figure 10. Clubroot 2008 and 2009 survey samples with disease indices of >30%, determined by bioassay, plotted over the proportion of land in oilseed rape production (note that post-project positive samples were received from Kent, near Dover and Somerset, near Bridgwater).

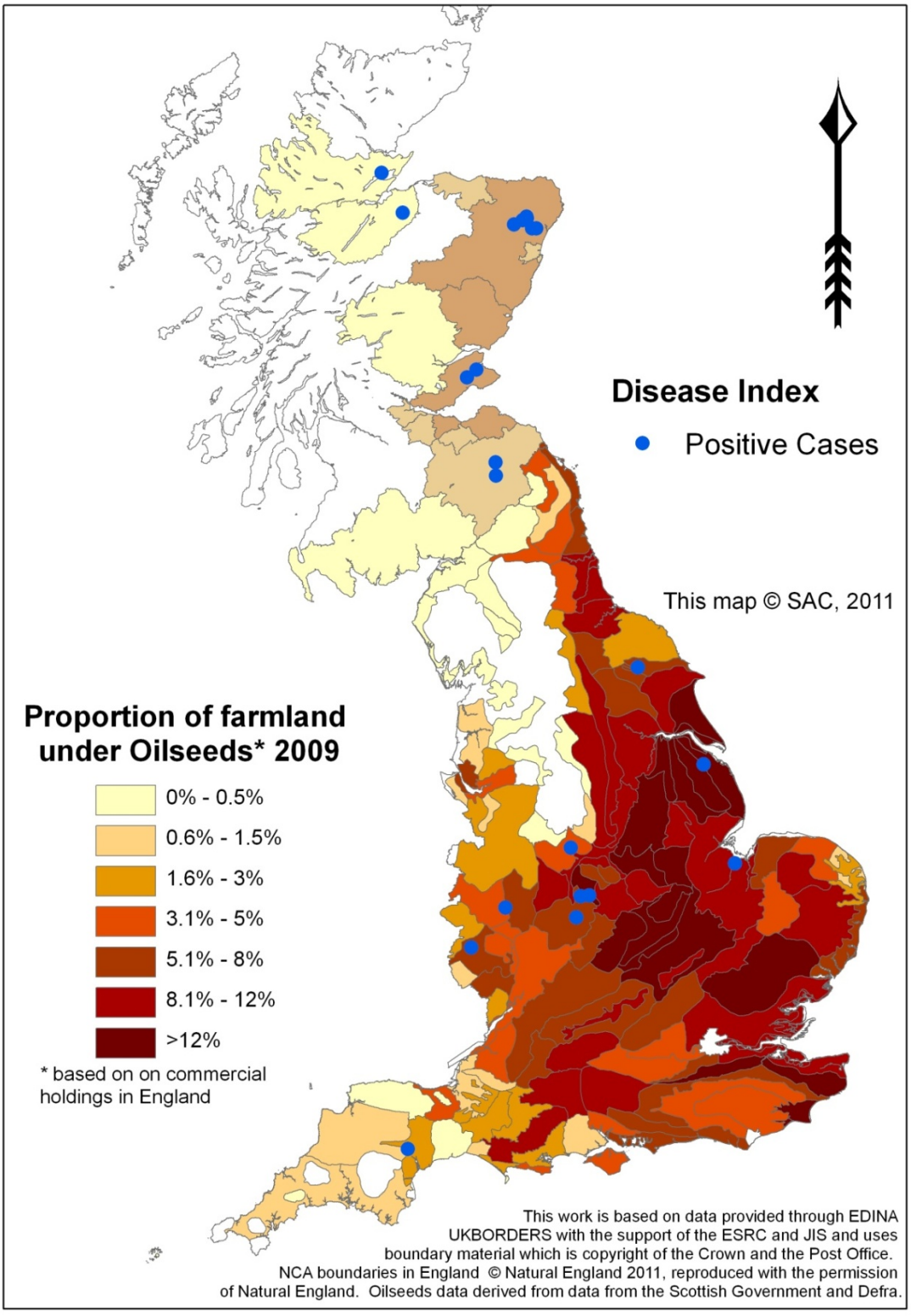

Figure 11. Positive clubroot 2008 and 2009 survey samples plotted over the proportion of land in oilseed rape production (note that post-project positive samples were received from Kent, near Dover and Somerset, near Bridgwater).

Figures 7 to 11 show the locations of the sites surveyed plotted over maps of the proportion of land in oilseed rape production. Clubroot infection is widely distributed throughout the UK and was detected in most production areas tested. The proportion of land in production differs significantly between Scotland and England and the incidence of clubroot infection is similar so no direct correlations could be drawn between infection and intensity of production. In general, the West Midlands and Aberdeenshire remain the regions that had the most numerous cases. However, results were somewhat biased by the location of participants in the survey: multiple samples were received for some areas such as Kent and very few for others.

3.3.2. Bioassay variety testing

The bioassay results, shown in table 6, indicated that Mendel was significantly better for clubroot control than all the other varieties tested and that there was no significant difference in susceptibility between the other varieties. The range in clubroot severity for the other varieties tested was 65.0 (for Flash) to 87.5 (for NPZ0527). The mean index (excluding Mendel and the un-inoculated control) was 74.1.

Table 6. Oilseed rape varieties tested for susceptibility to clubroot in a bioassay.

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

The intention had been to continue any promising tolerance results into field trials, funded by breeding companies, but this line of investigation was discussed with breeders and not pursued on the basis of the above results.

3.3.3. Field trial results

Clubroot disease in trials was assessed as both % incidence and % index which gives a measure of severity. One was a good predictor of the other and the correlation between the two measure is shown below in figure 12, and was highly significant (Pearson correlation value = 0.961 and P value <0.001). The % incidence value though did not differentiate samples from high disease severities so while the severity index kept increasing there was a flattening of incidence values for those samples at the top end of the index scale at this extreme, so severity (% index) is reported in the results and discussion that follow.

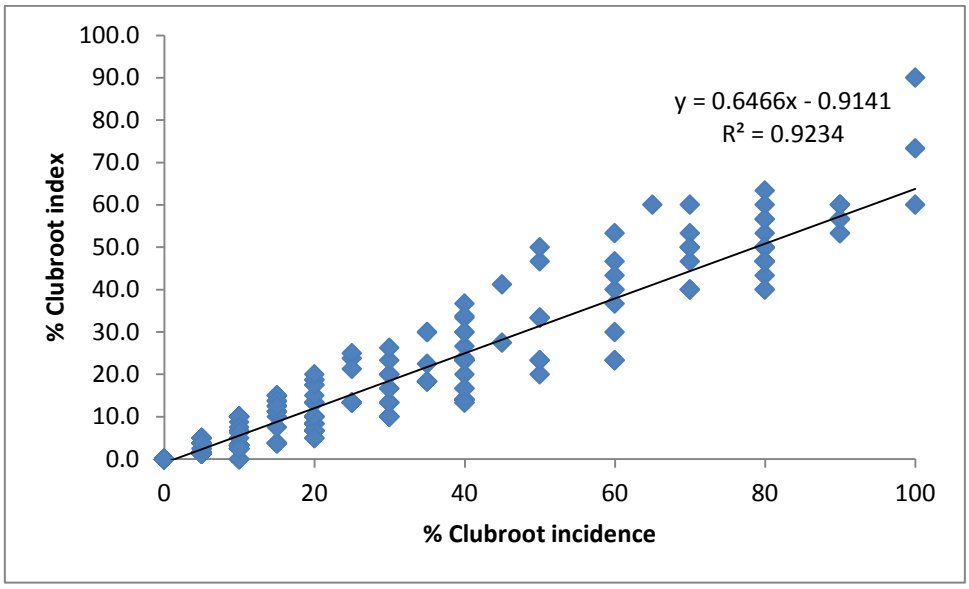

Figure 12. The correlation between % index and % incidence of clubroot in the field trial data set.

2007 – 2008 Field trials

Two field trials were carried out, one in Shropshire and one in Aberdeenshire. High levels of disease developed at the Shropshire site only. Disease levels, pH, and the efficacy of control measures either through the use of the resistant variety Mendel or through the use of soil treatments were variable and differed between the two sites. At the Aberdeen site clubroot indices were low in most plots, possibly as a result of a cold autumn which may have slowed disease development. The site tested positive in a bioassay prior to trial establishment (index = 22.7%) and had a history of severe clubroot infection, but this disease potential was never realised in the trial. The disease indices, were not significantly reduced by Calcium carbonate or Calcium cyanamide treatments of up to 8t/ha or 250 kg/ha respectively and, although there was a trend for some Calcium carbonate treatments to have the lowest clubroot scores, this was confounded by the

control plots also having low scores. There was also a trend for the Calcium cyanamide incorporated treatment to have higher clubroot levels than the non-incorporated treatment, as shown in table 7.

Table 7. Clubroot indices at 2008 Aberdeen site for Kommando and Mendel

Treatment Kommando	% Clubroot index September	% Clubroot index March	% Clubroot index May
1. Untreated control	1.33	0.00	0.00
2. Calcium carbonate 2 t/ha	2.50	5.00	1.67
3. Calcium carbonate 4 t/ha	0.00	0.00	0.00
4. Calcium carbonate 8 t/ha.	1.67	2.50	0.83
5. Calcium cyanamide 250 kg/ha	5.00	2.50	0.83
6. Calcium cyanamide 250kg/ha – not incorporated	0.83	17.5	5.83
7. Control with extra 50 kg/ha nitrogen	0.00	2.50	0.83
8. Boron 20kg/ha	2.50	15.0	5.00
Treatment Mendel			
1. Untreated control	0.00	2.50	0.83
2. Calcium carbonate 2 t/ha	2.33	0.00	0.00
3. Calcium carbonate 4 t/ha	6.67	10.0	3.33
4. Calcium carbonate 8 t/ha.	0.00	10.0	3.33
5. Calcium cyanamide 250 kg/ha	3.33	7.50	2.50
6. Calcium cyanamide 250kg/ha – not incorporated	2.50	5.00	1.67
7. Control with extra 50 kg/ha nitrogen	3.33	5.00	0.00
8. Boron 20kg/ha	5.00	17.5	0.83
LSD (5%) Treat	NS	NS	NS
LSD (5%) variety	NS	NS	NS
LSD (5%) Treat x variety	NS	NS	NS

NS – not significant at 5% level

There was no effect on clubroot severity from variety at this site and levels of clubroot in the Mendel were no lower than in the Kommando plots.

There were significant differences in yield, and Calcium carbonate was the highest yielding treatment, with a dose response evident to this treatment in the variety Mendel, but not in Kommando, as shown in table 8. None of the other treatments significantly improved yield.

Table 8. Yield (t/ha) corrected to 91% dry matter for Aberdeen 2008 site

	Kommando	**Mendel**
1. Untreated control	2.10	2.08
2. Calcium carbonate 2 t/ha	1.77	1.80
3. Calcium carbonate 4 t/ha	2.58	2.50
4. Calcium carbonate 8 t/ha.	2.53	2.81
5. Calcium cyanamide 250 kg/ha	1.97	1.83
6. Calcium cyanamide 250kg/ha – not incorporated	1.68	2.04
7. Control with extra 50 kg/ha nitrogen	2.04	2.27
8. Boron 20kg/ha	1.86	1.82
LSD (5%) Treat	NS	0.612
LSD (5%) variety	NS	NS
LSD (5%) Treat x variety	NS	NS

NS – not significant at 5% level

There were no significant correlations between clubroot disease and the soil parameters, pH and extractable calcium content. The soil pH and extractable calcium was measured at three points in the season. One measure was taken per replicate block, at sowing and later in the season samples were bulked across treatments but kept separate across the two varieties. These have been meaned for analysis and the standard deviation given in Table 9. An analysis of variance was applied treating the two varieties as replicate values but differences were not significant.

Table 9. Soil pH and extractable calcium (mg/l) for Aberdeen 2008 site

	Sowing		**February**		**Harvest**	
	pH	Ca	pH	Ca	pH	Ca
1. Untreated control	6.8	1858	5.95	1445	6.10	1650.0
2. Calcium carbonate 2 t/ha	-	-	6.45	1915	6.10	1380.0
3. Calcium carbonate 4 t/ha	-	-	6.40	1800	6.10	1500.0
4. Calcium carbonate 8 t/ha.	-	-	6.80	3785	6.00	1410.0
5. Calcium cyanamide 250 kg/ha	-	-	6.80	2385	6.60	2230.0
6. Calcium cyanamide 250kg/ha – not incorporated	-	-	6.15	1575	6.20	2140.0
7. Control with extra 50 kg/ha nitrogen	-	-	6.10	1445	5.95	2600.0
8. Boron 20kg/ha	-	-	6.05	1370	5.95	1440.0
St Dev	0.311	344	0.452	818	0.363	844
ANOVA	-	-	NS	NS	NS	NS

NS – not significant at 5% level

At the Shropshire site disease levels were very high and control was variable. There were no significant differences in disease control as a result of soil treatments, shown in table 10, although some non-significant trends were observed. Calcium carbonate at 4t/ha and 8 t/ha tended to reduce clubroot compared to the untreated control. Calcium cyanamide at 250 kg /ha not

incorporated also reduced clubroot by up to 50%. Severely affected plants died from autumn onwards and spring assessments were done on surviving plants.

At the Shropshire site significant control was noted with Mendel (56 - 85% control) compared to the susceptible Kommando. Phytophthora root rot (*Phytophthora megasperma*) caused a severe root rot and plant losses over-winter at this site. This disease was found at other wet or poorly drained sites with clubroot. Some clubroot sites may be affected by a combination of different pathogens and this would merit further study.

Table 10. Clubroot indices at 2008 Shropshire site for Kommando and Mendel

Treatment	**% Clubroot index**	**% Clubroot index**	**% Clubroot**
Kommando	**Dec**	**March**	**index**
			June
1. Untreated control	69.8	46.5	62.5
2. Calcium carbonate 2 t/ha	64.0	43.0	43.3
3. Calcium carbonate 4 t/ha	66.8	42.5	43.8
4. Calcium carbonate 8 t/ha	37.0	38.5	22.7
5. Calcium cyanamide 250 kg/ha	77.8	38.2	60.9
6. Calcium cyanamide 250kg/ha – not incorporated	80.2	47.2	32.8
7. Control with extra 50 kg/ha nitrogen	89.2	52.0	23.4
8. Boron 20kg/ha	40.2	27.8	40.6
Treatment			
Mendel			
1. Untreated control	8.8	16.2	3.1
2. Calcium carbonate 2 t/ha	4.0	19.0	3.1
3. Calcium carbonate 4 t/ha	5.5	7.2	2.3
4. Calcium carbonate 8 t/ha.	1.5	14.0	4.7
5. Calcium cyanamide 250 kg/ha	4.5	12.5	16.4
6. Calcium cyanamide 250kg/ha – not incorporated	12.8	22.0	7.8
7. Control with extra 50 kg/ha nitrogen	2.5	16.2	23.4
8. Boron 20kg/ha	7.0	28.8	13.3
LSD (5%) Treat	NS	NS	NS
LSD variety	13.6	9.31	13.7
P value	<0.001	<0.001	<0.001
LSD (5%) Treat x variety	NS	NS	NS

NS = non significant at 5% level

There was a significant yield benefit at this site from the Mendel resistance – yield in the Kommando untreated controls was only 1.04 t/ha compared to 3.41 in the Mendel (table 11 and figure 13). Only the 8 t/ha Calcium carbonate treatment showed a significant increase in pH and only the 8 t/ha of Calcium carbonate and Calcium cyanamide (not incorporated) treatments increased yield, although this was not statistically significant. Yields were negatively correlated with clubroot severity at both sites.

Table 11. Yield (t/ha) corrected to 91% dry matter for Shropshire 2008 site

	Kommando	**Mendel**
1. Untreated control	1.04	3.41
2. Calcium carbonate 2 t/ha	0.90	3.14
3. Calcium carbonate 4 t/ha	1.03	3.01
4. Calcium carbonate 8 t/ha.	2.14	3.14
5. Calcium cyanamide 250 kg/ha	0.86	3.49
6. Calcium cyanamide 250kg/ha – not incorporated	1.63	3.78
7. Control with extra 50 kg/ha nitrogen	0.63	2.97
8. Boron 20kg/ha	1.54	3.68
LSD (5%) Treat	NS	NS
LSD (1%) variety	NS	0.481
LSD (5%) Treat x variety	NS	NS

NS = not significant at 5% level

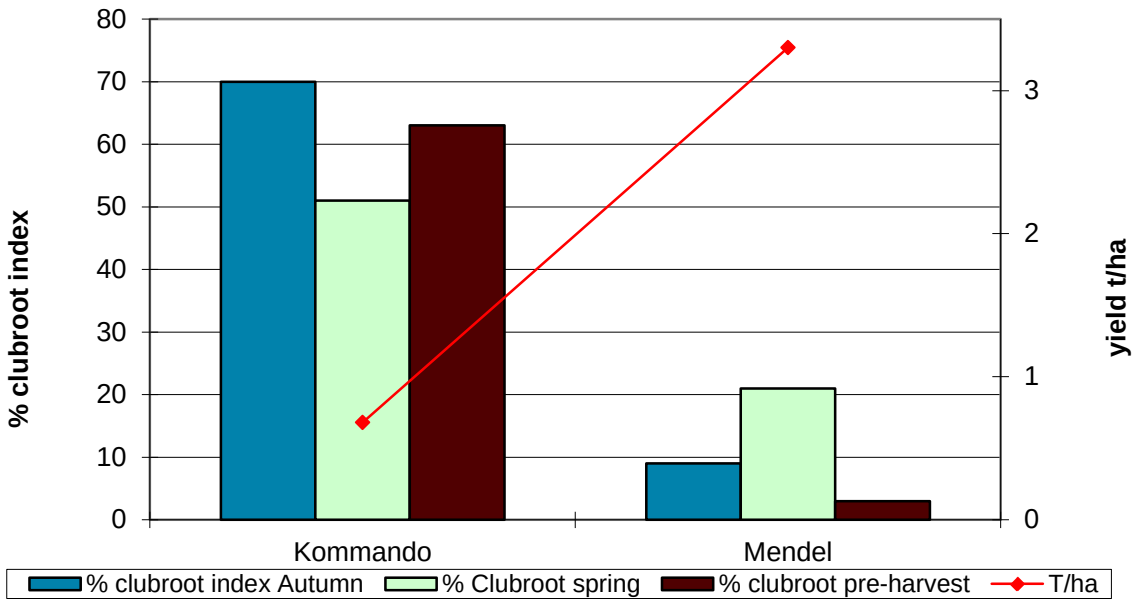

Figure 13. Clubroot control and yield advantage for the resistant variety Mendel, compared to the susceptible variety Kommando at the Shropshire 2007/2008 site in untreated control plots.

There were significant improvements in crop cover and vigour as a result of variety at this site. Crop cover as a % ground cover in March was 4% in untreated Kommando plots and 13.3% in the untreated Mendel plots (LSD = 1.91, *P* = 0.05). Vigour assessed on a 1-9 scale in March was 1.62 in the Kommando untreated control plots and 4.50 in the Mendel untreated control (LSD = 0.960, *P* = 0.001).

The pH in treatments was significantly raised by the higher rate Calcium carbonate treatments, shown in table 12 in spring and summer. Extractable calcium values were not raised significantly by treatment.

Table 12. Soil pH and extractable calcium (mg/l) for Shropshire 2007/2008 site.

	Sowing		**Feb**		**Harvest**	
	pH	Ca	pH	Ca	pH	Ca
1. Untreated control	6.5	1720	6.44	1910	6.42	1787
2. Calcium carbonate 2 t/ha			6.69	1655	6.65	1560
3. Calcium carbonate 4 t/ha			6.92	2394	7.06	1992
4. Calcium carbonate 8 t/ha.			7.18	2020	7.16	1890
5. Calcium cyanamide 250 kg/ha			6.26	1392	6.20	1482
6. Calcium cyanamide 250kg/ha – not incorporated			6.31	1531	6.46	1464
7. Control with extra 50 kg/ha nitrogen			6.79	1198	6.06	1196
8. Boron 20kg/ha			6.62	2100	6.79	1968
LSD (5%) Treat	-	-	0.254	NS	0.509	NS
P			<0.001		<0.001	

NS = non significant at 5% level

Despite the lack of treatment effects on disease in this trial there were significant correlations between pH and clubroot severity in spring and summer assessments (r = -0.371, *P* = 0.003 at harvest and r = -0.445, *P* = <0.001 in spring). There were also significant correlations between calcium content and disease severity at the same timings (r = -0.403, *P* = 0.001 at harvest and r = -0.309, *P* = 0.013).

2008-2009 Field trials

Two field trials were carried out one in Herefordshire and one in Aberdeenshire. Both sites showed low to moderate levels of disease. At the Aberdeen site the disease incidence and severity were low (generally less than 10%) There were significant treatment effects and the Calcium cyanamide unincorporated treatment had a significantly lower disease index compared to control plots in the

December assessment and the Calcium carbonate treatments had a lower index at the March assessment (table 13). No significant differences in plant vigour or counts were noted at this site. The Calcium cyanamide unincorporated treatment (250 kg/ha) had elevated calcium levels and the high rate Calcium carbonate treatment (8t/ha) and the Calcium cyanamide unincorporated treatment (also 250 kg/ha) had the highest pH values. A further treatment with soluble boron also had a trend to lower disease levels at the autumn timing. This trial was not yielded due to an error in an electronic data recorder at harvest. No significant difference in disease levels were noted between Kommando and Mendel. This is consistent with the results found in year one of the trial series at a different site in the Aberdeenshire area, and with results seen in a bioassay of soil on the site where any effect from volunteer 'non-Mendel' plants could be eliminated.

Table 13. Clubroot indices at 2008/2009 Aberdeenshire site for Kommando and Mendel

Treatment	**% Clubroot index**	**% Clubroot index**
Kommando	**Dec**	**March**
1. Untreated control	3.33	9.20
2. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	5.80	0.00
3. Calcium carbonate 4 t/ha	12.5	1.75
4. Calcium carbonate 8 t/ha.	8.33	2.50
5. Calcium cyanamide 250 kg/ha	15.0	10.8
6. Calcium cyanamide 250kg/ha – not incorporated	0.00	7.50
7. Control with extra 50 kg/ha nitrogen	12.5	1.70
8. Boron 20kg/ha	2.50	15.8
Mendel		
1. Untreated control	0.00	23.3
2. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	3.33	3.33
3. Calcium carbonate 4 t/ha	4.20	5.80
4. Calcium carbonate 8 t/ha.	5.00	5.80
5. Calcium cyanamide 250 kg/ha	8.33	0.00
6.Calcium cyanamide 250kg/ha – not incorporated	8.00	0.80
7. Control with extra 50 kg/ha nitrogen	16.7	4.20
8. Boron 20kg/ha	0.00	24.2
LSD (5%) Treat	2.63	5.26
LSD (1%) variety	NS	NS
LSD (5%) Treat x variety	NS	NS

NS = non significant at 5% level

When the treatment results for Mendel and Kommando were meaned there were significant treatment differences from the March assessment where all treatments significantly reduced clubroot severity with the exception of the soluble boron treatment. This was confounded though by the extra nitrogen control plots also having a very low clubroot severity and none of the treatments were significantly lower than this control treatment.

Table 14. Clubroot severity in December and March (% index), meaned across varieties Kommando and Mendel

Treatment	**% clubroot index December**	**% clubroot index March**
1. Untreated control	1.7	16.2
2. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	4.6	1.7
3. Calcium carbonate 4 t/ha	8.3	3.7
4. Calcium carbonate 8 t/ha.	6.7	4.2
5. Calcium cyanamide 250 kg/ha	11.7	5.4
6. Calcium cyanamide 250kg/ha – not incorporated	0.0	4.2
7. Control with extra 50 kg/ha nitrogen	14.6	2.9
8. Boron 20kg/ha	1.2	20.0
LSD Treat	NS	2.63
P		0.005

NS = non significant

There were no significant differences as a result of soil treatment in soil pH or extractable calcium (table 15).

Table 15. Soil pH and extractable calcium (mg/l) for Aberdeen 2008/2009 site

	Sowing		**Feb**		**Harvest**	
	pH	Ca	pH	Ca	pH	Ca
1. Untreated control	6.6	2407	7.6	3870.0	7.5	3725
2. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha			7.2	2490.0	6.75	2445
3. Calcium carbonate 4 t/ha			7.3	2540.0	7.2	3055
4. Calcium carbonate 8 t/ha.			7.6	6650.0	7.65	7210
5. Calcium cyanamide 250 kg/ha			7.0	2270.0	7.5	4015
6. Calcium cyanamide 250kg/ha – not incorporated			7.2	3900.0	7.85	14800
7. Control with extra 50 kg/ha nitrogen			6.9	2540.0	6.3	1860
8. Boron 20kg/ha			7.2	3120.0	7.8	5345
St Dev	0.198	243	-	-	-	-
ANOVA			NS	NS	NS	NS

NS = non significant at 5% level

As well as lack of treatment differences on pH and calcium there were no significant correlations between soil pH and clubroot severity.

The Herefordshire trial site was sown late on 24 September 2008 after a delayed harvest, however, by spring it still had slightly higher levels of clubroot than developed at the Aberdeen site. Clubroot symptoms in the autumn were undetectable visually, indicating late sowing may enable crops to escape early severe infection, despite the high level of infection noted in a pre-establishment bioassay. The incidence of clubroot in untreated plots Kommando plots was 32.5 % and in the Mendel plots 2.5% at a 29 June assessment (table 16 and fig. 12). Levels in March were lower, 13.8% and 0% respectively. When analysed over both varieties there were significant effects from soil treatments at the assessment made in June 2009 with significant reductions in disease incidence noted in the two Calcium cyanamide treatments (250 kg/ha incorporated and unincorporated) and the Calcium carbonate 8t/ha treatment compared to the extra nitrogen control. Calcium carbonate 4t/ha plus Calcium cyanamide 250 kg/ha as a mixed treatment also gave a significant reduction compared to control plots. There was significantly less clubroot in the extra nitrogen (50kg/ha, applied as Ammonium nitrate) control plots compared to the untreated control plots. This could relate to an increase in pH in the rhizosphere as a result of nitrogen application in the form of nitrate. It could also relate to more vigorous plants being better able to withstand

infection although this was not detected in vigour assessments. There were significant increases in pH in the spring following Calcium carbonate treatment at either 4 or 8 t/ha. There was significantly more available calcium in the Calcium cyanamide 250 kg/ha incorporated treatment – which differed from the Aberdeen result, where the unincorporated Calcium cyanamide was higher. There were, however no yield benefits associated with either varietal control or soil amendments (Table 17). Mendel yielded less than Kommando, although this difference was not statistically significant. Soluble boron was significantly lower in clubroot in June when compared to the untreated control, but not to the extra nitrogen treatment.

Table 16. Clubroot indices at 2008/2009 Hereford site for Kommando and Mendel

Treatment Kommando	% Clubroot index Dec	% Clubroot index March	% Clubroot index June
1. Untreated control	-	3.44	30.6
2. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	-	2.19	6.56
3. Calcium carbonate 4 t/ha	-	1.56	14.7
4. Calcium carbonate 8 t/ha.	-	0.31	4.69
5. Calcium cyanamide 250 kg/ha	-	2.19	4.67
6. Calcium cyanamide 250kg/ha – not incorporated	-	1.56	6.25
7. Control with extra 50 kg/ha nitrogen	-	2.81	15.3
8. Boron 20kg/ha	-	1.56	15.6
Mendel	-		
Untreated control	-	0.00	2.50
Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	-	1.25	0.00
Calcium carbonate 4 t/ha	-	0.94	0.00
Calcium carbonate 8 t/ha.	-	0.00	0.00
Calcium cyanamide 250 kg/ha	-	0.62	0.00
Calcium cyanamide 250kg/ha – not incorporated	-	0.31	0.00
Control with extra 50 kg/ha nitrogen	-	0.62	2.50
Boron 20kg/ha	-	0.31	0.00
LSD Treat		NS	7.42 (0.005)
LSD variety		0.59 (<0.001)	3.74 (<0.001)
LSD Treat x variety		NS	10.6 (0.040)

NS = non significant at 5% level

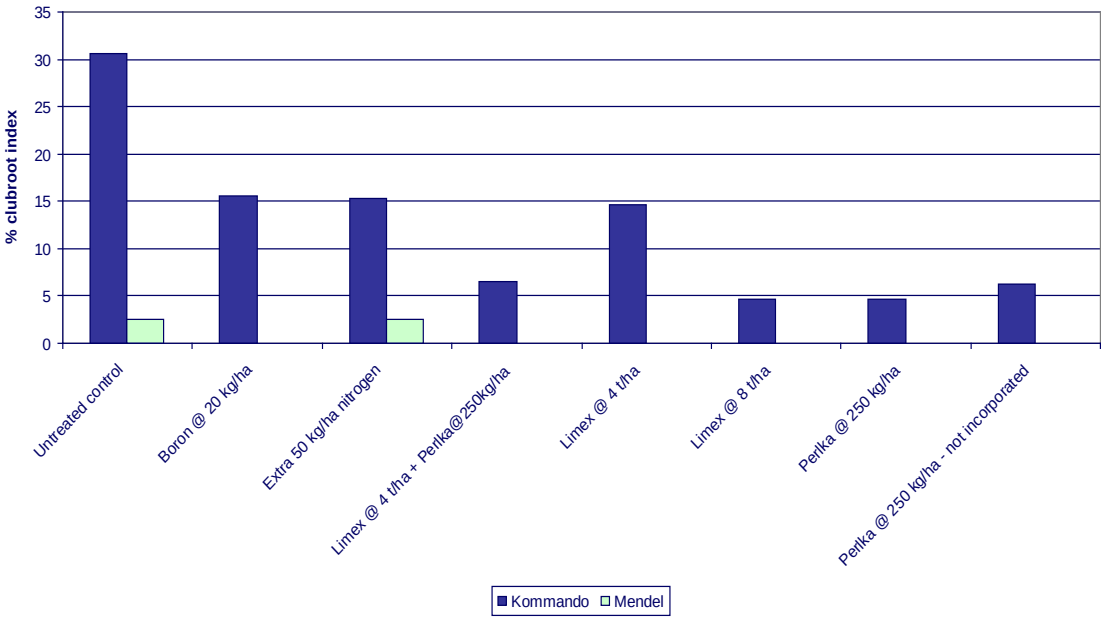

Figure 14. Clubroot disease index in 2008/09 Herefordshire site assessed in June 2009

There was a significant reduction in clubroot severity in June in the Mendel compared with Kommando (figure 14).

The control and yield benefit associated with Mendel was far stronger in year one where disease levels at the English site were far higher.

Table 17. Yield (t/ha) corrected to 91% dry matter for Hereford 2008/2009 site

Treatment	**Kommando**	**Mendel**
1. Untreated control	4.77	5.16
2. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	4.95	4.93
3. Calcium carbonate 4 t/ha	5.22	4.86
4. Calcium carbonate 8 t/ha.	4.97	4.64
5. Calcium cyanamide 250 kg/ha	5.60	4.90
6. Calcium cyanamide 250kg/ha – not incorporated	5.22	4.84
7. Control with extra 50 kg/ha nitrogen	4.68	4.57
8. Boron 20kg/ha	4.95	4.96
LSD Treat	NS	NS
P		

NS = not significant at 5% level

There were significant differences in crop cover and vigour in response to variety at this site in the autumn. Vigour and crop cover results are presented in the cross trial analysis at the end of this section.

There were significant differences in soil pH and extractable calcium values (table 18). Calcium carbonate at 8 t/ha was significantly higher in pH and calcium than Calcium cyanamide (not incorporated).

Table 18. Soil pH and extractable calcium (mg/l) for Herefordshire 2008/2009 site

	Sowing		**Feb**		**Harvest**	
	pH	Ca	pH	Ca	pH	Ca
1. Untreated control	6.10	999	5.82	940	5.90	982
2. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha			6.65	1400	6.76	1390
3. Calcium carbonate 4 t/ha			6.62	1471	6.80	1546
4. Calcium carbonate 8 t/ha.			7.40	2476	7.18	1882
5. Calcium cyanamide 250 kg/ha			6.05	1029	6.19	1180
6. Calcium cyanamide 250kg/ha – not incorporated			6.02	1050	6.11	1086
7. Control with extra 50 kg/ha nitrogen			5.92	1039	6.05	1082
8. Boron 20kg/ha			5.90	1084	6.05	1095
St Dev	0.258	67.7	-	-	-	-
LSD			0.37	620	0.35	252
P			<0.001	<0.001	<0.001	<0.001

There were also significant correlations between clubroot severity and soil pH (r = -0.499, *P* = 0.004 in spring and r = -0.394, P = 0.026 in June. Calcium also correlated with disease severity in spring assessments (r = -0.422, *P* = 0.016) but not in the June assessments.

2009–2010 Field trials

Two field trials were carried out, one in Warwickshire and one in Aberdeenshire in 2009/10, using the treatments that had demonstrated most potential in years one and two of the project and incorporating the susceptible variety Kommando and the two resistant varieties Cracker (then listed as coded variety NPZ0700) and Mendel. There were low to moderate levels of disease at the Warwickshire site in the autumn which then increased further in the spring. Levels at the Aberdeen site where higher in the autumn and severe by summer.

At the Aberdeen site the disease severity in March and June exceeded 50% in the untreated Kommando plots. No significant differences were noted at this Aberdeen site between treatments in terms of yield (Table 20) or disease severity (Table 19) although there was a trend for the combined treatment to be more effective in the March assessment this was not statistically significant. There were significant differences in plant vigour in December – Calcium cyanamid 250kg/ha had lower vigour than other treatments and compared to the untreated control. Because of high levels of volunteers (thought to be Kommando) the resistant variety treatments were abandoned at this site.

Table 19. Clubroot indices at 2009/2010 Aberdeen site for Kommando only

Treatment Kommando	**% Clubroot index Dec**	**% Clubroot index March**	**% Clubroot index August**
1. Untreated control	15.0	54.2	50.8
2. Calcium carbonate 4 t/ha	10.0	40.0	50.0
3. Calcium carbonate 8 t/ha.	8.33	44.2	46.7
4. Calcium cyanamide 250 kg/ha	8.33	55.0	55.8
5. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	13.3	44.2	52.5
6. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha	11.7	26.7	57.5
LSD Treat	NS	NS	NS

NS = non significant at 5% level

Table 20. Yield (t/ha) corrected to 91% dry matter at 2009/2010 Aberdeen site for Kommando only

Treatment Kommando	**Yield (t/ha) at 91% dry matter**
1. Untreated control	2.974
2. Calcium carbonate 4 t/ha	3.012
3. Calcium carbonate 8 t/ha.	2.930
4. Calcium cyanamide 250 kg/ha	2.99
5. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	3.16
6. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha	3.07
LSD Treat	NS

NS = non significant

There were significant treatment effects on soil pH and extractable calcium levels, shown in table 21. All treatments significantly raised soil pH and calcium and of these the Calcium carbonate treatments was most effective – significantly better than the Calcium cyanamide treatment in raising pH.

Table 21. Soil pH and extractable calcium (mg/l) for at 2009/2010 Aberdeen site

	Sowing		**Feb**		**Harvest (not assessed)**	
	pH	Ca	pH	Ca	pH	Ca
1. Untreated control	6.6	2128	6.10	1620		
2. Calcium carbonate 4 t/ha			6.60	2140		
3. Calcium carbonate 8 t/ha.			7.00	2620		
4. Calcium cyanamide 250 kg/ha			6.40	1900		
5. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha			6.70	2240		
6. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha			6.70	2240		
St Dev	0.281	459				
LSD			0.450	935		
P			<0.001	<0.001	-	-

There were no significant correlations between clubroot severity and pH or calcium content.

The Warwickshire trial site had lower levels of clubroot compared to the Aberdeen site. This was associated with dry conditions at sowing that limited early plant infection. The severity index of clubroot in untreated Kommando plots was 20% in spring and over 30% by June, compared to less than 5% in the Mendel and Cracker at March and June assessments (table 22). This level of infection in resistant varieties is lower than seen in 2007/2008 and similar to that seen in 2008/2009 trials in Shropshire and Herefordshire respectively and a significant reduction compared to Kommando. Significant control of clubroot was seen for soil amendments as seen in table 22. Calcium cyanamide, calcium carbonate and combinations of treatments were significant on all varieties. The most effective treatment in the March assessment was the Calcium cyanamde at 250 k/ha treatment. In the June assessment this was not significantly better than the Calcium carbonate at 4 t/ha treatment. The results for the Calcium carbonate treatments were variable as clubroot tended to be lower in the 4 t/ha treatment in March and was significantly lower in December and June. This could not be explained by misapplication as the soil results did show the

expected dose effect. Nor could it be explained by high inoculum levels in those plots as it was also observed as a trend across all the Cracker and Mendel varieties.

Table 22. Clubroot indices at 2009/2010 Warwickshire site for Kommando, Mendel and NPZ700

Treatment Kommando	**% Clubroot index Dec**	**% Clubroot index March**	**% Clubroot index June**
1. Untreated control	15.0	20.0	30.9
2. Calcium carbonate 4 t/ha	3.12	6.25	2.81
3. Calcium carbonate 8 t/ha.	10.94	10.94	10.6
4. Calcium cyanamide 250 kg/ha	5	0.94	0.94
5. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	3.12	1.25	4.38
6. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha	0.31	0.00	0.62
Mendel			
1. Untreated control	0.62	4.37	3.44
2. Calcium carbonate 4 t/ha	0.00	0.00	0.00
3. Calcium carbonate 8 t/ha.	0.62	1.25	2.19
4. Calcium cyanamide 250 kg/ha	0.00	0.00	0.00
5. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	0.00	0.00	0.00
6. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha	0.00	0.00	0.00
Cracker			
1. Untreated control	1.25	4.69	0.94
2. Calcium carbonate 4 t/ha	1.25	1.25	0.62
3. Calcium carbonate 8 t/ha.	0.31	0.00	2.19
4. Calcium cyanamide 250 kg/ha	0.00	0.00	0.00
5. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	0.94	1.25	0.00
6. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha	0.31	0.63	0.00
LSD Treat	2.00 (*P*=0.098)	2.72 (*P*= <0.001)	4.83 (*P*= <0.001)
LSD variety	1.42 (*P*= <0.001)	1.92 (*P*= <0.001)	3.42 (*P*= <0.001)
LSD Treat x variety	NS	4.72 (*P*= <0.001)	8.36 (*P*= <0.001)

NS = non significant at 5% level

There were significant yield benefits to the use of all soil treatments with the exception of Calcium cyanamide (table 23) on Kommando and Mendel. The pH at the site at sowing ranged between 6.5 and 7.0 in contrast to the Aberdeen site (5.6 – 6.2) which may be a factor in treatment success.

Table 23. Yield (t/ha) corrected to 91% dry matter Warwickshire 2009/2010 site

Treatment	**Kommando**	**Mendel**	**NPZ700**
1. Untreated control	3.52	4.17	4.45
2. Calcium carbonate 4 t/ha	4.31	4.42	4.57
3. Calcium carbonate 8 t/ha.	4.44	4.67	4.77
4. Calcium cyanamide 250 kg/ha	3.50	4.22	4.10
5. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha	4.50	4.56	4.72
6. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha	4.44	5.05	4.56
LSD Treat	LSD variety		LSD Treat x variety
0.422 (*P*=0.002)	0.299 (*P*= 0.011)		NS

NS = non significant at 5% level

At the harvest assessment there were significant rises in pH and calcium content of soils in response to treatment. Calcium carbonate at 8 t/ha was the most effective treatment.

Table 24. Soil pH and extractable calcium (mg/l) for 2009/2010 Warwickshire site

	Sowing		**Feb**		**Harvest**	
	pH	Ca	pH	Ca	pH	Ca
1. Untreated control	6.50	1720	6.66	2955	6.42	2246
2. Calcium carbonate 4 t/ha			7.00	2403	6.92	2672
3. Calcium carbonate 8 t/ha.			6.74	2896	7.14	3129
4. Calcium cyanamide 250 kg/ha			6.33	2110	6.42	2212
5. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha			7.18	4872	6.72	2496
6. Calcium carbonate 4 t/ha + calcium cyanamide 250 kg/ha + Boron 20kg/ha			6.62	2312	6.82	2725
LSD			-	-	0.301	1009
P			NS	NS	<0.001	<0.001

NS = non significant at 5% level

There were no significant correlations between disease severity and soil pH and calcium content.

Field trials – cross site combined analysis

In the following tables soil treatments are coded as follows in table 25. The first column lists abbreviated names that are used subsequently; these are followed by the harvest years in which each treatment was applied. Table 25 provides a treatment summary.

Table 25. Abbreviated treatment names used in cross site analysis and treatment summary

Control	**08**	**09**	**10**	**Untreated control**
ExtraN	08	09	-	Control with extra 50 kg/ha N to balance Perlka N
Limex2	08	-	-	Limex 2 t/ha
Limex4	08	09	10	Limex 4 t/ha
Limex8	08	09	10	Limex 8 t/ha
Perlkai	08	09	-	Perlka 250kg/ha: incorporated in soil
Perlkan	08	09	10	Perlka 250kg/ha: not incorporated
Solubor	08	09	-	Boron as Solubor (20.8% boron) 20kg/ha
Limex4Perlkai	-	09	-	Limex 4 t/ha + Perlka 250 kg/ha: Perlka incorporated
Limex4Perlkan	-	-	10	Limex 4 t/ha + Perlka 250 kg/ha: Perlka not incorporated
Limex4PerlkanSQ	-	-	10	Limex 4 t/ha + Perlka 250 kg/ha + Solubor +Quicksilver: Perlka not incorporated

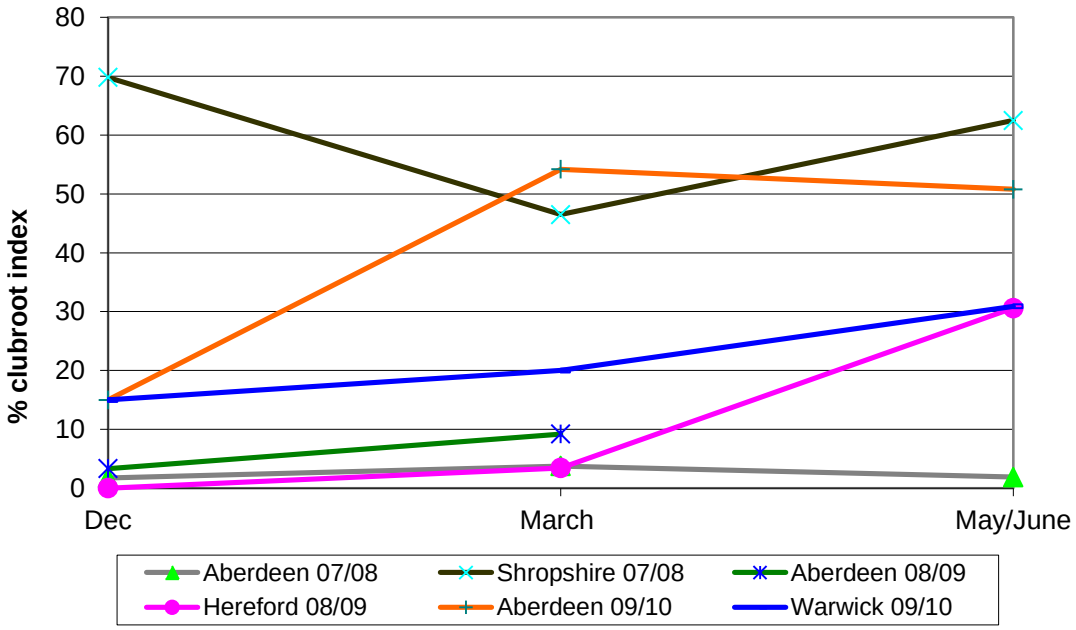

Figure 15. Clubroot epidemic progress at the different field sites and seasons

Clubroot disease severity in the autumn of sowing and subsequent disease development in spring and summer differed widely between sites. The two most severe epidemics were seen at the Aberdeen site in 2009/2010 and the Shropshire site 2007/2008, but the disease progress curves were very different, with the Aberdeen 2009/2010 far lower in the autumn. The characteristics of the different sites are summarised in table 26 and the disease epidemics in figure 15.

Table 26. Field sites characterised by disease, autumn weather and treatment effects

Factor / Site	Aberdeen 2007/ 2008	Shropshire 2007/ 2008	Aberdeen 2008/ 2009	Hereford-shire 2008/ 2009	Aberdeen 2009/ 2010	Warwick-shire 2009/ 2010
Soil type	Sandy loam	Silty clay	Sandy loam	Sandy loam	Sandy loam	Medium loam
Clubroot epidemic	Very low all season	Very severe from early on	Low all season	Low in autumn and medium in spring	Moderate in autumn and severe in summer	Low in autumn and moderate in spring
Autumn conditions	Cool and drier than average	Wet, warmer soils	Cool and very wet, early frosts	Late drilled, wet	Wet, cool, but crop grew well	Dry at sowing. Early onset of winter
Soil treatment effect	No	No	Yes	Yes	No	Yes
Variety effect	No	Yes	No	Yes	-	Yes
Yield effect	Yes	Variety only	No	No	No	Yes
Sowing date	28 08 07	24 08 07	29 08 08	21 09 08	24 08 09	02 09 09
pH pre trial	6.8	6.5	6.6	6.1	6.6	6.5
Calcium pre-trial (mg/l)	1857	1720	2407	999	2127	1720
Clubroot severity pre-trial (bioassay)	22.6	35.0	7.1	83.0	24.6	11.3

Yield summary across sites

There was a significant correlation between clubroot severity at harvest and yield across all sites and varieties (P=0.001, r = -0.472), shown in Figure 16. Yield loss can be measured by the slope of the line. When there is no disease (% index=0), the (predicted) yield is around 4t/ha (the intercept = 3.85t/ha). At % index=100: the (predicted) yield is ~1t/ha. So the increase from 0 to100% caused a (predicted) loss of 3t/ha, or 3/100 = 0.03 t/ha per % increase in the clubroot index.

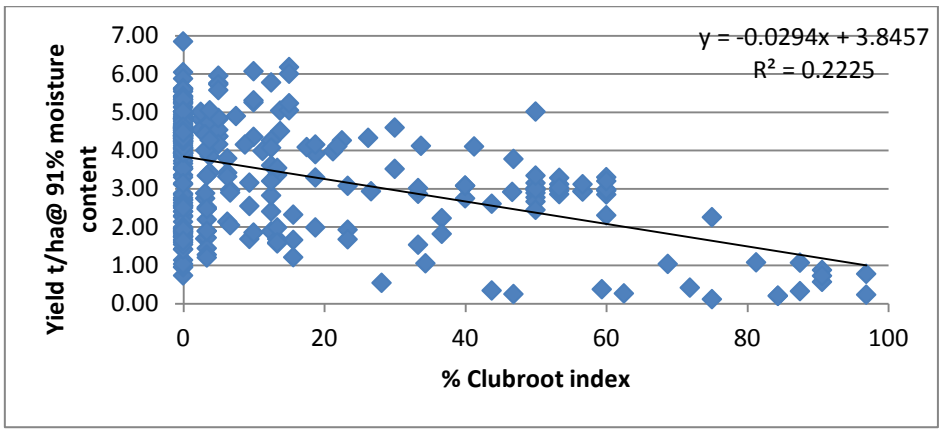

Figure 16. Correlation between yield and clubroot severity across all varieties and sites showing a 0.03 t/ha loss per each 1% increase in disease severity.

When the varieties were analysed separately this yield loss did not alter significantly as shown in Figures 17, 18 and 19. Losses in Mendel were slightly lower than in Kommando and losses in Cracker did not significantly correlate with disease levels as there were few data points, and these only at one site.

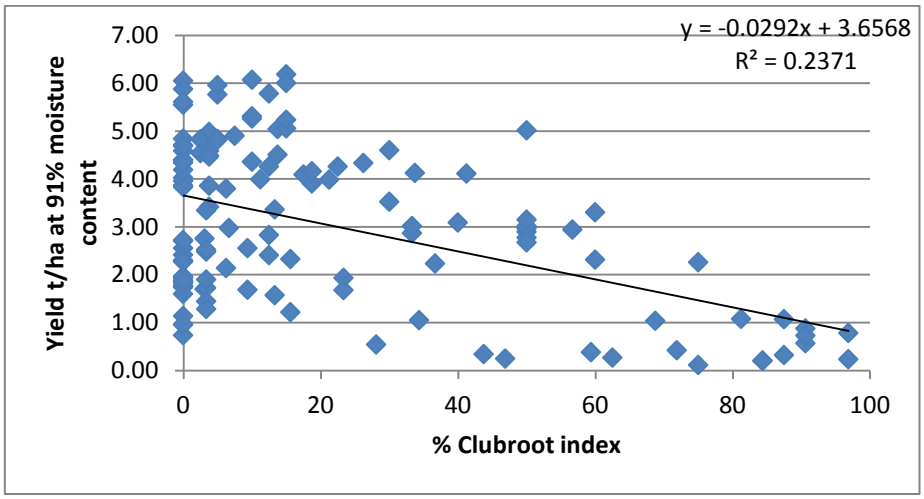

Figure 17. Correlation between yield and clubroot severity in the variety Kommando across all sites and sites, showing a 0.03 t/ha loss per each 1% increase in disease severity.

The correlation between disease severity at harvest and the yield for Kommando was also very highly significant (r = -0.465, *P* <0.001).

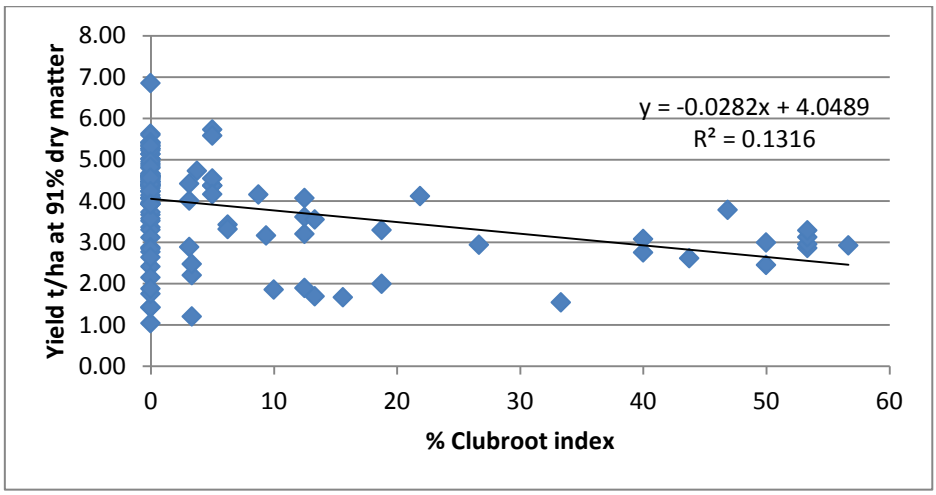

Figure 18. Correlation between yield and clubroot severity in the variety Mendel across all sites and sites, showing a 0.028 t/ha loss per each 1% increase in disease severity.

The correlation between disease severity at harvest and the yield for Mendel (Figure 18) was highly significant (r = -0.296, *P* =0.001). The correlation between disease severity at harvest and the yield for Cracker was not significant (r = 0.296, *P* =0.161). This was only trialled and yielded at one site – Warwickshire 2009/2010. At this site disease was often absent in the assessments so there are few infected examples to chart disease loss from, and these were often at very low disease indices because clubroot control was very effective.

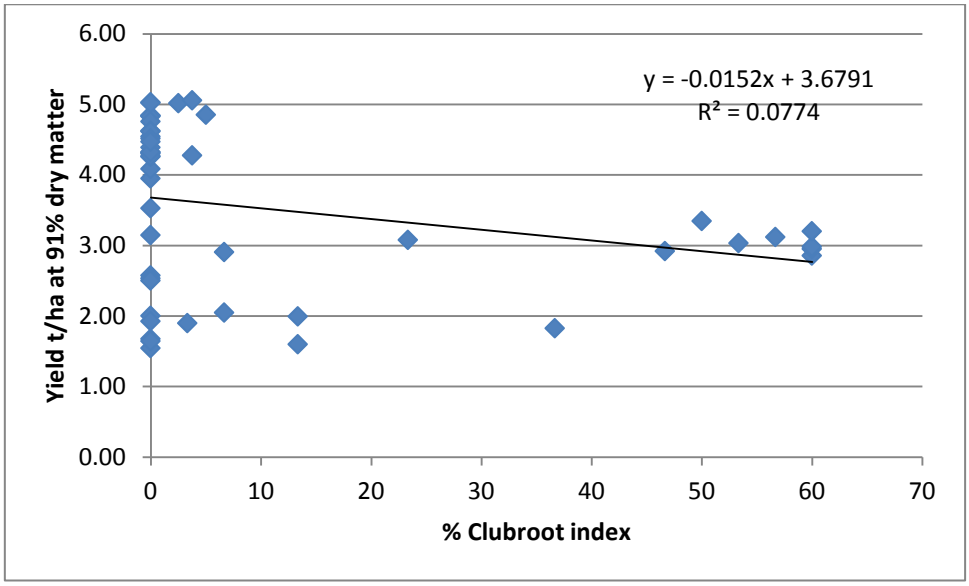

Figure 19. The lack of correlation between yield and clubroot severity in the variety Cracker at Warwickshire site 2009/2010

Yield data are not available for Aberdeen 2009; for Aberdeen 2010, yields are available only for Kommando: the varietal element of this trial was abandoned because the plots were overrun by volunteer oilseed rape plants. Mean yields over replicates are as follows in table 27 for the available combinations of geographic area, harvest year, variety and treatment.

Table 27. Yield summary across sites

	Treatment	Abdn 08	Abdn 09	Abdn 10	Shrop 08	Here 09	Warw 10
Kommando	Control	2.10	–	2.97	1.04	4.77	3.52
	ExtraN	2.04	–	–	0.63	4.68	–
	Limex2	1.77	–	–	0.89	–	–
	Limex4	2.58	–	3.01	1.03	5.22	4.31
	Limex8	2.53	–	2.93	2.14	4.97	4.44
	Perlkai	1.97	–	–	0.86	5.09	–
	Perlkan	1.68	–	2.99	1.63	5.22	3.50
	Solubor	1.86	–	–	1.54	4.95	–
	Limex4Perlkai	–	–	–	–	4.95	–
	Limex4Perlkan	–	–	3.16	–	–	4.50
	Limex4PerlkanSQ	–	–	3.07	–	–	4.45
Mendel	Control	2.08	–	–	3.69	5.16	4.17
	ExtraN	2.27	–	–	3.14	4.57	–
	Limex2	1.80	–	–	3.01	–	–
	Limex4	2.50	–	–	3.14	4.86	4.42
	Limex8	2.81	–	–	3.49	4.64	4.68
	Perlkai	1.83	–	–	3.78	4.90	–
	Perlkan	2.04	–	–	2.98	4.84	4.22
	Solubor	1.82	–	–	3.41	4.96	–
	Limex4Perlkai	–	–	–	–	4.93	–
	Limex4Perlkan	–	–	–	–	–	4.55
	Limex4PerlkanSQ	–	–	–	–	–	5.05
Cracker	Control	–	–	–	–	–	4.45
	ExtraN	–	–	–	–	–	–
	Limex2	–	–	–	–	–	–
	Limex4	–	–	–	–	–	4.58
	Limex8	–	–	–	–	–	4.77
	Perlkai	–	–	–	–	–	–
	Perlkan	–	–	–	–	–	4.10
	Solubor	–	–	–	–	–	–
	Limex4Perlkai	–	–	–	–	–	–
	Limex4Perlkan	–	–	–	–	–	4.72
	Limex4PerlkanSQ	–	–	–	–	–	4.57

Split-plot analyses of the individual trials showed no evidence of special effects, except for the Aberdeen 2007/2008 site.

Highly significant variety effects were found in Shropshire 2007/2008 and Warwickshire 2009/2010. In both trials, Kommando gave the lowest mean yields for all treatments. Only in the latter trial were there significant treatment effects (*P*= 0.02).

A combined analysis of yield over trials was also carried out, omitting Aberdeen 2008/2009, Aberdeen 2009/2010 and the variety Cracker. Because of the incompleteness of the data, only the trial-by-variety interaction was included. This interaction was found to be very highly significant, along with the trial, variety and treatment effects. Predicted means for treatment and trial-by-variety are shown in Tables 28 and 29.

Table 28. Predicted yield means for soil treatments

	All 4 trials		**England: all years**	
	Mean	**LSD vs control**	**Mean**	**LSD vs control**
Control	3.30	—	3.74	—
ExtraN	3.19	0.36	3.47	0.31
Limex2	3.00	0.42	3.41	0.63
Limex4	3.58	0.33	3.90	0.27
Limex8	3.70	0.33	3.91	0.27
Perlkai	3.28	0.36	3.85	0.31
Perlkan	3.24	0.33	3.77	0.27
Solubor	3.26	0.36	3.84	0.31
Limex4Perlkai	3.39	0.52	3.81	0.33
Limex4Perlkan	3.83	0.54	4.20	0.43
Limex4PerlkanSQ	4.05	0.54	4.42	0.43

The Calcium carbonate 8 t/ha treatment had the largest predicted yields of any of the solo treatments, with a significant yield improvement of almost 5%. The combined treatments gave significantly higher predicted yields than the untreated control, with the exception of the incorporated Calcium cyanamide + Calcium carbonate treatment. The three way combined mix of Calcium carbonate+ Calcium cyanamide + boron had a predicted mean yield of almost 20% higher than the untreated control.

Table 29. Predicted yield means for combinations of trial and variety

	All 4 trials		England: all years	
	Kommando	**Mendel**	**Kommando**	**Mendel**
Aberdeen 2008	2.19	2.26	—	—
Shropshire 2008	1.34	3.45	1.33	3.44
Herefordshire 2009	5.05	4.93	5.04	4.92
Warwickshire 2010	3.94	4.34	3.98	4.37
Ave LSD within trials	0.24		0.33	
Ave LSD between trials	0.76		0.86	

There was a significant yield advantage to the Mendel variety at the Shropshire 2008 and Warwickshire 2010 sites (mean predicted yield increase of 250% and almost 10% respectively).

The site means and the predicted means were used to calculate a cost benefit to treatment. During the course of the project, prices for oilseed rape varied widely and so three prices were used: £250 (table 30), £350 (table 31) and £450 (table 32) per tonne. Treatments costs were as follows: Perlka was costed at £131, £133 and £138 per hectare at the rate used (250 kg/ha) for the 2007/2008, 2008/2009 and 2009/2010 seasons respectively. Limex70 was costed at £12 per tonne delivered and spread and Solubor at £2 per kg. With increasing prices very small increases in yield became economically viable but some treatments gave a yield depression which meant that economic losses increased as prices increased.

Table 30. Cost benefit £/ha analysis at £250 per tonne oilseed rape at 91% dry matter – shaded boxes represent a positive return on treatment cost

	Treatment	**Abdn 08**	**Abdn 09**	**Abdn 10**	**Shrop 08**	**Here 09**	**Warw 10**
Kommando	Control	0	-	0	0	0	0
	ExtraN	-63	-		-15	-54	-
	Limex2	-106	-		-61	-	-
	Limex4	72*	-	-38	-50	82	149*
	Limex8	12	-	-106	179	-28	134*
	Perlkai	-163	-		-176	-34	-
	Perlkan	-236	-	-126	16	-1	-136
	Solubor	-100	-		85	22	-
	Limex4Perlkai	-	-		-	-117	-
	Limex4Perlkan	-	-	-131	-	-	66*
	Limex4PerlkanSQ	-	-	-194	-	-	13*
Mendel	Control	0	-	-	0	0	0
	ExtraN	-1	-	-	-1	-181	-
	Limex2	-94	-	-	-94		-
	Limex4	57	-	-	57	-108	14
	Limex8	86	-	-	-194	-211	32*
	Perlkai	-194	-	-	-193	-181	-
	Perlkan	-141	-	-	-141	-196	-119
	Solubor	-105	-	-	-105	-75	-
	Limex4Perlkai	-	-	-	-	-222	-
	Limex4Perlkan	-	-	-	-	-	-84
	Limex4PerlkanSQ	-	-	-	-	-	1*
Cracker	Control	-	-	-	-	-	-
	ExtraN	-	-	-	-	-	-
	Limex2	-	-	-	-	-	-
	Limex4	-	-	-	-	-	-15
	Limex8	-	-	-	-	-	-16
	Perlkai	-	-	-	-	-	-
	Perlkan	-	-	-	-	-	-219
	Solubor	-	-	-	-	-	-
	Limex4Perlkai	-	-	-	-	-	-
	Limex4Perlkan	-	-	-	-	-	-111
	Limex4PerlkanSQ	-	-	-	-	-	-189

* Only these values based on statistically different (minimum 5% level) yields

Table 31. Cost benefit analysis (£/ha) at £350 per tonne oilseed rape at 91% dry matter– shaded boxes represent a positive return on treatment cost

	Treatment	**Abdn 08**	**Abdn 09**	**Abdn 10**	**Shrop 08**	**Here 09**	**Warw 10**
Kommando	Control	0	-	0	0	0	0
	ExtraN	-70	-	-	-192	-56	-
	Limex2	-140	-	-	-76	-	-
	Limex4	120*	-	-34	-52	134	228*
	Limex8	54	-	-110	289	-2	226*
	Perlkai	-177	-	-	-194	5	-
	Perlkan	-278	-	-124	75	51	-138
	Solubor	-124	-	-	135	48	-
	Limex4Perlkai	-	-	-	-	-92	-
	Limex4Perlkan	-	-	-113	-	-	164*
	Limex4PerlkanSQ	-	-	-184	-	-	106*
Mendel	Control	0	-	-	0	0	0
	ExtraN	18	-	-	-241	-234	-
	Limex2	-122	-	-	-262	-	-
	Limex4	99	-	-	-240	-132	40
	Limex8	160	-	-	-166	-257	82*
	Perlkai	-219	-	-	-100	-201	-
	Perlkan	-145	-	-	-380	-222	-114
	Solubor	-131	-	-	-138	-89	-
	Limex4Perlkai	-	-	-	-	-239	-
	Limex4Perlkan	-	-	-	-	-	-46
	Limex4PerlkanSQ	-	-	-	-	-	89*
Cracker	Control	-	-	-	-		
	ExtraN	-	-	-	-		
	Limex2	-	-	-	-		
	Limex4	-	-	-	-		-2
	Limex8	-	-	-	-		16
	Perlkai	-	-	-	-		
	Perlkan	-	-	-	-		-254
	Solubor	-	-	-	-		
	Limex4Perlkai	-	-	-	-		
	Limex4Perlkan	-	-	-	-		-85
	Limex4PerlkanSQ	-	-	-	-		-177

* Only these values based on statistically different (minimum 5% level) yields

Table 32. Cost benefit analysis (£/ha) at £450 per tonne oilseed rape at 91% dry matter– shaded boxes represent a positive return on treatment cost

	Treatment	**Abdn 08**	**Abdn 09**	**Abdn 10**	**Shrop 08**	**Here 09**	**Warw 10**
Kommando	Control	0	-	0	0	0	0
	ExtraN	-76	-	-	-233	-58	-
	Limex2	-172	-	-	-92	-	-
	Limex4	168*	-	-30	-52	186	308*
	Limex8	98	-	-114	399	25	318*
	Perlkai	-190	-	-	-212	44	-
	Perlkan	-320	-	-122	134	103	-140
	Solubor	-148	-	-	185	72	-
	Limex4Perlkai	-	-	-	-	-67	-
	Limex4Perlkan	-		-94	-	-	262*
	Limex4PerlkanSQ	-		-174	-	-	199*
Mendel	Control	0	-	-	0	0	0
	ExtraN	37	-	-	-296	-287	-
	Limex2	-150	-	-	-330		-
	Limex4	141	-	-	-296	-156	64
	Limex8	232	-	-	-186	-303	133*
	Perlkai	-244	-	-	-91	-221	-
	Perlkan	-149	-	-	-451	-248	-109
	Solubor	-157	-	-	-166	-103	-
	Limex4Perlkai	-	-	-		-256	-
	Limex4Perlkan	-	-	-		-	-8
	Limex4PerlkanSQ	-	-	-		-	177
Cracker	Control	-	-	-	-	-	-
	ExtraN	-	-	-	-	-	-
	Limex2	-	-	-	-	-	-
	Limex4	-	-	-	-	-	10
	Limex8	-	-	-	-	-	48*
	Perlkai	-	-	-	-	-	-
	Perlkan	-	-	-	-	-	-289
	Solubor	-	-	-	-	-	-
	Limex4Perlkai	-	-	-	-	-	-
	Limex4Perlkan	-	-	-	-	-	-58
	Limex4PerlkanSQ	-	-	-	-	-	-165

* Only these values based on statistically different (minimum 5% level) yields

Cross site analysis of clubroot severity

Clubroot incidence and clubroot severity (as %) were assessed throughout the seasons in the trial series. The data suggest that, in each trial, the two measures of clubroot varied in similar ways with soil treatments, varieties and assessment occasions. The probability distributions of incidence and severity are difficult to model well, since they are positively skewed with non-negligible proportions of values at the extremes of their ranges: the square roots of these values are analysed here. Individual-trial analyses of both variables for March in Aberdeen 2009/2010 and the three trials in the West Midlands show that variety effects are highly significant in all four (with Kommando more susceptible than Mendel or Cracker). There is also highly significant dependence on treatment in the two 2009/10 trials.

Combined analyses over the four trials are reported here for the March assessments. Again including only the trial-by-variety interaction, the trial, variety and treatment effects for clubroot *incidence* in March are found to be very highly significant, but the interaction itself is not. Tables of predicted means for treatment and trial-by-variety (on the square-root scale) are shown below in Tables 33 and 34. Average standard errors of differences are 0.56 for treatment means and 0.51 for trial-by-variety means.

Fitting a similar model for clubroot *severity* in March, the trial and variety effects are very highly significant, and the treatment effects and trial-by-variety interaction are highly significant. Tables of predicted means (also on the square-root scale) are as follows. Average standard errors of differences are 0.43 for treatment means and 0.45 for trial-by-variety means.

Table 33. Predicted treatment means for clubroot incidence and severity in March

	Incidence	**Severity**
Control	4.44	3.56
ExtraN	4.52	3.52
Limex2	3.82	3.18
Limex4	3.29	2.81
Limex8	3.35	2.71
Perlkai	4.00	3.25
Perlkan	3.61	3.09
Solubor	3.42	2.94
Limex4Perlkai	4.70	3.57
Limex4Perlkan	3.08	2.56
Limex4PerlkanSQ	2.55	1.90
SED	0.56	0.43

Calcium carbonate at 8 t/ha was the most effective solo treatment, with a cross site predicted mean control of around 25%. The combined Calcium carbonate plus Calcium cyanamide plus boron treatment was the most effective treatment overall – predicted mean control was over 45%.

Table 34. Predicted variety means for clubroot incidence and severity in March

Incidence	**Kommando**	**Mendel**	**Cracker**
Aberdeen 10	8.11	7.11	7.03
Shropshire 2008	6.57	3.47	–
Herefordshire 2009	2.15	0.58	–
Warwickshire 2010	2.14	0.68	0.77
SED 0.51			
Severity	**Kommando**	**Mendel**	**Cracker**
Aberdeen 10	6.75	6.02	5.61
Shropshire 2008	6.05	3.10	–
Herefordshire 2009	1.00	0.22	–
Warwickshire 2010	1.98	0.58	0.65
SED = 045			

The varietal effects were significant, with the exception of the Aberdeenshire sites. At the English sites in the trial series control as a consequence of varietal resistance ranged from 50% at the Shropshire site to over 95% at the Herefordshire site.

Dependence of clubroot incidence and severity on pH and calcium

Records were made of soil pH and calcium concentration (mg/l), since they were thought to influence clubroot development. They were recorded at various levels for different combinations of trial and occasion, as follows in table 35.

Table 35. Soil sampling summary

	Abdn08	**Abdn09**	**Abdn10**	**Shrop08**	**Here09**	**Warw10**
Sowing	replicate	replicate	replicate	–	replicate	replicate
Spring	–	–	treatment	sub-plot	sub-plot	sub-plot
Harvest	–	sub-plot	–	sub-plot	sub-plot	sub-plot

Clubroot incidence and severity in November/December might be related to pH and calcium before sowing, but with the latter available only at the replicate level, their influence is confounded with replicate effects. One could, though, regard variation in these two covariates as explaining the replicate effects, but the required data are recorded only for Aberdeen 2008/2009 and 2009/2010 and Warwickshire 2009/2010. None of the trials has data at harvest on both soil and clubroot.

Data at sub-plot level on incidence and severity in both March and June are available for the English trials, along with pH and calcium recorded in spring. Apart from the differences already observed between trials and between varieties, clubroot incidence and severity appear to decline strongly with increased calcium concentration and weakly with increased pH.

In combined analyses of the West Midlands trials with treatment effects omitted from the model, both incidence and severity in March show highly significant reductions as soil calcium increases, and significant reductions with increasing pH: neither variable has a significant effect in addition to the other. Calcium has a significant effect on incidence in addition to the treatments, but neither has a significant additional effect on severity.

Clubroot incidence and severity in June both show highly significant reductions with increasing calcium and pH when treatment effects are omitted from the model. These remain highly significant when treatments are included, except for the effect of calcium on incidence.

Crop vigour

Crop vigour was recorded (on a 1-9 scale) in March in the West Midlands trials: the mean values are as follows.

Table 36. Summary of crop vigour data

Variety	Treatment	Shrop 08	Here 09	Warw 10
Kommando	Control	1.62	5.25	5.25
	ExtraN	1.62	5.25	–
	Limex2	1.25	–	–
	Limex4	2.00	5.50	5.88
	Limex8	3.50	6.00	5.25
	Perlkai	2.06	5.75	–
	Perlkan	3.44	5.50	5.88
	Solubor	2.19	5.50	–
	Limex4Perlkai	–	6.00	–
	Limex4Perlkan	–	–	6.12
	Limex4PerlkanSQ	–	–	6.25
Mendel	Control	4.50	5.50	5.25
	ExtraN	5.25	5.50	–
	Limex2	3.00	–	–
	Limex4	4.12	6.25	5.62
	Limex8	4.75	6.50	5.38
	Perlkai	5.25	6.50	–
	Perlkan	5.00	6.00	5.75
	Solubor	2.75	6.00	–
	Limex4Perlkai	–	6.25	–
	Limex4Perlkan	–	–	5.88
	Limex4PerlkanSQ	–	–	5.75
Cracker	Control	–	–	5.12
	ExtraN	–	–	–
	Limex2	–	–	–
	Limex4	–	–	5.38
	Limex8	–	–	5.62
	Perlkai	–	–	–
	Perlkan	–	–	6.00
	Solubor	–	–	–
	Limex4Perlkai	–	–	–
	Limex4Perlkan	–	–	5.88
	Limex4PerlkanSQ	–	–	5.62

Split-plot analyses of individual trials find very highly significant variety effects for Shropshire 2007/2008 and Herefordshire 2008/2009, and significant treatment effects in Warwickshire 2009/2010 (P=0.012).

A combined analysis of the three trials including only the trial-by-variety interaction finds this interaction and the treatment effects to be very highly significant. Predicted means for treatment and trial-by-variety are as follows, with average standard errors of differences equal to 0.31 for treatment means and 0.56 for trial-by-variety means.

Table 37. Mean predicted vigour for treatments

Treatment	Mean predicted vigour
Control	4.26
ExtraN	4.26
Limex2	3.28
Limex4	4.68
Limex8	4.79
Perlkai	4.91
Perlkan	4.87
Solubor	4.37
Limex4Perlkai	4.93
Limex4Perlkan	5.08
Limex4PerlkanSQ	4.99
SED	0.31

Highest mean vigour was predicted for the mixed Calcium carbonate + Calcium cyanamide (not incorporated). Calcium cyanamide treatments had the highest vigour of the solo treatments, and there was a vigour dose effect to the three rates of Calcium cyanamide applied.

Table 38. Predicted mean vigour from varietal resistance

	Kommando	Mendel	Cracker
Shrop 08	2.37	4.48	-
Here 09	5.54	6.01	-
Warw 10	5.58	5.41	5.41
SED = 0.56			

Varietal resistance increased vigour at the Shropshire site in 2007/2008 and the Herefordshire site the following year. The varietal effect on vigour was not seen at the Aberdeen sites or at the Warwickshire site in 2010.

3.3.4. Climate change impact on disease severity

Climate matching

The current (2012) climate for Bacup in Lancashire was matched with the current, 2030 and 2050 projected climates (A1B Global Climate Model) using the CLIMEX 'Match Climates' function (Figure 20).

Maps showing the match of soil moisture between the dates of 1st October and 4th March to Bacup are shown below (Figure 20). The areas that have similar soil moisture to the current Bacup soil moisture do not change much after the 2030 and 2050 climate change projections are applied. Some areas in southern England and north-west Scotland will have a lower soil moisture match to the current Bacup soil moisture in 2030 and 2050.

Clubroot CLIMEX model

The clubroot species model within CLIMEX provides a range of indices as outputs to describe various stresses and parameters. An index called the Ecoclimatic Index (EI) gives an overall measure of favourableness of the location for a species to establish. Figure 21 shows a map of the EI for clubroot under the current climate and the climates projected for 2030 and 2050 under the A1B Global Climate Change Model (Kriticos *et al*., 2012). EI values < 10 are considered to be marginal for clubroot establishment, and values >20 are considered to be very favourable for clubroot establishment. Under projected climate change for 2030 and 2050 more areas in the UK become ecoclimatically suitable for clubroot, particularly in northern England and Scotland (Figure 21).

A table of key outputs for 3 different locations: Bacup, Lancashire; Brechin, Angus; and Halstead, Essex, summarises the changes under current and projected climates (Table 39).

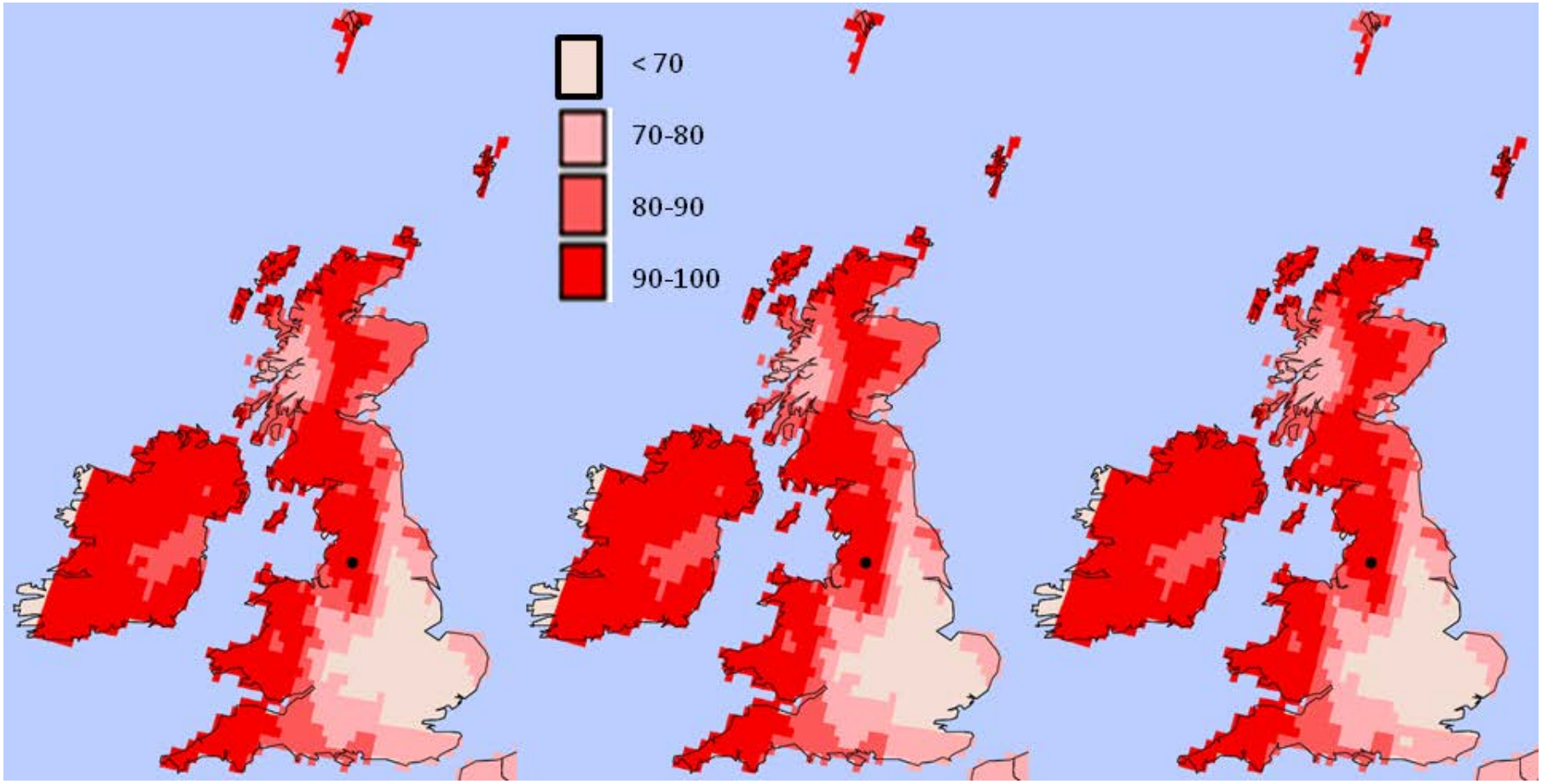

Figure 20. Soil moisture match (<70% to 100%) between 1st October and 4th March of the current climate of Bacup, Lancashire (•) and the rest of the UK under the current climate (left), projected 2030 climate (middle) and projected 2050 climate (right).

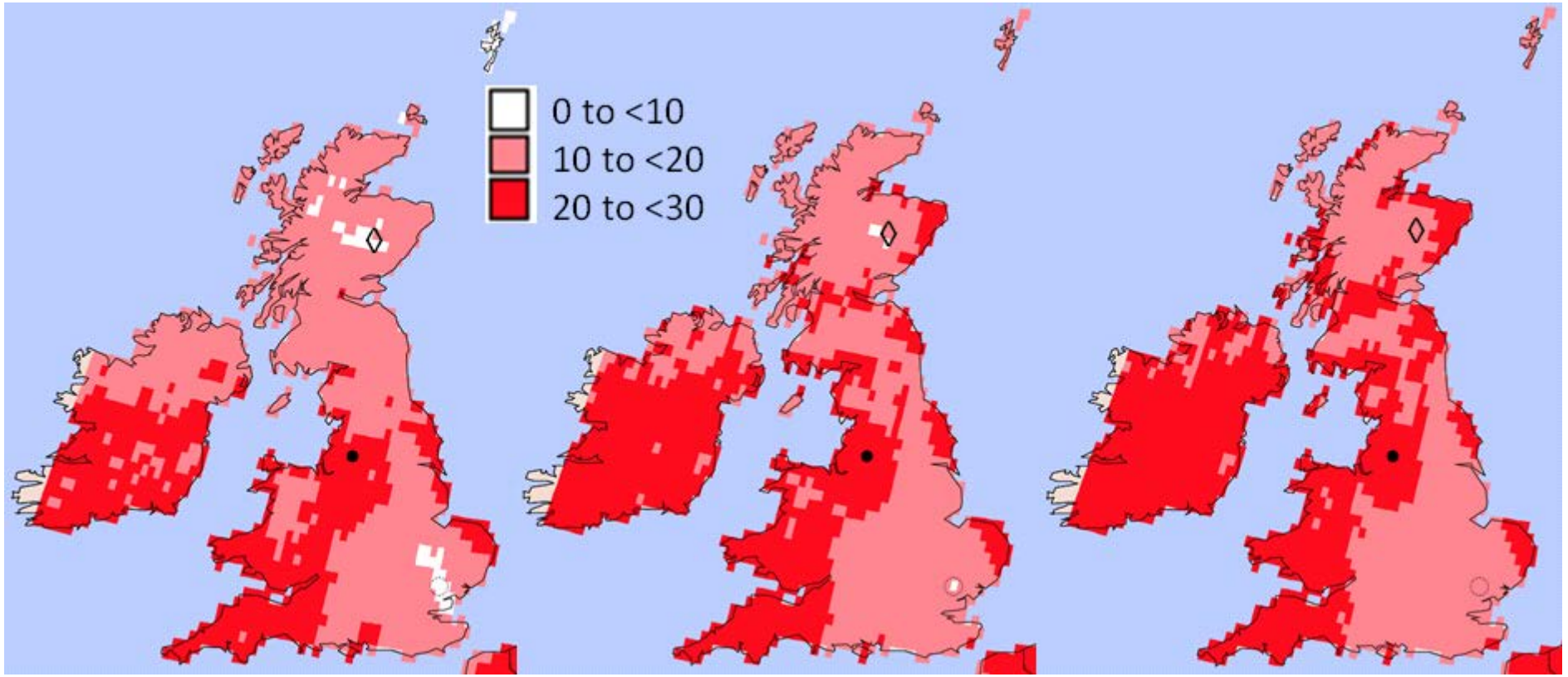

Figure 21. Ecoclimatic Index (EI) for clubroot under the current climate (left), projected 2030 climate (middle) and projected 2050 climate (right). The higher the index the greater the ecoclimatic suitability for clubroot establishment. Bacup, Lancashire (•); Brechin, Angus (◊); and Halstead, Essex (◌).

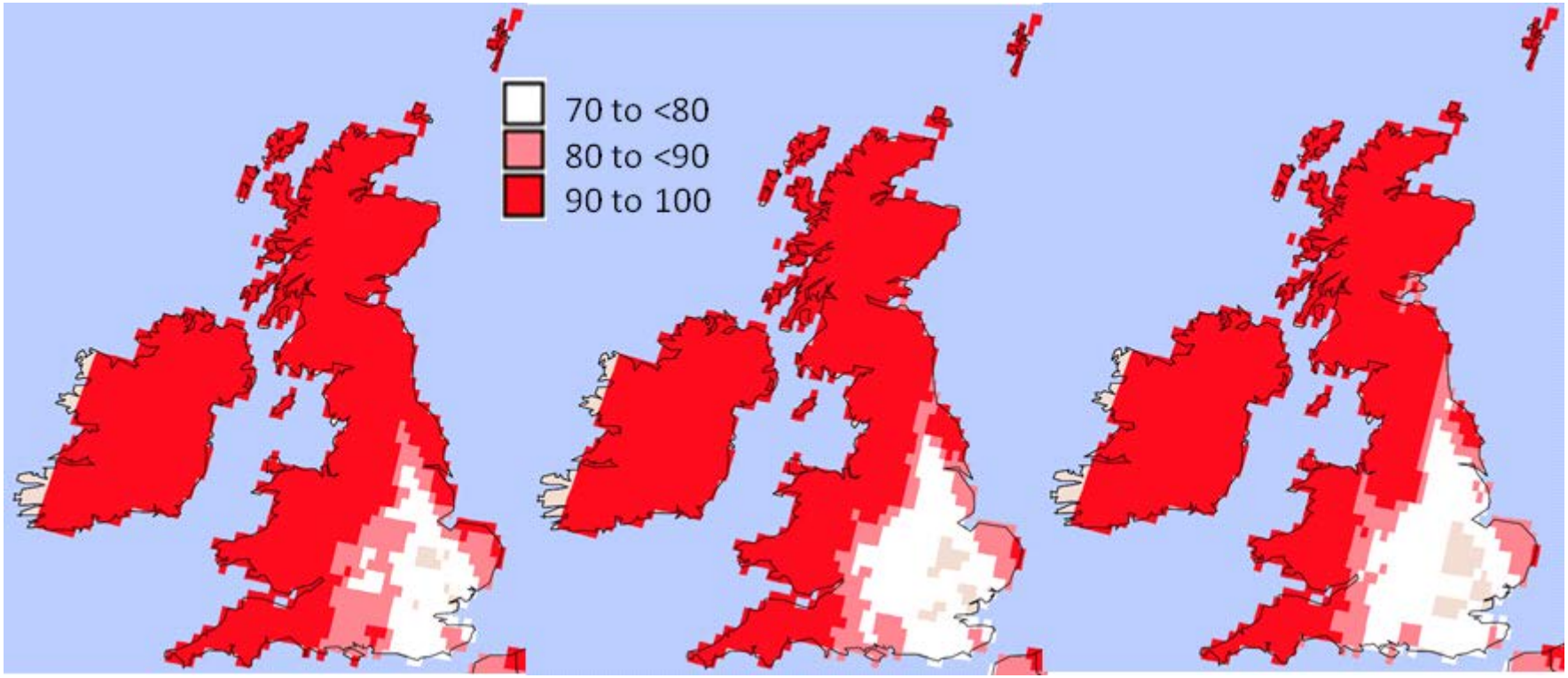

Figure 22. Moisture Index for clubroot under the current climate (left), projected 2030 climate (middle) and projected 2050 climate (right). The higher the index the greater the moisture suitability for clubroot establishment.

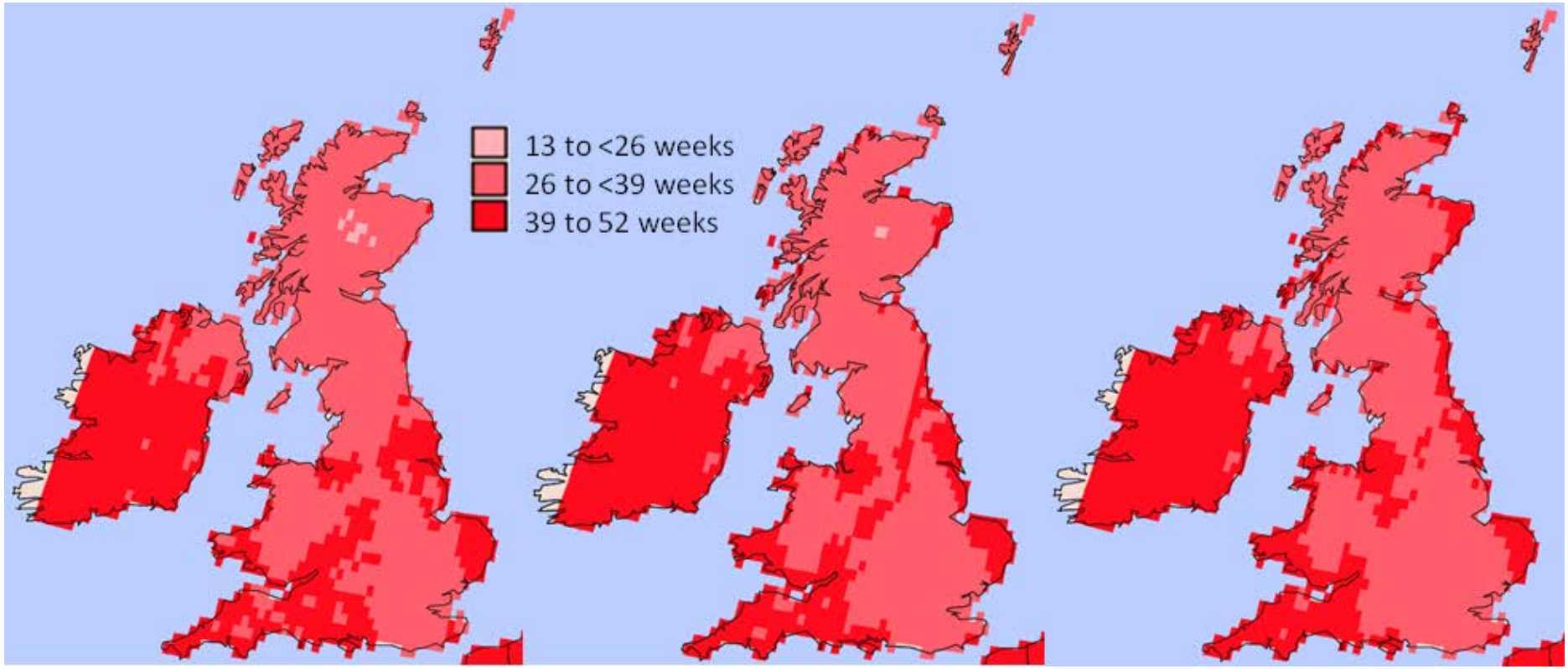

Figure 23. Weeks of positive growth per annum for clubroot under the current climate (left), projected 2030 climate (middle) and projected 2050 climate (right).

Table 39. Output from the clubroot CLIMEX model of selected indicies for 3 different locations and under the current, 2030 and 2050 climate.

Index	**Bacup, Lancs**			**Halstead, Essex**			**Brechin, Angus**		
	Current	**2030**	**2050**	**Current**	**2030**	**2050**	**Current**	**2030**	**2050**
Ecoclimatic index	21	24	26	8	9	10	9	12	14
Moisture Index	100	100	100	70	69	69	100	100	100
Temperature Index	21	24	26	26	29	30	9	12	14
Degree days	921	1043	1116	1123	1247	1322	411	537	617
Weeks of positive growth	36	36	38	29	29	30	28	30	31
Potential No. of generations	3.07	3.48	3.72	3.74	4.16	4.41	1.37	1.79	2.06

For Bacup, as climate changes from current to projected 2030 and 2050 climates, the Ecoclimatic Index (EI) increases (Table 39; Figure 21), primarily due to a high Moisture index (Table 39; Figure 22) coupled with increasing temperature (increase in Temperature index) that accelerates clubroot development (Table 39). There is an increase in the Potential No. of generations and in the Weeks of positive growth of clubroot (Table 39; Figure 23).

For Brechin, the current climate is too cool to allow much clubroot development (Temperature index of 9; 411 day degrees; Table 39) despite available moisture (Moisture index of 100, Figure 21), but under projected climate change the Temperature index increases, as does Weeks of positive growth to give potentially 2 generations of clubroot per annum (Table 39; Figure 23).

For Halstead, the low EI in the current climate (Figure 21) is primarily due to a relatively low moisture index (Figure 22; Table 1). Under projected climate change, the Moisture index doesn't alter (Table 39), and whilst the Temperature index increases, to allow for the potential for over 4 generations of clubroot per annum, the lack of moisture (unless crops get irrigated) keeps the suitability for clubroot relatively low (EI 10 or less) (Figures 21 & 22; Table 39).

The differences between Bacup, Brechin and Halstead in terms of soil moisture and the periods of positive clubroot development above a base temperature of 8°C are shown in figure 24.

Halsted in Essex has a considerable drop in soil moisture during the summer months (Figure 24) which has a significant effect on its ecoclimatic suitability for clubroot (Figure 21). However, if crops are irrigated then this soil moisture deficit will be overcome and allow clubroot development to occur at a rapid rate, as the temperature index is relatively high (29-30), allowing for potentially up to 4 cycles of clubroot a year (Table 39), beginning at the end of March and stretching to November (Figure 24).

Both Bacup and Brechin have relatively high soil moisture, even with climate change (Figures 22 & 24). Consequently there is enough soil moisture to allow clubroot to develop in the presence of suitable host plants. Any additional moisture applied via irrigation will only make conditions even more suitable for clubroot. The temperature index increases for both locations under climate change (Table 39), and the degree days above 8°C increase allows clubroot development to occur for longer during the year (Figure 24). At Bacup weeks of positive clubroot growth increase from 36 to 38 weeks, stretching from early April to the end of October (Figure 24). At Brechin, an extra 3 weeks of positive growth occurs by 2050 (28 to 31 weeks), beginning in early May through to mid-October (Figure 24).

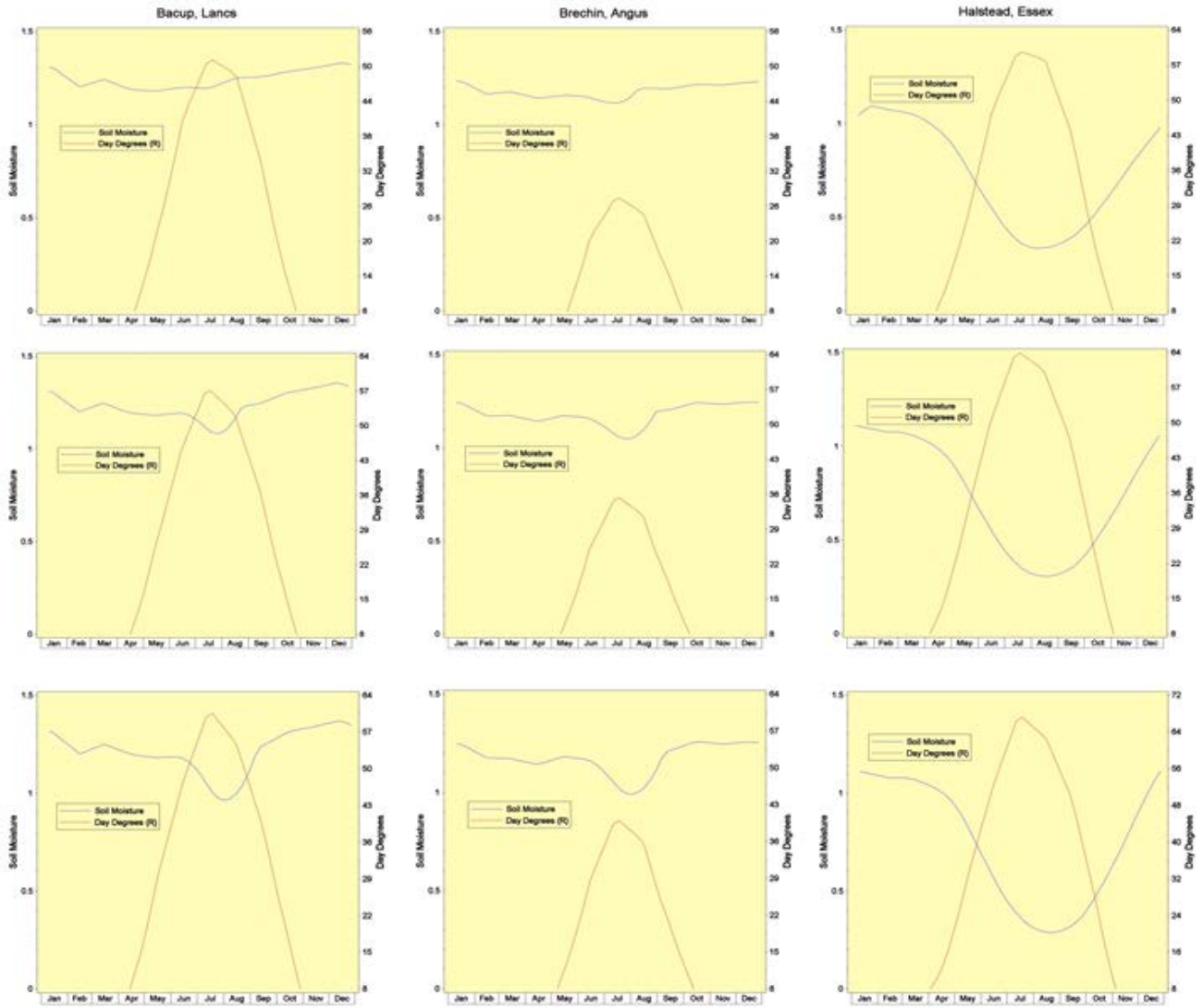

Figure 24. Outputs from the clubroot CLIMEX model. Graphs of the soil moisture and day degrees above the base temperature of 8°C for clubroot development for Bacup, Lancs; Brechin, Angus; and Halsted, Essex. Current climate (top), 2030 climate (middle) and 2050 climate (bottom).

3.3.5. Real-time PCR detection of clubroot from soil and plant material

Primers and probes were designed by aligning similar sequence data to design highly specific primers and probes that only amplified only the target *P. brassicae* sequence. In addition to checking the sequence database a large number of resting spores were also additionally sequenced to check for the uniformity of the target sequences. The results of this exercise has shown that the ITS sequence used for the design of this assay is highly conserved in all clubroot isolates both in the database and from our further sequences. The chosen probes and primers were checked using primer express software for any secondary product formation. The specificity of all primers and probes used was also checked using the National Centre for Biotechnology Information BLAST server and confirmed by PCR testing against a panel of fungi of related species and microbes which could be found in the soil.

The absence of signals and PCR products in real-time PCR or gel electrophoresis respectively, confirmed the specificity of the assays. The threshold of detection for this assay was 0.1 pg of *P. brassicae* DNA in the presence of 50 ng of Chinese cabbage DNA. This is more sensitive than assays designed previously. The graph in Figure 25 shows the standard curve and amplification plots achieved in each of the experiments.

The assay worked well for nearly all of the test soil samples spiked with *P. brassicae* DNA. However, the results obtained when using the soil samples obtained during this project were very mixed, when compared to the data obtained from the *in-planta* bioassay. Neither of the testing systems appears to be accurate in predicting the presence of clubroot disease in oilseed rape, as demonstrated in Figures 26 and 27 showing the Pearson correlation between the clubroot index from the field and bioassays against the pg of *P. brassicae* DNA. These results show that there is no significant correlation between the real-time PCR data and the bioassay infection ($r = 0.011$, $P = 0.953$). Figure 28 shows the same lack of correlation for the $\log_{10}$ of the DNA quantity ($r = 0.134$, $P = 0.953$). The correlation between the clubroot index from the field and bioassay is significant with an R^2 value of 0.416, compared to 0.216 (correlation between pg of *P. brassicae* DNA and bioassay results) and 0.018 (correlation between $\log_{10}$ of *P. brassicae* DNA and bioassay results). Clubroot spores may be present in soil but not cause disease unless the weather conditions are suitable for infection or suppressive factors are present.

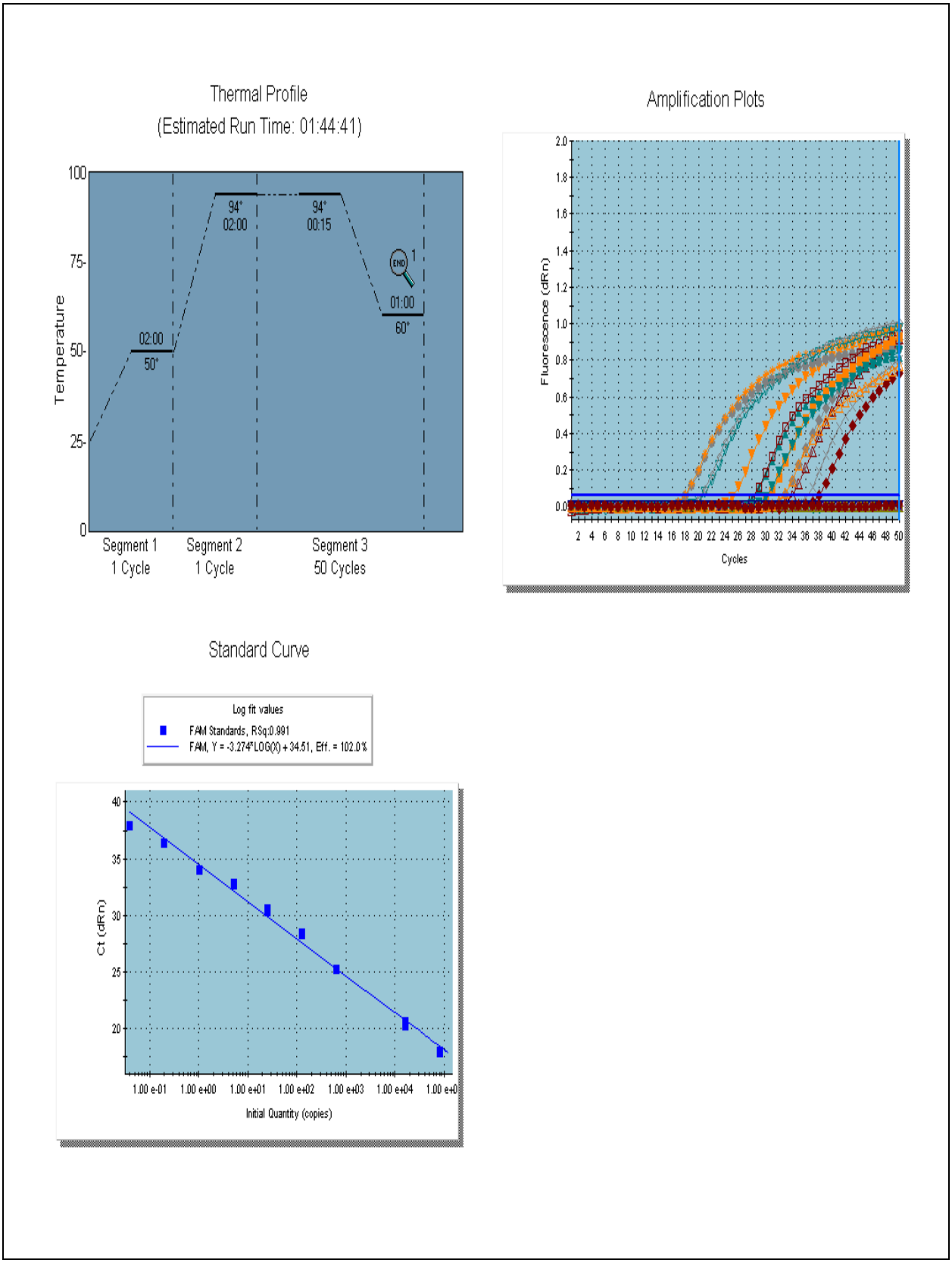

Figure 25. The standard curve and amplification plots for the real-time PCR assay.

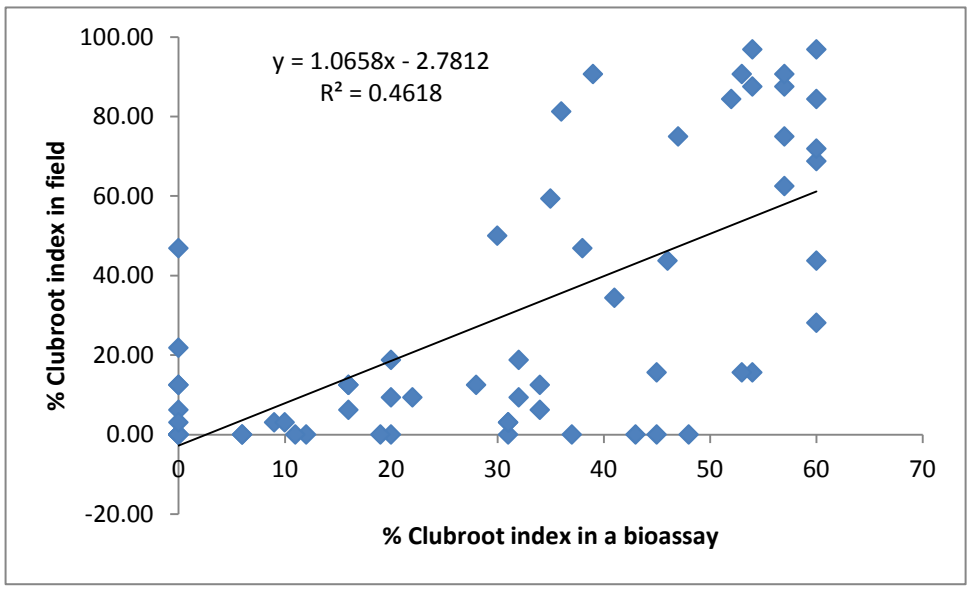

Figure 26. The correlation between clubroot index in the field and in a bioassay for the Shropshire 2008 harvest assessments.

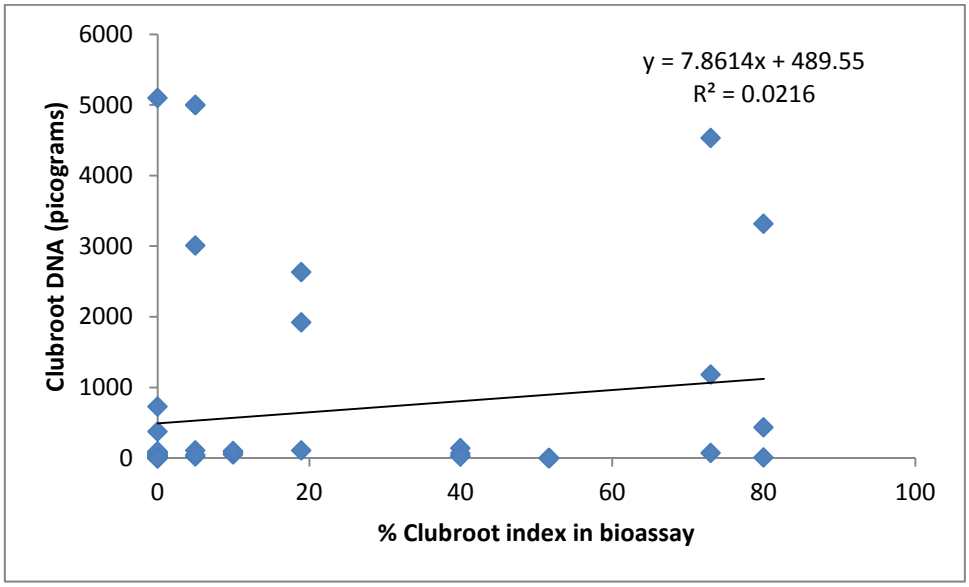

Figure 27. The lack of correlation between PCR tests (pictograms of DNA) and plant infection from survey soils.

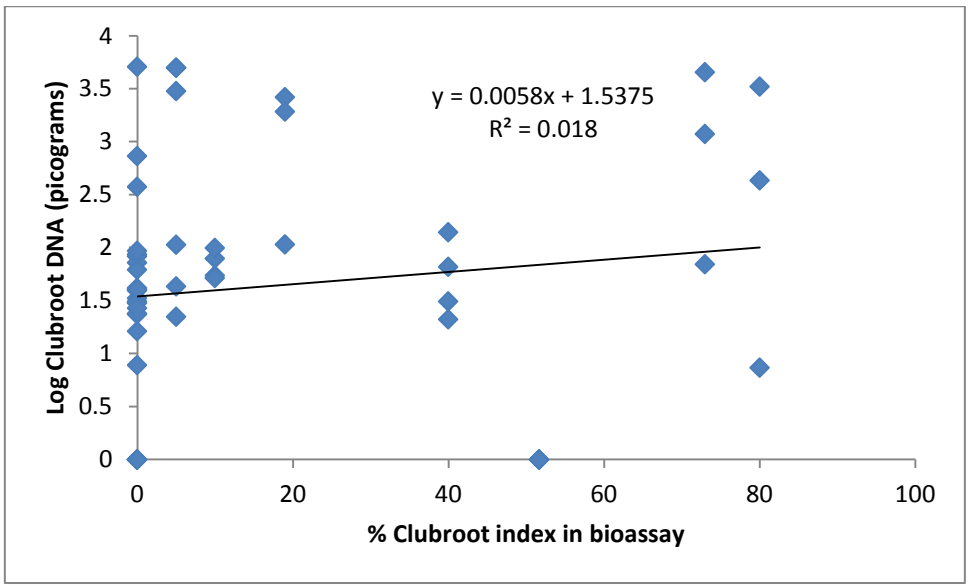

Figure 28. The lack of correlation between Log_{10} DNA quantity (pictograms) and plant infection from survey soils.

3.4. Discussion

A survey of oilseed rape fields carried out in Scotland in 2008 and 2009 showed that over half of oilseed rape fields were infected with clubroot. Soil samples were also taken from England, but mainly from farms where clubroot was already known to be present, however, a few new cases of clubroot were identified on farms that had not seen symptoms. Previously up to 2% of randomly sampled crops in England and Wales have shown clubroot symptoms (CropMonitor data) which may underestimate lower risk situations. The survey undertaken for this project showed similar rates of infection for England and Scotland – 54% and 50% respectively. However, these results may be an overestimation of risk as samples were taken from sites with a known/suspected clubroot problem. The positive sites were distributed throughout the oilseed rape growing areas surveyed. The survey data could not directly support a link with cropping intensity as the proportion of total land in Scotland in oilseed rape production is far lower than in England, but rotation lengths are frequently short with 1 in 2 rotations being common in areas in the north east of Scotland where alternative break crops are unreliable. In England, many OSR crops have been grown on 1in 2 or 1 in 3 rotations for 10-15 years.

As clubroot can persist in soils for upwards of 15 years (Wallenhammer, 1996). Rotation length is a critical factor in disease severity and short rotations increase the problem. The decline in clubroot index (using the same index calculation as this project report) over subsequent years in Wallenhammer's 1996 Swedish work is reproduced in figure 29. Wallenhammer also reported a decline in severity in clubroot with increasing rotation lengths, reproduced in figure 30. By using the yield loss figures generated in this current project and plotting these against the declines noted by Wallenhammer it would appear that rotation lengths of 1 in 4 are no better than 1 in 3 in terms of

yield loss. There was some improvement in expected yield on a 1 in 5rotation but even at rotations of 1 in 8 almost 50% of total yield could be lost.

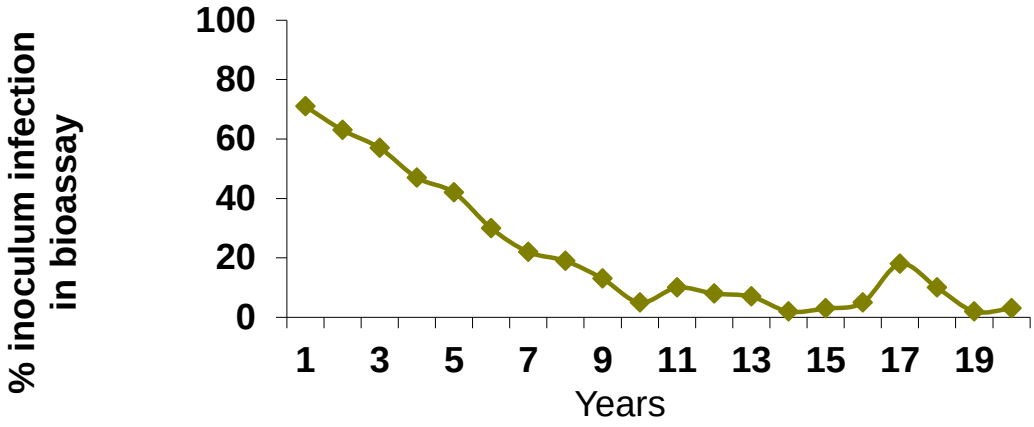

Figure 29. After Wallenhammer (1996) showing the decline of clubroot in Swedish soils, with a half-life of 3.7 years

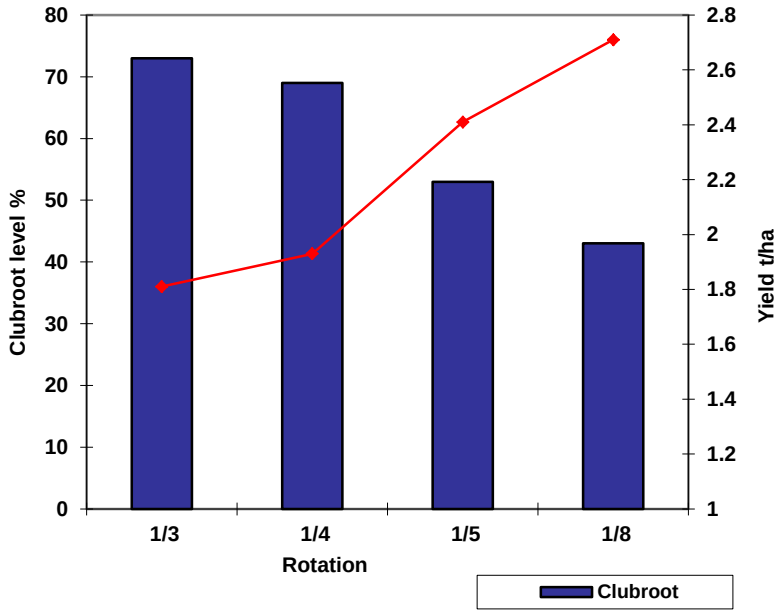

Figure 30. After Wallenhammer (1996) showing the clubroot severity in fields following the previous crop of oilseed rape, with crop yield overlaid assuming losses of 0.03 t/ha per % severity of clubroot in a 4 t/ha crop.

The current project provided data on a range of clubroot epidemics at the six sites in the trial series. The combined data set was used to establish yield losses to clubroot which equated to 0.03 t/ha per % point of clubroot severity. This was established with data from all three varieties trialled, Kommando, Mendel and Cracker, although there were very few data points for Cracker which was only trialled at one of the six sites in a single season. Yield losses in Mendel were similar to those in Kommando. This is counterintuitive for a resistant variety but probably reflects the lower yield

potential and weaker agronomic attributes of this variety at sites where it is infected. There have been a few commercial cases of very severe clubroot in susceptible varieties where the whole crop was affected and complete crop loss occurred as crops were ploughed in before spring cropping. More frequently, clubroot causes the loss of plants in small patches and surrounding plants may compensate for small plant losses. Where clubroot patches are evident, spot treatment with lime or calcium oxide during the growing season can be beneficial in controliing 'hot spots' within the field to safeguard future oilseed rape cropping.

The variability of clubroot resistance as a control strategy was clearly demonstrated at the sites. Mendel and Cracker share the same resistance mechanism, a single major gene although Cracker has a higher yield and better foliar disease resistance than Mendel which is now somewhat outclassed (see HGCA Recommended List data). Little is known or understood about the distribution of clubroot strains throughout the UK, but work by Stewart (2007) which followed established principles of differentiating clubroot strains by the host plants they will infect, demonstrated a wide range of different strains in the UK. Not all of these strains are controlled by the host resistance present in Cracker and Mendel and at sites where these varieties have been grown several times in the back rotation strains of clubroot have built up which can infect these varieties. No clubroot resistance or tolerance was noted in other varieties screened that did not contain the specific resistance mechanism in Mendel and Cracker.

Poor control of clubroot was seen in trials around the Aberdeenshire area where Mendel has been commonly used in the past. Better control was seen at the West Midland sites but even here it was variable and ranged from 50% control in Shropshire in 2008 to over 95% control at sites in Herefordshire and Warwickshire. It did not always carry a yield advantage even where disease control was observed. At the very high disease pressure Shropshire site (shown in figure 31) there were significant yield benefits to using Mendel, despite the poorer control it offered relative to other West Midland's sites. At the following season's Herefordshire site Mendel showed very good control of clubroot but disease pressure was so low that there was no yield advantage, reflecting the inherently lower yield of Mendel. However, there should be a benefit from lower clubroot inoculum production on resistant varieties, which will be evident in future cropping. Cracker offers much better yield potential (HGCA Recommended list) but this also gives the industry cause for concern. Mendel is generally planted only in situations where growers have an established and significant clubroot problem in a field. Cracker is being widely grown even in fields with no history of clubroot which means that strains of clubroot which can overcome this resistance mechanism will be widely selected.

Figure 31. Shropshire field trial site showing Kommando plot in the foreground and Mendel in the background, spring 2008.

The use of the variety Mendel in the bioassays showed the strength of varietal resistance in managing this disease. The level of control noted was 63.7% compared to the mean of other varieties tested. Whilst this level of control is encouraging it should be noted that it is by no means complete and implies that a portion of the clubroot population which was sourced from a high pressure site in Fife can overcome the Mendel resistance. This is indicative of the pressure the 'Mendel' gene is under and of the need for new forms of varietal resistance to clubroot. Further erosion of this varietal resistance through the continued widespread use of this gene and resultant selection for strains of clubroot able to overcome it would be of major concern to the industry. Little is known about the pathotypes within the clubroot population with regards to virulence on Mendel or varieties using the same resistance. Previously strains were characterised by the range of species they infected (Buczacki *et al*., 1975) which was a somewhat limited system. The use of molecular markers as a method of characterising strains of clubroot virulent of Mendel (or any following resistant varieties developed) and thereby identifying sites at risk would aid individuals and the industry. The development of alternative forms of resistance to clubroot would greatly assist future cropping.

Clubroot severity is known to be linked to pH and soil calcium content (Donald and Porter, 2009) and significant control and yield benefits can be achieved in vegetable brassica crops through the use of soil amendments which raise pH or calcium ion content. Harling (2006) reported the improved disease control potential of finer and more available forms of lime and demonstrated that control as best when spikes in pH and calcium following treatment coincided with the infection period for clubroot when spore balls had germinated. From this work two products in particular had

potential – LimeX (a Calcium carbonate by-product of the sugar industry) and Perlka (a Calcium cyanamide product) and these were selected for trialling in oilseed rape. In the course of this work though the Calcium cyanamide product has ceased to be promoted for use on oilseed rape as, at the application rate used for clubroot control, it adds 50 kg of nitrogen per hectare which contravenes Defra guidelines on limiting nitrogen applications to 30kg/ha to crops in the autumn. These are to be reviewed in 2013 but at present the use of this product will not be pursued. Banding of applications to crops grown in wide rows merits further investigation.

The use of soil amendments to manage clubroot in oilseed rape in the current trial series gave variable results in the trial series. Control ranged from 0% at some sites to 95% control for combined treatments in the final seasons' trial at a Warwickshire site. This variability in control made data analysis difficult but using predicted means from the across site analysis, there were significant treatment effects at the three West Midlands sites and the 2010 Aberdeenshire site. Calcium carbonate at 8 t/ha was the most effective solo treatment, with a mean control level of 25% compared to an untreated control. A combined treatment of Calcium carbonate at 4 t/ha + Calcium cyanamide 250 kg /ha + Boron 20 kg/ha gave a predicted mean control of 45%. This was better than the two way mixture without boron which gave 30% control. Soluble boron alone had a predicted mean control of 18%. Best yield increase was seen at sites with a moderate infection severity of clubroot and treatment effects were generally absent at sites with very low infection levels, as would be anticipated, and were poor at sites with a very high infection severity where treatments were overwhelmed for example the Shropshire 2007/2008 site or the Aberdeenshire 2009/2010 site.

Yield benefits in response to treatment were also highly variable across sites and were noted at only two of the six trials. A cross site analysis using predicted means, however, established that there were yield benefits to some of the treatments. Two of the Aberdeen sites were excluded from this: one trial was not yielded and one did not include the varietal element of the trial design. Using these predicted means Calcium cyanamide gave a mean increase in yield of 15% compared to an untreated control and the three way mixture of Calcium carbonate at 4 t/ha + Calcium cyanamide 250 kg/ha + Boron 20 kg/ha gave the largest (20%) increase to yield. Soluble boron alone lifted yield by a mean of 12%.

Despite the variability in control seen between sites some important correlations were demonstrated. There was a strong correlation between calcium content and clubroot severity and between pH and clubroot severity, as shown in figure 32.

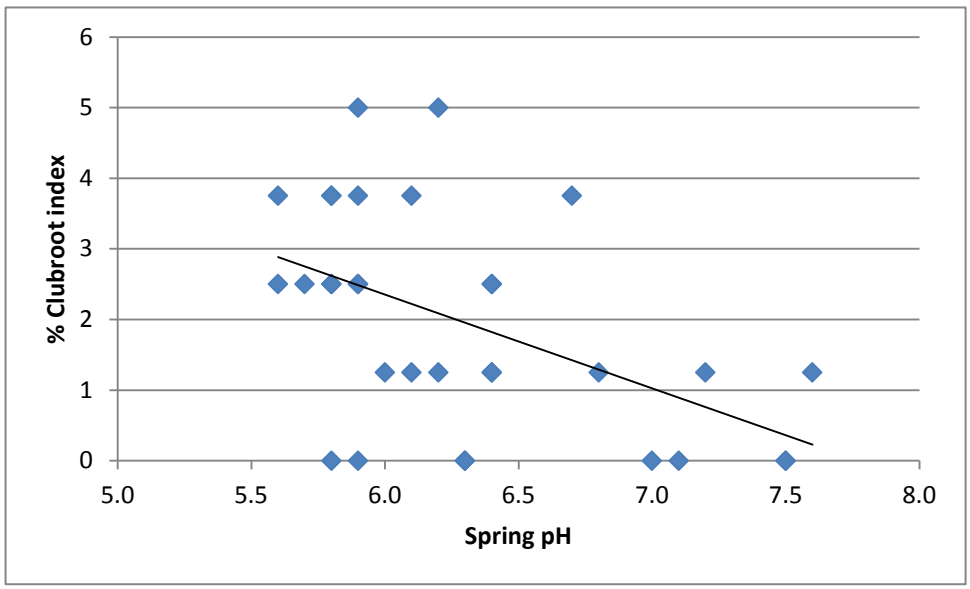

Figure 32. The correlation between clubroot severity and soil pH in the spring assessments at the Herefordshire 2008/2009 site.

There were also significant correlations between clubroot seveity and yield such that pH and calcium content also correlated with yield. Significant improvement in vigour in response to soil amendments was also noted at some sites: of these Calcium carbonate at 4 t/ha had the best predicted mean vigour of the solo treatments and the combined Calcium carbonate 4 t/ha + Calcium cyanamide 250 kg/ha had the best predicted mean vigour of the combined products.

The variability in responses across sites should not be ignored. Cost benefits were applied to treatments and these were often very low or negative for the specific trial data concerned. The principles of using soil amendments to manage clubroot severity over longer rotations could not be costed in this trial series but is an important consideration.

The trial series demonstrated that neither varietal resistance nor the use of soil amendments offers a single sustainable solution to managing clubroot in short rotations. Soil amendments gave very variable control in differing soil types and disease scenarios and the data illustrated the difficulties in altering soil pH or nutrient levels by enough to significantly attribute disease control effects to treatments. However, over the trial series the data showed that they could reduce clubroot severity by up to 25%, in the case of Calcium carbonate. The level of control from varietal resistance (50-95%) was greater and was established in the cross site analysis as being more significant than control from soil amendments (25 – 45% from the most effective amendments). The option to use varietal resistance is likely to have lower cost and fewer environmental implications, but the build up of strains of clubroot that can overcome the resistance mechanism is likely if growers should rely wholly on varietal resistance as a strategy to manage clubroot. New forms of varietal resistance may become available and this would improve the sustainability of varietal resistance as

a strategy. However, the varieties screened in this project did not identify any potential new resistance.

Clubroot development has been very variable from year to year and severe attacks are associated with wet conditions in late August and September whilst soils are still warm (>15°C). In dry autumns, few symptoms are seen in the autumn though plants may show clubroot galls in the following spring. Early sown crops are more prone to clubroot than later sowings and September sowing could be used to reduce the risk of severe infection. Climate change predictions of increasing autumn and spring temperatures in the 2030s and 2050s indicate that the UK climate will become more conducive to clubroot development in crops. Available soil moisture and temperature are the primary drivers of clubroot development in the presence of a host-plant. The modelling work undertaken in this project that takes into account the optimal and minimal temperature and soil parameters for clubroot development identified UK areas particularly suitable for clubroot. Results suggest that larger areas of the UK could become suitable for clubroot in the future. Soil moisture will continue to be a barrier for clubroot in some areas, however, if crops are irrigated this barrier will be overcome and soil moisture and temperatures will be optimal for clubroot development. Clubroot will be able to develop on winter oilseed rape crops for a longer part of the growing season than currently, in 2030 and 2050 and this could lead to significant effects on the rape crop as well as multiplying the level of clubroot inoculum in the soil.

Accurate prediction of extreme weather events is more uncertain but clubroot is observed to spread in flood waters and this is a common reason that fields around an infected source become infected themselves. Increased incidences of flooding events could therefore be further cause for concern. Effects from rotational choices are likely to be far more significant than climate changes *per se* in driving disease pressure on farms. Increased profitability in oilseed rape crops encourages tight rotations and increased acreages which increases the likelihood of further issues with clubroot. Oilseed rape is also important in farm rotations as a break crop to cereals and alternative break crops are often not as profitable, or can be very unreliable particularly in the far north of the UK where alternatives like legumes can be difficult to grow successfully and to harvest. Ideally alternative break crops should be sought so that the time between susceptible crops can be extended.

Testing soils for clubroot, identifying infected fields and limiting the spread of infection on farm remains an important part of a clubroot management strategy. Although a DNA based test would speed up this process for growers the difficulties of extracting and quantifying clubroot in soils in a molecular assay could not be overcome in the course of this project. The molecular assay developed in this project worked well when quantifying the amount of clubroot DNA in spiked soil samples; however, there was a lack of correlation in the DNA in test samples with plant infection

(bioassay) results. This lack of correlation in results may be partially due to the detection threshold of the assay being higher than the amount of inoculum found in the field but it may still cause significant disease in the field, however, this is not the only explanation. Soil is a complicated source of microbes with a high level of microbial diversity and particle types. The DNA extraction method developed as part of this project uses a large amount of initial soil sample and it was found when carrying out this protocol the quality and quantity of DNA extracted from the soil tended to differ with each of the soil types. This is normal with a DNA extraction, but in soil this can be potentially problematic as the amount of PCR inhibitors found in soil can differ significantly. DNA extraction is difficult for soils containing clay (Andersen *et al.,* 1998; Braid *et al.,* 2003) due to the binding of DNA strands to clay soil particles (Cai *et al.,* 2006). Additionally, extra-cellular DNA binds to and is co-purified with soil humic substances, which inhibit the activity of enzymes such as restriction endonucleases and DNA polymerase (Cai *et al.,* 2006). Although clay-bound DNA can be PCR-amplified in the absence of inhibitors (Alvarez *et al.,* 1998), it is often the case that inhibitors are present in the soil environment, among them bilirubin, bile salts, urobilinogens and polysaccharides. Of these inhibitors, humic substances have been found to be the most recalcitrant (Tebbe *et al.,* 1993). During this project we used PVP to remove some of the inhibitors, however, this can also have an effect of reducing the DNA quantity for the sample, as well has not removing all of the potential humic acid that could be present (Jia *et al.,* 2006). Off the shelf extraction kits have been tried in previous work at SAC (unpublished) but because they use only very small quantities of soil and a sporeball is relatively large, we found a very poor correlation between the DNA quantified and the detection in bioassays and so we worked on larger and more representative soil volumes. This suggests that soil is not a good source material for the accurate detection of clubroot when using PCR and detection via bioassay plants might be a method to get around these difficulties.

The other aspect that has to be considered when detecting a pathogen in a soil is the inability to differentiate between living and dead material. Whilst DNA can be extracted and amplified, this current procedure is not an accurate way of assessing the potential of this inoculum to cause active disease in crops. It is still possible to extract DNA from archive samples of *Ramularia collo-cygni* DNA and amplify it using PCR from the Rothamsted Hoosfield spring barley archive from grain and straw samples despite the pathogen is no longer viable (Fountaine *et al.,* 2009).

The most accurate way to assess for the development of clubroot from soil samples is to set up a bioassay as described in this report with Chinese cabbage and grow these in the sample soil for shortened period and then do a PCR detection assay from the root tissues to assess the quantity of *P. brassicae* in the Chinese cabbage plants. This will then accurately detect for actively growing clubroot pathogen without the risk of false negatives which may occur due to the presence of humic acids. The procedure for plant DNA extraction is also considerably quicker to perform than

that of the soil extraction procedure. The alternative is to further develop the DNA extraction method from bulk soils without losing the quantity of the original sample. Detection of low clubroot infestations is an important area for investigation as it is not known whether clubroot is present and building up gradually on farms where clubroot has not been found.

3.4.1. Conclusions

Clubroot causes significant yield loses in oilseed rape and these can equate to more than half of potential yield on a site with severe infection and occasionally crop failure. No single strategy of using either varietal resistance or soil amendments offers complete and reliable control. The risks of clubroot increase when brassicas are grown in short rotations, whether these are winter or spring crops or oilseed rape, vegetable or forage brassicas. Basic husbandry to use lime to maintain soil pH and have suitable rotations have been neglected. Preventative action with lime will be beneficial in managing this disease where disease pressure is low. Varietal resistance is likely to be very successful initially but will quickly be eroded in short rotations when there is a build up of clubroot strains that can overcome the current genetic resistance. Using soil amendments was less successful on oilseed rape than has been reported on vegetable crops and this may be due to differences in clubroot strains between the crop types but is more likely to be due to the ability to time treatments and resultant pH and calcium rises closely to clubroot germination in transplanted vegetable crops. In drilled oilseed rape crops there is likely to be a short delay between soil treatment application and crop germination which will then stimulate the clubroot to germinate (emergence can range from one to three weeks), and in this time there may have been declines in pH and calcium spikes that were there at drilling.

Control was very variable with soil amendments but general principles were established. There was a clear link between raised pH and calcium content in soils and declines in clubroot severity, and over the trial series as a whole modest reductions in disease and yield benefits were noted. The use of such products as part of the overall strategy for infected fields on farm should therefore be considered. The calcium carbonate in LimeX 70 has a very fine particle size and acts quickly because of the large surface of the particles. Other formulations of calcium carbonate with larger particle sizes may be slower acting and require different rates of application. Some cultivation of the seedbed is required so that the lime is mixed into the soil. Where routine liming is required, application can be made at a point in the rotation where soil cultivation takes place so additional incorporation costs are minimised. Tight rotations and a prediction of warmer and wetter autumn and spring temperatures will further increase clubroot pressure in oilseed rape crops

The impact and cost of clubroot in a single season on farm is relatively simple to calculate. The impact in the longer term could be more damaging and future work to properly cost different rotational strategies with regards to the frequency of use of oilseed (and other susceptible crops)

would be useful to the industry. It would indicate if the long term profitability of the crop is threatened by the current frequency of this crop in farm rotations. This would allow growers to make crop and input choices based on a long term strategy for a field and farm.

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APPENDIX

Statistics

Data are analysed on the following variables.

Sclerotinia (% plot affected) at harvest

Light leaf spot (% plot affected) in March and at harvest

Crop coverage (% plot cover) in March

Sclerotinia

Values for Sclerotinia (% plot affected) were recorded at harvest in the trials Aberdeen 2009/2010 (for Kommando only), Shropshire 2007/2008 and Warwickshire 2009/2010. Sclerotinia was not noted in the other three trials.

Mean percentages are as follows in table 40. The distribution shows strong positive skewness and a large proportion of zeros, so that analyses of variance based on an assumption of Normality are not appropriate. It is clear, though, that incidence is much higher in Aberdeenshire 2009/2010 trial than in the other two trials, where significant control from the combined Calcium cyanamide and calcium carbonate +boron treatment was noted.

Table 40. Mean percentage sclerotinia in trials.

Variety	Treatment	Abdn 08	Abdn 09	Abdn 10	Shrop 08	Here 09	Warw 10
Kommando	Control	–	–	12.5	0.38	–	1.3
	ExtraN	–	–	–	0.3	–	–
	Limex2	–	–	–	0.38	–	–
	Limex4	–	–	9.3	0.0	–	1.0
	Limex8	–	–	11.5	0.0	–	0.8
	Perlkai	–	–	–	0.5	–	–
	Perlkan	–	–	9.8	0.38	–	0.5
	Solubor	–	–	–	0.3	–	–
	Limex4Perlkan	–	–	15.0	–	–	0.12
	Limex4PerlkanSQ	–	–	9.0	–	–	0.62
Mendel	Control	–	–	–	0.12	–	0.12
	ExtraN	–	–	–	0.3	–	–
	Limex2	–	–	–	0.3	–	–
	Limex4	–	–	–	0.12	–	0.38
	Limex8	–	–	–	0.0	–	0.62
	Perlkai	–	–	–	0.38	–	–
	Perlkan	–	–	–	0.38	–	0.3
	Solubor	–	–	–	0.0	–	–
	Limex4Perlkan	–	–	–	–	–	0.5
	Limex4PerlkanSQ	–	–	–	–	–	0.8
Cracker	Control	–	–	–	–	–	0.8
	ExtraN	–	–	–	–	–	–
	Limex2	–	–	–	–	–	–
	Limex4	–	–	–	–	–	0.38
	Limex8	–	–	–	–	–	0.3
	Perlkai	–	–	–	–	–	–
	Perlkan	–	–	–	–	–	0.0
	Solubor	–	–	–	–	–	–
	Limex4Perlkan	–	–	–	–	–	0.3
	Limex4PerlkanSQ	–	–	–	–	–	0.5

Light leaf spot (LLS)

Light leaf spot (% plot affected) was recorded in March only in the 2007/2008 Shropshire trial. Mean percentages were as shown below in table 41.

Table 41. Mean % plot affected with light leaf spot in March in Shropshire 2007/2008 trial

Treatments	Kommando	Mendel
Control	13.8	23.8
ExtraN	12.5	22.5
Limex2	11.8	21.3
Limex4	11.3	21.3
Limex8	13.8	23.8
Perlkai	16.3	27.5
Perlkan	13.0	22.5
Solubor	13.8	21.8

The distribution is positively skewed, but the logarithm of LLS+0.5 is roughly normal. A split-plot analysis shows no evidence of a trend over columns or of a treatment effect, but there is very strong evidence of a difference between the variety effects.

Light leaf spot was recorded at harvest except in the Hereford 2008/2009 trial: the mean percentages are as follows in table 42.

Table 42. Mean light leaf spot % plot affected at harvest

		Abdn 08	Abdn 09	Abdn 10	Shrop 08	Here 09	Warw 10
Kommando	Control	3.00	2.50	2.75	3.25	–	0.38
	ExtraN	4.50	4.50	–	2.50	–	–
	Limex2	2.50	–	–	3.00	–	–
	Limex4	3.00	2.00	2.50	3.50	–	0.25
	Limex8	2.50	3.00	2.75	2.50	–	0.31
	Perlkai	3.50	2.75	–	3.00	–	–
	Perlkan	2.75	3.25	3.00	2.25	–	0.50
	Solubor	3.50	3.25	–	2.25	–	–
	Limex4Perlkai	–	4.00	–	–	–	–
	Limex4Perlkan	–	–	3.00	–	–	0.12
	Limex4PerlkanSQ	–	–	3.25	–	–	0.19
Mendel	Control	2.50	3.50	3.25	1.75	–	0.25
	ExtraN	4.75	4.75	–	3.00	–	–
	Limex2	3.00	–	–	1.75	–	–
	Limex4	2.75	4.25	2.50	2.00	–	0.19
	Limex8	3.25	3.50	1.50	2.25	–	0.50
	Perlkai	3.75	2.75	–	2.25	–	–
	Perlkan	4.25	4.50	3.00	1.75	–	0.44
	Solubor	5.25	2.75	–	2.00	–	–
	Limex4Perlkai	–	3.50	–	–	–	–
	Limex4Perlkan	–	–	5.00	–	–	0.31
	Limex4PerlkanSQ	–	–	3.00	–	–	0.44
Cracker	Control	–	–	3.75	–	–	0.12
	ExtraN	–	–	–	–	–	–
	Limex2	–	–	–	–	–	–
	Limex4	–	–	2.75	–	–	0.25
	Limex8	–	–	2.00	–	–	0.19
	Perlkai	–	–	–	–	–	–
	Perlkan	–	–	4.75	–	–	0.00
	Solubor	–	–	–	–	–	–
	Limex4Perlkai	–	–	–	–	–	–
	Limex4Perlkan	–	–	3.25	–	–	0.19
	Limex4PerlkanSQ	–	–	3.50	–	–	0.25

Split-plot analyses of the individual trials show no evidence of a trend over rows or columns, and the Aberdeenshire trials give little evidence of treatment or variety effects. The West Midlands trials both have significant variety effects (P=0.01 and P=0.02 for Shropshire 2007/2008 and Warwickshire 2009/2010).

A combined analysis of log of light leaf spot at harvest for all five trials including only the trial-by-variety interaction finds this interaction to be highly significant, with highly significant trial effects and significant variety and treatment effects (tables 43 and 44). A similar combined analysis of only the Shropshire 2007/2008 and Warwickshire 2007/2010 data finds highly significant trial, variety and treatment effects. Tables of predicted means for treatment and trial-by-variety (on the $\log_{10}$ scale) are as follows. Average standard errors of differences are 0.045 (0.058 for English sites) for treatment means and 0.090 (0.049) for trial-by-variety means.

Table 43. Log_{10} treatment means for light leaf spot

Treatments	All 5 trials	WMid only
Control	0.361	0.150
ExtraN	0.466	0.208
Limex2	0.337	0.144
Limex4	0.358	0.163
Limex8	0.366	0.172
Perlkai	0.372	0.195
Perlkan	0.369	0.124
Solubor	0.377	0.107
Limex4Perlkai	0.405	–
Limex4Perlkan	0.359	0.120
Limex4PerlkanSQ	0.374	0.145

Table 44. Log_{10} variety means for light leaf spot

All 5 trials		Kommando	Mendel	Cracker
	Abdn 08	0.518	0.577	–
	Abdn 09	0.509	0.568	–
	Abdn 10	0.459	0.469	0.496
	Shrop 08	0.496	0.402	–
	Warw 10	-0.110	-0.085	-0.192

WMid only		Kommando	Mendel	Cracker
	WMid 08	0.490	0.396	–
	WMid 10	-0.115	-0.090	-0.197

Crop coverage

Crop coverage (in % plot area) was recorded in March in all the West Midlands trials, and in the Aberdeen 2010 trial. The mean values show a very large difference between the Shropshire 2008 trial (with very high clubroot indices) and the other 3 trials.

Table 45. Summary of crop coverage data

		Abdn 08	Abdn 09	Abdn 10	Shrop 08	Here 09	Warw 10
Kommando	Control	–	–	100	4.0	83.8	90.5
	ExtraN	–	–	–	3.5	83.8	–
	Limex2	–	–	–	3.8	–	–
	Limex4	–	–	100	4.8	90.0	92.5
	Limex8	–	–	100	6.0	90.0	90.5
	Perlkai	–	–	–	4.5	87.5	–
	Perlkan	–	–	100	8.3	86.3	93.8
	Solubor	–	–	–	8.0	80.0	–
	Limex4Perlkai	–	–	–	–	90.0	–
	Limex4Perlkan	–	–	100	–	–	93.8
	Limex4PerlkanSQ	–	–	100	–	–	95.0
Mendel	Control	–	–	100	13.0	87.5	90.3
	ExtraN	–	–	–	9.8	91.3	–
	Limex2	–	–	–	13.5	–	–
	Limex4	–	–	100	10.5	95.0	91.8
	Limex8	–	–	100	16.0	95.8	91.0
	Perlkai	–	–	–	13.5	92.5	–
	Perlkan	–	–	100	14.5	90.0	91.8
	Solubor	–	–	–	13.0	93.8	–
	Limex4Perlkai	–	–	–	–	92.5	–
	Limex4Perlkan	–	–	100	–	–	91.8
	Limex4PerlkanSQ	–	–	100	–	–	95.0
Cracker	Control	–	–	100	–	–	90.5
	ExtraN	–	–	–	–	–	–
	Limex2	–	–	–	–	–	–
	Limex4	–	–	100	–	–	89.3
	Limex8	–	–	100	–	–	91.8
	Perlkai	–	–	–	–	–	–
	Perlkan	–	–	100	–	–	93.8
	Solubor	–	–	–	–	–	–
	Limex4Perlkai	–	–	–	–	–	–
	Limex4Perlkan	–	–	100	–	–	92.5
	Limex4PerlkanSQ	–	–	100	–	–	93.5