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Control of clubroot (*Plasmodiophora brassicae*) in oilseed rape using varietal resistance and soil amendments

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Abstract

Clubroot is a major threat to global brassica production. It has been an increasing problem in UK oilseed rape (OSR) crops due to the persistence of the soil-borne pathogen responsible for disease, *Plasmodiophora brassicae*, exacerbated by close rotations. Field surveys in the UK in the years 2007-2010 showed that clubroot was present in all areas of the UK where OSR was grown with 52% of the selected sites testing positive. Varietal resistance and soil amendment with calcium carbonate, calcium cyanamide and boron alone or in various combinations applied before sowing were assessed for the potential to manage clubroot. Soil amendments gave variable control between sites and years but showed some potential as part of a clubroot management strategy. Varietal resistance remained the more effective management option providing 50 – 95% disease control at three sites in England. However, this control was not consistently effective at sites in Scotland where resistant OSR varieties have been heavily used in rotations. Yield losses were demonstrated at 0.03 t ha^{-1} for every 1% increase in clubroot severity in the susceptible variety Kommando. Yield losses were only slightly lower per 1% increase in clubroot severity for the resistant variety Mendel at 0.028 t ha^{-1} despite lower disease levels. Losses in affected crops can therefore equate to over 50% of potential yield in severely infected crops. Soil testing for clubroot and lengthening rotations are important to the long term management of clubroot as varietal resistance and soil amendments can reduce clubroot severity but provide inconsistent results.

1. Introduction

Clubroot is the most damaging disease of brassica crops globally occurring in more than 60 countries where typical yield losses are in the range of 10-15% (Dixon, 2009a) but can reach in excess of 80% (Pageau et al., 2006; Hwang et al., 2011a). The disease is caused by the soil-borne root pathogen *Plasmodiophora brassicae* which can remain dormant in soils as long-lived resting spores for upwards of 15 years (Hwang et al., 2012b). The estimated half life of *P. brassicae* resting spores is 3.7 years (Wallenhammar, 1996) hence the disease is a major issue when brassica crops are grown in short rotations. Furthermore clubroot can infect and survive on cruciferous weeds in arable rotations. The introduction of oilseed rape (OSR) into rotations in the UK in the mid 1970s further increased the risk of disease spread and build up. OSR is grown on a little under 700,000 hectares of land in the UK (Anon, 2014a) approximately 37,000 hectares of which are in Scotland (Anon, 2014b). As break crop choices become increasingly limited OSR has become particularly important in arable rotations in the north of the UK. OSR 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 clubroot multiplying and spreading across farms as has been observed across the major OSR and vegetable brassica growing regions of the world (Dixon, 2009a) including recently in Canada (Hwang et al., 2012b).

Clubroot is a disease typically associated with warm wet soils (Dixon, 2009b). Infection begins when resting spores germinate and motile zoospores swim through available soil water towards the host roots. This process is thought to be stimulated by root exudates (Rashid et al., 2013). During the primary infection phase the zoospores infect root hairs where they can multiply and go on to spread and form secondary infections in root cells (Webster, 1986; Hwang et al., 2011b). Pathogen development within root cortical cells leads to changes in root hormone balance resulting in hypertrophy and formation of galls which present as the typical clubroot symptom (Ludwig-Müller et al., 2009; Siemens et al., 2006). The galls form strong metabolic sinks which alter the source-sink relationship and result in nutrients being transported to the roots leading to reduced green leaf area and consequent growth and yield penalties (Ludwig-Müller et al., 2009; Mitchell and Rice, 1979; Keen and Williams, 1969). Clubroot spread can occur via contaminated soil transferred on machinery wheels and although there is some limited movement of the resting spores through the soil profile, spread following field flooding is commonly noted (Dixon, 2009b). Disease symptoms often first appear in patches in the crop but can become distributed throughout the field in subsequent

years. In vegetable cropping land is often rented and soil testing for the disease prior to field selection allows infected sites to be rejected (Dixon, 2009a). However, clubroot-free land is a diminishing resource and rejecting infested fields for OSR cultivation adds additional pressures to the holding's crop rotation strategy, as well as having financial implications. In recent years, reports of serious problems with clubroot infection in OSR and yield losses in commercial crops have increased.

Control of clubroot is particularly difficult due to the persistence of the pathogen in soil (Wallenhammar, 1996). Some fungicides have shown some control of clubroot (Peng et al., 2014; 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 for OSR. Faced with an infected field, growers will usually opt to drill a clubroot resistant OSR variety which not only reduces disease levels in the crop but also has the potential to reduce inoculum levels in the field (Hwang et al., 2012a). Although numerous clubroot resistance sources have been found in brassica crops worldwide (Diederichsen et al., 2009) all clubroot resistant OSR in the UK contains a single major gene resistance source derived from the variety Mendel (Diederichsen et al., 2006; http://www.hgca.com/varieties/hgca-recommended-lists/winter-oilseed-rape-2015-16.aspx). However, this source of resistance is not effective against all pathotypes of clubroot present in the UK and significant disease can develop where it has been used several times in a rotation indicating this source of resistance is unlikely to be durable (Diederichsen et al., 2006; Werner *et al*., 2008; Oxley, 2007).

Soil amendments using non-synthetic compounds have potential for use in clubroot management strategies. 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 (Donald et al., 2004; Myers and Campbell, 1985; Niwa et al., 2007; Tremblay et al., 2005). A direct effect from calcium and pH on resting spores has been shown in previous research (Donald and Porter, 2009; Myers and Campbell, 1985) but only at extremes of low inoculum and high values for these two parameters. Timing of soil amendment application, such as lime products (Harling, 2006), is critical and the immediate three or four days following transplanting of vegetable brassicas has been identified as the most important (Webster, 1986). Control offered by raised pH and calcium, although potentially significant, is not complete. Even distribution in soil is also a key factor and this can be hard to achieve, which allows clubroot infection to occur in pockets of lower pH and available calcium. Other

nutrients such as boron may also have an effect on clubroot (Donald and Porter, 2009). 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 (Myers and Campbell, 1985; Webster, 1986).

Managing clubroot infections is essential to maintain OSR as a viable break crop in the UK. The aim of this study was to assess the incidence and severity of infestation in UK OSR crops, to screen varieties for resistance and to test the efficacy of soil amendments and the effectiveness of varietal resistance to control clubroot in different environments. Together these factors can be used to develop sustainable management strategies for clubroot in UK OSR agriculture.

2. Methods and materials

2.1. Survey of oilseed rape fields

In 2008, 2009 and 2010 a survey of commercial OSR fields in Scotland and England was undertaken. A total of 96 samples were collected, 42 from England, two from Wales and 52 from Scotland. Sampling was not random with fields selected by growers and agronomists (ADAS and SAC). Sites in England were mainly taken from known infected farms that were still growing winter OSR in the South East, South West, Midland and Northern regions. Fifty cores were taken to a depth of 15-20 cm from each sampling area (c. 2.5 ha) in autumn or winter to give a soil sample of approximately 2 kg. Cores were collected at regular intervals in a "W" pattern. Each soil had large stones and plant material removed before being mixed by hand and used to fill seed trays for bioassay testing. Seed trays with drainage holes (20 x 14.5 x 5.5 cm) were filled with each sample soil and placed into a larger tray with no drainage holes (33.5 x 21 x 5.5 cm). Positive, soil from a previously determined heavily infected site, and negative, John Innes No. 2 potting compost pH 5.5, control samples were set up with every batch of tests. Twenty seedlings of untreated Chinese cabbage variety SB1 Kilo were planted per tray. Trays were then put on raised benches in a glasshouse and watered daily by pouring water into the larger trays without holes. The glasshouse air temperature was set at 18^{o}C. After six weeks, roots were assessed and scored for clubroot infection on a four point scale where 0 was uninfected, 1 = slight clubbing, 2 = moderately clubbed and 3 = severely clubbed (Kuginuki et al., 1999). A disease severity index was

calculated using the following formula:- Index = ((1*slight) + (2* moderate)+ (3* severe)) * (100/3*number of plants assessed).

2.2 OSR variety clubroot resistance bioassay

A bioassay of 31 OSR varieties (Table S1) going through the UK National or Recommended list testing system at the time plus a Chinese cabbage control were used to test for varietal resistance to clubroot. The same seed tray set was used as for the field survey experiments except each tray was filled with John Innes No. 2 potting compost, pH 5.5. An isolate of *Plasmodiophora brassicae,* prepared from clubroot infested soil from Cupar, Scotland, was used as inoculum for the bioassays. To inoculate the compost, a 50 mL clubroot resting spore suspension containing 10^5 spores mL^{-1} was poured over the top of the soil to give a final concentration of around 10^4 spores g $soil^{-1}$. The spore suspension was prepared from clubroot galls collected from Chinese cabbage plants grown in infested soil for six weeks. Galls were washed free of soil, homogenised, filtered through eight layers of muslin using 20 – 25 mL tap water and centrifuged at 100 x g. Pellet debris was discarded and the supernatant spun at 6000 x g for 15 minutes to pellet the resting spores. Spores were re-suspended in de-ionised water and a haemocytometer used to count the number of spores in 25 mL solution. Tested varieties were sown into inoculated soil at 25 seeds per tray. After six weeks growth in a glasshouse at 18°C, plant roots were assessed for clubbing using the 0-3 scale and a disease severity index calculated as described previously.

2.3 OSR field trial assessments of clubroot control by soil amendments and varietal resistance

Field trials were established in the autumns of 2007, 2008 and 2009 at sites with a history of severe clubroot. There were two sites per season, one in Aberdeenshire (all years) and one in Shropshire (2007-2008), Herefordshire (2008-2009) and Warwickshire (2009-2010). These sites were selected to provide a geographical spread of UK OSR growing regions that are typically at high risk of clubroot infection. Details of each site are presented in Table S2. Crops were managed by farmers and their agronomists and grown with commercial applicants of nitrogen, other nutrients and pesticides including foliar fungicides. 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 (Table 1). The soil amendments evaluated were a precipitated calcium carbonate product ($CaCO_3$, LimeX70, British Sugar, Peterborough, UK) and a calcium cyanamide product ($CaCN_2$, Perlka, AlzChem, Trostberg, Germany). $CaCO_3$was applied to plots before cultivation and incorporated. $CaCN_2$ was applied after soil preparation for drilling and then shallow incorporated in two of the treatments and not incorporated for a third treatment (Table 1). Boron applied as Solubor (20.8% boron, BORAX, California, USA) was also evaluated as a soil drench amendment using a knapsack sprayer. In each trial there was an untreated control treatment and in 2007-2008 and 2008-2009 a further control treatment with additional ammonium nitrate added was included to balance the 50 kg ha^{-1} of nitrogen in the $CaCN_2$ product. Other inputs were as per local practice. OSR varieties assessed were Kommando, which carries no known resistance to clubroot, and Mendel and Cracker which are clubroot resistant. Cracker was kindly made available to the project by LS Plant Breeding (Cambridge, UK) in the 2009-2010 trial.

The soil amendments evaluated in this first trial season (2007-2008) are indicated in Table 1. The same protocol as year one was broadly followed in the second trial season (2008-2009) but the lowest rate of $CaCO_3$ (2 t ha^{-1}) was replaced with a combined treatment of $CaCO_3$and $CaCN_2$ (Table 1). In the final years trial (2009-2010) only the most effective treatments from year one and two were selected as a second clubroot resistant variety, Cracker, was introduced, and tested alongside Mendel and Kommando. However due to high levels of OSR volunteers at the Aberdeenshire site the varietal component to the trial had to be abandoned and the Kommado treatments only were assessed and analysed at this site in 2009-2010. Throughout the season clubroot development was monitored with disease assessments made in the Autumn, Spring and just before harvest using the 0-3 scale (Kuginuki et al., 1999). Clubroot severity was calculated as described for the glasshouse bioassays. At harvest plant survival was recorded and yield was assessed and adjusted to 91% dry matter. Soil available calcium and pH measurements were assessed using commercial services (http://www.sruc.ac.uk/info/120148/analytical_services/645/soils).

2.4 Statistical analysis

Unless stated otherwise data were analysed using GenStat v13 (Payne et al., 2009). Differences in varietal resistance in glasshouse bioassays were assessed using general linear modelling (GLM) with variety as the factor. Yield response, clubroot severity and soil pH

and calcium levels were analysed for each site separately. Treatment and variety were used as factors for GLM to assess the effects on yield response, soil pH and calcium. Clubroot severity data was LOGIT transformed as previously described (McGrann et al., 2014) prior to linear mixed modelling of repeated measures analysis. Factors for the linear mixed model included scoring date, treatment and variety. Correlation analyses between different variables were performed using Pearson correlation in Minitab v16 (Minitab, 2010).

3. Results

3.1 Survey results (bioassay method)

From a total of 96 samples received from locations across the UK during the two years of the study 57% of the soils from 2008 tested positive for clubroot in the glasshouse assay in 2008 whereas 40% of the samples were positive in 2009. Infection levels across English and Scottish samples were similar – 50% in the Scottish samples and 52% in the English samples (26 positives out of 52 Scottish samples, and 22/42 English samples). The samples from England were received from mainly known clubroot infested farms but the disease was detected in one farm with no previous history of clubroot. Two positive samples were received from Wales. Mean clubroot infection index after bioassay testing was 26.6% with a range of 1.7 to 100%. Soil analysis indicated the mean pH of soils was 6.6 with a range of 5.6 – 8.2 and the mean extractable calcium level was 2564 mg L^{-1} with a range of 894 – 21800. There was no significant correlation between disease index and soil pH (r = 0.223, P = 0.168) or extractable calcium levels (r = -0.1303, P = 0.525) in the surveyed soils (Fig. S1).

Many samples that tested postive for clubroot but had low disease indices in the bioassay were from fields where clubroot had not previously been reported in the crop indicating the disease was present at subclinical levels (Fig S2). Based on maps highlighting the proportion of land across Scotland and England under OSR production in 2009 the survey indicated that clubroot infestation is widely distributed in Scotland but may be more localised throughout England although it was detected in most production areas tested (Fig. S3). Overall, the highest number of cases of clubroot was reported from the West Midlands and Aberdeenshire (Fig. S3).

3.2 Varietal response to clubroot

The variety Mendel had the lowest average clubroot severity of 26.9% which was significantly lower than all the other varieties tested ($P < 0.001$; Fig. 1). Clubroot severity for the other varieties tested ranged from 64.95% (for PR46W14) to 87.47% (for Emerson). When Mendel was excluded from the analysis no significant differences in clubroot severity were observed between the other OSR varieties or Chinese cabbage plants used a positive control (Fig. S4) for clubroot infection ($P = 0.171$).

3.3 Field trial results

Clubroot was assessed in trials as both disease incidence (%) and disease index (%) which gives a measure of severity. One was a good predictor of the other and the correlation between the two measures was highly significant (Fig 2; Pearson correlation value = 0.961 and $P < 0.001$). However, disease incidence values did not differentiate samples with high disease severities so while the severity index kept increasing there was a plateauing of incidence values for those samples at the top end of the index scale. Therefore the disease index was used to assess treatment efficacy in each field trial.

3.3.1 Field trials 2007 – 2008

Two field trials were carried out, one in Shropshire and one in Aberdeenshire. High levels of disease developed at the Shropshire site only (Fig. 3a). Disease levels 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 Shropshire site disease levels were very high and control was variable. Severe galling was noted on the tap roots of plants from autumn onwards resulting in plant loss over winter. Spring assessments were done on surviving plants. There were no statistically significant differences in disease control as a result of soil treatments ($P = 0.264$), although some non-significant trends were observed. $CaCO_3$ at 4t ha^{-1} and 8 t ha^{-1} typically reduced clubroot severity compared to the untreated control and the $CaCN_2$ at 250 kg ha^{-1} not incorporated treatment also reduced clubroot by up to 50% in some plots (Fig. 3a). There was a significant ($P < 0.001$) effect of variety on the clubroot severity at the Shropshire site with Mendel exhibiting 56 - 85% less disease compared to the susceptible Kommando (Fig. S5). Reduced clubroot severity in

Mendel resulted in significantly increased yields for this variety compared to Kommando (Fig. 4; $P < 0.001$). None of the treatments significantly affected yield ($P = 0.400$). Phytophthora root rot (*Phytophthora megasperma*) was also observed at the Shropshire site causing a severe root rot and plant losses over-winter and in early spring.

Levels of clubroot were low at the Aberdeenshire site in most plots, possibly as a result of a cold autumn which may have slowed disease development (Fig. 3b). 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. Treatments ($P < 0.001$) and variety ($P = 0.002$) had significant effects on clubroot severity (Fig. 3b). However the effects of different treatments were not consistent across varieties or scoring dates with significant interactions between treatments and both scoring date ($P < 0.001$) and variety ($P = 0.031$) observed. There were significant differences in yield between the treatments ($P = 0.008$) but not between varieties ($P = 0.563$) at the Aberdeenshire site. $CaCO_3$ treatment at 8 t ha^{-1} was the highest yielding treatment ($P = 0.034$). None of the other treatments significantly improved yield (Fig. 4a).

3.3.2 Field trials 2008 - 2009

Field trials were carried out at a site in Herefordshire and one in Aberdeenshire. Both sites showed low to moderate levels of clubroot. The Herefordshire trial site was sown late on 24 September 2008 after a delayed cereal harvest but in spring it still had slightly higher levels of clubroot than observed at the Aberdeenshire site. Visual clubroot symptoms were not observed in the autumn, indicating that late sowing may enable crops to escape early severe infection. Throughout the trial clubroot severity was significantly lower in Mendel than in Kommando plots (Fig. 3c; $P < 0.001$). There were significant effects from soil treatments ($P = 0.001$) on clubroot severity with the most significant reductions in disease observed in the June assessment ($P = 0.042$). The largest effects compared to the untreated control were observed in the two $CaCN_2$ treatments (250 kg ha^{-1} incorporated $P < 0.001$ and unincorporated $P = 0.002$), $CaCO_3$ 8t ha^{-1} treatment ($P < 0.001$) and $CaCO_3$ 4t ha^{-1} alone ($P = 0.03$) or plus $CaCN_2$ 250 kg ha^{-1} as a mixed treatment ($P < 0.001$). Despite reductions in clubroot severity no significant yield benefits were associated with either varietal control ($P =$

0.151) or soil amendments ($P = 0.275$). Mendel yielded less than Kommando, although this difference was not statistically significant (Fig. 4b).

At the Aberdeenshire site disease severity was low. Overall there were no significant effects of treatment ($P = 0.588$) or variety ($P = 0.353$) on clubroot severity in the trial. However a significant interaction between the effect of treatment and scoring date was observed ($P = 0.001$). The mixed $CaCO_3$ (4t ha^{-1}) plus $CaCN_2$ (250 kg ha^{-1}) treatment ($P = 0.012$) and the $CaCN_2$ unincorporated treatment ($P = 0.051$) had a significantly a lower disease index compared to control plots in the December assessment and the extra nitrogen treatment ($P = 0.036$) had a lower index at the March assessment (Fig. 3d). No yield assessments were recorded at this trial site due to an error in an electronic data recorder at harvest.

3.3.3 Field trials 2009 – 2010

Trials were carried out in Warwickshire and Aberdeenshire in 2009-2010, testing the treatments that had demonstrated most potential in years one and two of the project and adding a second clubroot resistance variety Cracker (then listed as coded variety NPZ0700) to the susceptible Kommando and resistant Mendel used in previous trials. The Warwickshire trial site had lower levels of clubroot compared to the Aberdeenshire site and this was associated with dry conditions at sowing that limited early plant infection. Clubroot severity was significantly lower in both of the resistant varieties compared to the susceptible Kommando ($P < 0.001$) with disease severity at 20% in untreated Kommando plots in March and over 30% by June, compared to less than 5% in the Mendel and Cracker at same assessments (Fig. 3e). Disease levels were also reduced by the soil amendments ($P < 0.001$) although a significant interaction between variety and treatment were observed ($P = 0.002$). The $CaCN_2$, $CaCO_3$ and combinations of these treatments significantly reduced clubroot severity on all varieties (Fig. 3e). Yield was significantly affected by both treatment ($P < 0.001$) and variety ($P = 0.002$) at the Warwickshire site (Fig. 4c). All treatments significantly increased yield in susceptible variety Kommando and despite yields being higher in the treated plots of both resistant varieties the only statistically significant effects were observed with Mendel for the $CaCN_2$, $CaCO_3$ and boron combination amendments Mendel ($P = 0.006$).

At the Aberdeenshire site clubroot severity in March and June exceeded 50% in the untreated Kommando plots. No significant differences were noted at the Aberdeenshire site between treatments in terms of disease severity (P = 0.141; Fig. 3f) or yield (Fig. 4c) although there was a trend for the combined treatment to be more effective in the March assessment (Fig. 3f). Because of the high levels of OSR volunteer plants thought to be the susceptible variety Kommando, in the trial plots at the Aberdeenshire site collecting data from the resistant variety treatments was abandoned at this site.

3.3.4 Effect of soil amendments on soil pH and calcium levels

Pre-trial soil pH levels ranged from pH 6.8 at Aberdeenshire in 2007-2008 to pH 6.1 at Herefordshire in 2008-2009 and calcium levels varied from 999 mg L^{-1} at Herefordshire in 2008-2009 to 2407 at Aberdeenshire 2008-2009 (Table S2). Initial clubroot levels were assessed by disease severity bioassays and indicated the sites had varying degrees of clubroot ranging from 7.1% at Aberdeenshire 2008-2009 to 83% at Herefordshire in 2008-2009. Although the lowest pH and soil calcium levels at the Herefordshire in 2008-2009 were associated with the highest initial clubroot levels no other obvious patterns between disease and these soil parameters were observed. The effect of various soil treatments on pH and calcium levels was assessed either in the spring or at harvest but due to variation in the responses observed at each site trial data was analysed separately. Measurements were made at the Aberdeenshire sites in 2007-2008 or 2009-2010 but due to incomplete data sets these sites were not analysed.

At the Shropshire site in 2007-2008 pH levels assessed in the spring were significantly increased by the addition of $CaCO_3$ at 4 or at 8 t ha^{-1} ($P < 0.001$) but significantly lowered by the extra nitrogen treatment (P = 0.006). Calcium levels in spring were significantly reduced by the application of extra nitrogen (P = 0.001) and $CaCN_2$ (P = 0.011) compared to the untreated plots. By harvest pH levels remained significantly higher in the $CaCO_3$ at 4 (P = 0.001) or 8 t ha-1 ($P < 0.001$) treatments and calcium levels were significantly lower in the extra nitrogen (P = 0.015) plots (Fig. 6a+S7a). In 2008-2009 at the Herefordshire site $CaCO_3$ applied at 4 t ha^{-1} with or without the addition of $CaCN_2$ and 8 t ha^{-1} ($P < 0.001$) all significantly raised pH levels measured in the spring or at harvest (Fig. S6b). The same three $CaCO_3$-based amendments had also increased calcium levels measured at both spring and harvest (Fig. S7b; $P < 0.01$). Calcium and pH levels were only recorded at harvest at the Aberdeenshire site in 2008-2009. At this site pH was significantly lowered by extra nitrogen

($P < 0.001$), 4 t ha^{-1} $CaCO_3$ ($P = 0.009$) with or without $CaCN_2$ ($P < 0.001$) but boron ($P = 0.009$) and unincorporated $CaCN_2$ ($P = 0.003$) treatments significantly raised pH (Fig. S6c). Calcium levels were lower in the extra nitrogen plots ($P = 0.027$) but higher in the 8 t ha^{-1} $CaCO_3$ ($P < 0.001$), unincorporated $CaCN_2$ ($P < 0.001$) and boron ($P = 0.019$) treatments (Fig. S7c). Harvest measures were also only taken at the Warwickshire site in 2009-2010. At this site all of the $CaCO_3$-based treatments significantly increased soil pH (Fig. S6d; $P < 0.01$) whereas only the 4 t ha^{-1}, 8 t ha^{-1} and the 4 t ha^{-1} plus $CaCN_2$ and boron treatments significantly increased calcium levels (Fig. S7d; $P < 0.01$).

3.3.5 Yield penalties associated with severity of clubroot infection

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 Aberdeenshire site in 2009-2010 and the Shropshire site 2007-2008, but the disease progress curves were very different, with disease at the Aberdeenshire 2009-2010 trial far lower in the autumn (Fig. 3). There was a highly significant correlation ($P = 0.001$, r = -0.472) between clubroot severity at harvest and yield across all sites and varieties (Fig. 5a). Clubroot-related yield loss can be measured by the slope of the line. When there is no disease (clubroot severity index = 0%), yield is predicted to be 3.85 t ha^{-1}. When clubroot severity index reaches 100% yield is predicted to be approximately 1 t ha^{-1} (Fig. 5a). Therefore an increase in clubroot severity from 0 to100% results in a predicted loss of 3 t ha^{-1} or a 0.03 t ha^{-1} loss per 1% increase in the clubroot index. When the varieties were analysed separately this yield loss per unit disease did not alter significantly (Fig. 5). Yield losses in Kommando (Fig. 5b; r = -0.465, $P < 0.001$) were 0.03 t ha^{-1} whereas losses in Mendel (Fig. 5c; r = -0.296, $P = 0.001$) were slightly lower at 0.028 t ha^{-1}. Clubroot associated yield losses in Cracker (Fig. 5d) did not significantly correlate (r = 0.296, $P = 0.161$) with disease levels but this is likely due to the limited data for this variety from a single trial site in 2009-2010.

Other factors that could influence yield in these trials include soil calcium and pH levels. A small but significant increase in yield was associated with elevated soil calcium levels at harvest (Fig. 5e; r = 0.140, $P = 0.048$) whereas no significant effect on yield was associated with changes in coil pH (Fig. 5f; r = 0.061, $P = 0.392$). Clubroot severity was significantly reduced by both increased soil calcium (Fig. S8a; r = 0.147, $P = 0.017$) and pH (Fig. S8b; r = 0.236, $P < 0.001$) at harvest.

4. Discussion

Clubroot causes significant yield loses in OSR and other brassica crops across the world which can equate to more than half of potential yield on a site with severe infection and, occasionally, crop failure (Dixon, 2009a; Hwang et al., 2012b). A survey of OSR fields carried out in Scotland in 2008 and 2009 showed that over half of the OSR fields tested were infected with clubroot. Soil samples were also taken from England and a few from Wales, mainly from farms where clubroot was already known to be present, but a few new cases of clubroot were identified. The increasing proportion of clubroot infected land in the UK has serious consequences for brassica growers when combined with the risks of clubroot levels further increasing when brassicas are grown in short rotations, whether these are winter or spring crops or OSR, vegetable or forage brassicas. In Canada spread of the disease across the major brassica growing regions has been rapid resulting in clubroot becoming established as a serious threat to production (Hwang et al., 2012b). Therefore improved management of clubroot is critical and the effectiveness of different control strategies needs to be assessed.

The severity of clubroot epidemics varied across sites and years (Fig. 3). The low level of clubroot at several sites was associated with unfavourable conditions for clubroot infection. *P. brassicae* infection is associated with high soil moisture and warm soil temperatures (Dixon, 2009b). Particularly cool and or dry weather conditions were noted in the autumn at the Aberdeenshire sites in 2007/2008 and 2008/2009 which may have contributed to the low disease epidemics observed at these sites compared to the very wet 2009/2010 trial which had high clubroot levels (Fig. 3b+d+f; Table S2). In England the most severe epidemics were during 2007/2008 and 2008/2009 in Shropshire and Herefordshire which are traditionally wetter regions of the country (Table 3a+c; Table S2). The lower disease levels in Herefordshire in 2008/2009 may have been influenced by the delayed sowing date of the trial that year. Clubroot severity is typically influenced by the previous cropping history of the field with shortened crop rotations having potentially severe consequences for clubroot management (Peng et al., 2014). The most severely diseased Shropshire site had been sown with WOSR the previous season (2006/2007) and again two years before that (2004/2005; Table S2). All of the other sites where previous cropping histories for the three previous seasons were available had WOSR in a 1 in 2 or 1 in 3 rotations. These limited rotations are likely to exacerbate clubroot problems in the long term particularly in seasons where environmental conditions are conducive to disease.

The variability of varietal resistance as a control strategy for clubroot was demonstrated in field sites across the UK and is indicative of the discrete populations of *P. brassicae* that are present within infested fields (Strehlow et al., 2014). Particularly poor control of clubroot was seen in the Scottish trials around the Aberdeenshire area where the clubroot resistant OSR variety Mendel has been commonly used in the past. Better disease control was seen at sites in England but this was variable and ranged from 50% control in Shropshire (2007-2008) to over 95% control at sites in Herefordshire (2008-2009) and Warwickshire (2009-2010). However, the use of Mendel did not always carry a yield advantage even where disease control was observed. Under high disease pressure at the Shropshire site there were significant yield benefits to using Mendel, despite the poorer control it offered relative to other West Midland's sites (Fig. 5). In the following season Mendel at the Herefordshire site 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. The combined data set established that 0.03 t ha^{-1} yield losses were associated with each 1% increase in clubroot severity. Losses in Mendel were similar per 1% increase in disease severity to those in Kommando. Losses were therefore less as disease levels were often lower but the finding of a common yield loss slope was counterintuitive for a resistant variety. The lack of a yield benefit Mendel relative to Kommando in several trials, despite lower clubroot levels reflects the lower yield potential and weaker agronomic attributes of this variety. Still there should also be an additional benefit from lower clubroot inoculum production on resistant varieties, which will be evident in future cropping (Hwang et al., 2012a).

Mendel was superseded on the UK recommended list in 2013-2014 (http://www.hgca.com/varieties/hgca-recommended-lists/rl-archive-2013-14.aspx) but was generally planted only in situations where growers have an established and significant clubroot problem. Newer clubroot resistant varieties such as Cracker are now widely grown even in fields with no history of clubroot due to improved yield potential. Clubroot resistance in Mendel and Cracker is derived from the same single major gene source suggesting that pathotypes of *P. brassicae* which can overcome this resistance mechanism are likely to be selected (Diederichsen et al., 2006; Werner et al., 2008). *P. brassicae* pathotype variation has been observed across the world. Pathotype 3 predominates in Canada however pathotypes 2, 5, 6 and 8 have also been reported (Xue et al., 2008), whereas at least seven pathotypes were observed in France (Some et al., 1996). There is limited information

regarding the genetic variation of *P. brassicae* pathotypes in the UK but experiments using differential brassica host plants indicated that the UK populations consists of diverse pathotypes (Buczacki *et al.*, 1975; Stewart, 2007). Not all of these pathotypes are controlled by the resistance in varieties such as Cracker and Mendel. *P. brassicae* populations are typically genetically diverse with multiple pathotypes present within an infected field (Manzanares-Dauleux et al., 2001; Strehlow et al., 2014). Consequently at sites where these varieties have been grown several times in the rotation it is likely that resistance-breaking pathotypes of clubroot will have been selected for as has been observed in other European countries (Diederichsen et al., 2009). This is indicative of the pressure the 'Mendel' gene is under and of the need for new forms of varietal resistance to clubroot. No clubroot resistance or tolerance was noted in other varieties screened that did not contain the specific resistance mechanism in Mendel and Cracker (Fig. 1). Further erosion of this varietal resistance through the continued widespread use of this gene and resultant selection for clubroot pathotypes able to overcome it would be of major concern to the industry.

Cloning of the clubroot resistance gene, *Crr1a,* from Chinese cabbage confirmed that *Crr1a* was an NBS-LRR-TIR type gene (Hatakeyama et al., 2013). NBS-LRR type resistance genes are often associated with race-specific resistance that can be quickly eroded through the evolution of novel pathotypes in the field (Brown, 2015). *Crr1a* confers race-specific resistance to *P. brassicae* isolates from Japan and is not effective against more virulent isolates (Hatakeyama et al., 2013). This together with the co-localisation of other clubroot resistance loci with NBS-LRR genes (Zhang et al., 2014) suggests that current varietal resistance to clubroot is unlikely to be durable as observed in the UK. The development of alternative forms of resistance to clubroot would greatly assist future cropping not only in the UK but worldwide (Diederichsen et al., 2009).

Clubroot severity is known to be linked to pH and soil calcium content (Donald and Porter, 2009). 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 (Donald et al., 2004; Myers and Campbell, 1985; Niwa et al., 2007; Tremblay et al., 2005). The use of soil amendments to manage clubroot in OSR demonstrated variable results in field trials. Treatment applications and methods were designed to test practical options for use on farms where establishment by minimal cultivation techniques are favoured. Treatments were applied shortly before sowing but may be less effective than treatments with lime that are

incorporated and mixed into soil by ploughing and cultivation. Control ranged from 0% at some sites to 95% control for combined treatments in the Warwickshire trial in 2009-2010 (Fig. 4). Highest yield increases was seen at sites with a moderate clubroot severity and treatment effects were generally absent at sites with very low infection levels and were poor at sites with a very high infection severity such as the Shropshire 2007-2008 site or the Aberdeenshire 2009/2010 site. Using soil amendments was less successful on OSR (Knox et al., 2015) than has been reported on vegetable crops in Australia and Japan (Donald et al., 2004; Niwa et al., 2007). This may be due to differences in clubroot pathotypes between the crop types but is more likely due to the ability to time treatments and resultant pH and calcium rises closely to clubroot germination in transplanted vegetable crops (Donald et al., 2004; Niwa et al., 2007; Tremblay et al., 2005). In drilled OSR crops there is likely to be a short delay between soil treatment application and crop germination which will then stimulate clubroot germination and in this time there may have been declines in pH and calcium spikes that were there at drilling. However, for winter crops soil temperatures typically decrease after sowing to the stage where they become unfavourable for clubroot infection (Dixon, 2009b). As such a small burst of calcium activity early post-sowing may be effective in delaying *P. brassicae* infection sufficiently to lower overall disease levels in the crop. Yield benefits in response to treatment were also highly variable across sites, only noted at two of the five trials where yield was assessed. Despite this variation across trial sites elevated soil calcium measured at harvest was significantly associated with increased yields (Fig. 5e) although no such effect was observed for elevated pH levels (Fig. 5f) even though both factors significantly reduced clubroot severity (Fig. S8). However, the harvest soil analyses do no reflect short term effects on pH and calcium that may have occur due to the soil amendments and could have unresolved effects on disease development. No association between clubroot severity and soil calcium content or pH was observed with the survey samples (Fig. S1) possibly due to confounding effects associated with the sampling techniques used or to the multiple other major influencers of disease severity such as weather, soil type, previous cropping and other disease pressures that also varied between sites. Further investigation of how WOSR yield responses are influenced by the interactions between various environmental factors, soil chemistry and the control afforded by soil amendments and resistant varieties will provide further insights into improve clubroot management strategies.

The trials 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 variable control at differing locations and disease pressure highlighting the difficulties of using these products alone to control clubroot. Using varietal resistance is likely to have lower cost and fewer environmental implications, but the build up of resistance-breaking clubroot pathotypes is likely if resistance alone is used as a strategy to manage the disease. This combined with limited sources of durable clubroot resistance on a global scale indicates that alternative strategies are necessary to manage this disease worldwide. Soil amendments raised pH and calcium content in soils and were associated with declines in clubroot severity in some trials. Although 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 managing clubroot on infected fields should be considered. Where routine liming is required, application can be made at a point in the rotation where soil cultivation takes place so that additional incorporation costs are minimised. Future clubroot management strategies should integrate soil testing for the disease and good field drainage together the traditional use of crop rotations and varietal resistance supplemented with targeted applications of soil amendments where clubroot patches are evident and mapped, or where clubroot issues have been recorded previously. Combining targeted application of soil amendments, as needed, with other control measures may be beneficial in controlling ‘hot spots’ within the field to safeguard future OSR cropping.

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References

Anonymous, 2014a. Farming Statistics Provisional crop areas, yields and livestock populations At June 2014 - United Kingdom. (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/364157/structure-jun2013prov-UK-16oct14.pdf).

Anonymous, 2014b. Results from the June 2014 Scottish Agricultural Census. Scottish Government. (http://www.gov.scot/Publications/2014/10/6277).

Brown JKM. 2015. Durable resistance of crops to disease: A Darwinian perspective. *Annual Review of Phytopathology* 53, 513–39.

Buczacki ST, Toxopeus H, Mattusch P, Johnston TD, Dixon GR, Hobolth LA. 1975. Study of physiologic specialization in *Plasmodiophora brassicae*: proposals for attempted rationalization through an international approach. *Transactions of the British Mycological Society* 65, 295-303.

Diederichsen, E, Beckmann, J, Schondelmaier, J, Dreyer, F. 2006. Genetics of clubroot resistance in *Brassica napus* 'Mendel'. *Acta Horticulturae* 706, 307-311.

Diederichsen E, Frauen M, Linders EG, Hatakeyama K, Hirai M. 2009. Status and perspectives of clubroot resistance breeding in crucifer crops. *Journal of Plant Growth Regulation* 28, 265-281.

Dixon GR. 2009a. The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. *Journal of Plant Growth Regulation* 28, 194-202.

Dixon GR. 2009b. *Plasmodiophora brassicae* in its environment. *Journal of Plant Growth Regulation* 28, 212-228.

Donald C, Porter I. 2009. Integrated control of clubroot. *Journal of Plant Growth Regulation* 28, 289-303.

Donald EC, Lawrence JM, Porter IJ. 2004. Influence of particle size and application method on the efficacy of calcium cyanamide for control of clubroot of vegetable brassicas. *Crop Protection* 23, 297-303.

Harling R, 2006. Clubroot control using novel and sustainable methods. Final reports for DEFRA project HH3227TFV

Hwang SF, Ahmed HU, Strelkov SE, Gossen BD, Turnbull GD, Peng G, Howard RJ. 2011a. Seedling age and inoculum density affect clubroot severity and seed yield in canola. *Canadian Journal of Plant Science* 9**1,** 183-190.

Hwang SF, Ahmed HU, Zhou Q, Strelkov SE, Gossen BD, Peng G, Turnbull GD. 2011b. Influence of cultivar resistance and inoculum density on root hair infection of canola (*Brassica napus*) by *Plasmodiophora brassicae*. *Plant Pathology* 60, 820-829.

Hwang SF, Ahmed HU, Zhou Q, Strelkov SE, Gossen BD, Peng G, Turnbull GD. 2012a. Assessment of the impact of resistant and susceptible canola on *Plasmodiophora brassicae* inoculum potential. *Plant Pathology* 61, 945-952.

Hwang SHEA, Strelkov SE, Feng JIE, Gossen BD, Howard RJ. 2012b. *Plasmodiophora brassicae*: a review of an emerging pathogen of the Canadian canola (*Brassica napus*) crop. *Molecular Plant Pathology* 13, 105-113.

Keen NT, Williams PH. 1969. Translocation of sugars into infected cabbage tissues during clubroot development. *Plant Physiology* 44, 748-754.

Knox, OGG, Oghoro, CO, Burnett, FJ, Fountaine, JM. 2015. Biochar increases soil pH, but is as ineffective as liming at controlling clubroot. *Journal of Plant Pathology* doi: 10.4454/JPP.V97I1.016

Kuginuki Y, Yoshikawa H, Hirai M. 1999. Variation in virulence of *Plasmodiophora brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *European Journal of Plant Pathology* 105, 327-332.

Ludwig-Müller J, Prinsen E, Rolfe SA, Scholes JD. 2009. Metabolism and plant hormone action during clubroot disease. *Journal of Plant Growth Regulation* 28, 229-244.

Manzanares-Dauleux MJ, Divaret I, Baron F, Thomas G. 2001. Assessment of biological and molecular variability between and within field isolates of *Plasmodiophora brassicae*. *Plant Pathology* 50, 165-173.

McGrann GRD, Stavrinides A, Russell J, Corbitt MM, Booth A, Chartrain L, Thomas WT, Brown JKM. 2014. A trade off between *mlo* resistance to powdery mildew and increased susceptibility of barley to a newly important disease, Ramularia leaf spot. *Journal of Experimental Botany* 65, 1025-1037.

Minitab. 2010. *Minitab 16 statistical software*. Minitab Inc., State College, Pennsylvania, USA.

Mitchell DT, Rice KA. 1979. Translocation of 14C-labelled assimilates in cabbage during club root development. *Annals of Applied Biology* 92, 143-152.

Myers DF, Campbell RN. 1985. Lime and the control of clubroot of crucifers: Effects of pH, calcium, magnesium, and their interactions. *Phytopathology* 75, 670-673.

Niwa R, Kumei T, Nomura Y, Yoshida S, Osaki M, Ezawa T. 2007. Increase in soil pH due to Ca-rich organic matter application causes suppression of the clubroot disease of crucifers. *Soil Biology and Biochemistry* 39, 778-785.

Oxley, S. Clubroot disease of oilseed rape and other brassica crops. 2007 Technical Note TN602. Scottish Agricultural College.

Pageau D, Lajeunesse J, Lafond J. 2006. Impact de l'hernie des crucifères [*Plasmodiophora brassicae*] sur la productivité et la qualité du canola. *Canadian Journal of Plant Pathology* 28, 137-143.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. 2009. GenStat for Windows (12th Edition) Introduction. . *VSN International, Hemel Hempstead.*

Peng G, Lahlali R, Hwang SF, Pageau D, Hynes RK, McDonald MR, Gossen BD, Strelkov SE. 2014. Crop rotation, cultivar resistance, and fungicides/biofungicides for managing clubroot (*Plasmodiophora brassicae*) on canola. *Canadian Journal of Plant Pathology* 36, 99-112.

Rashid A, Ahmed HU, Xiao Q, Hwang SF, Strelkov SE. 2013. Effects of root exudates and pH on *Plasmodiophora brassicae* resting spore germination and infection of canola (*Brassica napus* L.) root hairs. *Crop Protection* 48, 16-23.

Siemens J, Keller I, Sarx J, Kunz S, Schuller A, Nagel W, Schmulling T, Parniske M, Ludwig-Müller J. 2006. Transcriptome analysis of Arabidopsis clubroots indicate a key role for cytokinins in disease development. *Molecular Plant-Microbe Interactions* 19, 480-494.

Some A, Manzanares MJ, Laurens F, Baron F, Thomas G, Rouxel F. 1996. Variation for virulence on *Brassica napus* L. amongst *Plasmodiophora brassicae* collections from France and derived single-spore isolates. *Plant Pathology* 45, 432-439.

Stewart K. 2007. Conventional and novel treatments for control of clubroot in brassicas. *PhD Thesis*. University of Edinburgh.

Strehlow B, de Mol F, Struck C. 2014. History of oilseed rape cropping and geographic origin affect the genetic structure of *Plasmodiophora brassicae* populations. *Phytopathology* 104, 532-538.

Tremblay N, Belec C, Coulombe J, Godin C. 2005. Evaluation of calcium cyanamide and liming for control of clubroot disease in cauliflower. *Crop Protection* 24, 798-803.

Wallenhammar A. 1996. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. *Plant Pathology* 45, 710-719.

Webster MA. 1986. pH and nutritional effects on infection by *Plasmodiophora brassicae* Wor., and on clubroot symptoms. *PhD Thesis*. University of Aberdeen.

Werner S, Diederichsen E, Frauen M, Schondelmaier J, Jung C. 2008. Genetic mapping of clubroot resistance genes in oilseed rape. *Theoretical and Applied Genetics* 116, 363-372.

Xue S, Cao T, Howard RJ, Hwang SF, Strelkov SE. 2008. Isolation and variation in virulence of single-spore isolates of *Plasmodiophora brassicae* from Canada. *Plant Disease* 92, 456-462.

Zhang T, Zhao Z, Zhang C, Pang W, Choi SR, Lim YP, Piao Z. 2014. Fine genetic and physical mapping of the *CRb* gene conferring resistance to clubroot disease in Brassica rapa. *Molecular Breeding* 34, 1173-1183.

Table legends

Table 1 Soil amendments used in field trials.

	Field trial year		
	2007-2008	2008-2009	2009-2010
	Untreated control	Untreated control	Untreated control
	Calcium carbonate 2 t ha^{-1}		
	Calcium carbonate 4 t ha^{-1}	Calcium carbonate 4 t ha^{-1}	Calcium carbonate 4 t ha^{-1}
	Calcium carbonate 8 t ha^{-1}.	Calcium carbonate 8 t ha^{-1}.	Calcium carbonate 8 t ha^{-1}.
	Calcium cyanamide 250 kg ha^{-1}	Calcium cyanamide 250 kg ha^{-1} [a]	Calcium cyanamide 250 kg ha^{-1}
	Calcium cyanamide 250kg ha^{-1} – not incorporated	Calcium cyanamide 250kg ha^{-1} – not incorporated	
	Control with extra 50 kg ha^{-1} nitrogen	Control with extra 50 kg ha^{-1} nitrogen	
	Boron 20 kg ha^{-1}	Boron 20kg ha^{-1}	
		Calcium carbonate 4 t ha^{-1} + calcium cyanamide 250 kg ha^{-1} [a]	Calcium carbonate 4 t ha^{-1} + calcium cyanamide 250 kg ha^{-1}
			Calcium carbonate 4 t ha^{-1} + calcium cyanamide 250 kg ha^{-1} + Boron 20 kg ha^{-1}
Total	8 treatments x 2 varieties x 4 replicates = 64 plots	8 treatments x 2 varieties x 4 replicates = 64 plots	6 treatments x 3 varieties x 4 replicates = 72 plots
OSR varieties	Kommando (S) [b] Mendel (R)	Kommando (S) [b] Mendel (R)	Kommando (S) [b] Mendel (R) Cracker (R)

[a] Calcium cyanamide shallow incorporated after soil preparation

[b] Varietal resistance status against clubroot R = resistance, S = susceptible

Figure legends

Figure 1 Clubroot infection severity (%) on oilseed rape elite varieties. Bars indicate standard error.

Figure 2 Correlation between clubroot severity index and disease incidence from field trial data collected from sites located in the UK 2007-2010

Figure 3 Effect of soil amendments and varietal resistance on clubroot severity at different field trials located in the UK 2007-2010. a). Shropshire 2007-2008, b). Aberdeenshire 2007-2008, c). Herefordshire 2008-2009, d) Aberdeenshire 2008-2009, e). Warwickshire 2009-2010, f). Aberdeenshire 2009-2010. Bars indicate standard error.

Figure 4 Effect of soil amendments and varietal resistance on oilseed rape yields (91% dry matter t ha^{-1}) at different field trials located in the UK 2007-2010. a). 2007-2008, b). 2008-2009, c). 2009-2010. Light grey bars = trials located in Aberdeenshire, Scotland. Dark grey bars = trials located in Shropshire, Herefordshire and Warwickshire, England, in 2007-2008, 2008-2009 and 2009-2010, respectively. Bars indicate standard error.

Figure 5 Correlation between oilseed rape yields (91% dry matter t ha^{-1}) and clubroot severity (%), soil calcium and pH at harvest across all trial sites 2007-2010. a). all varieties, b). Kommando (susceptible), c). Mendel (resistant), d). Cracker (resistant – one years data from the Warwickshire site 2009-2010), e). soil calcium mg L^{-1} at harvest, f). soil pH at harvest.

Supplementary information legends

Table S1. Oilseed rape varieties tested for susceptibility to clubroot in glasshouse bioassay.

Table S2 Field sites characteristics autumn weather and measurements of pre-trial pH, calcium and clubroot severity

Figure S1 Correlation between clubroot disease severity index (%) scores from survey site soil samples and (a) pH and (b) extractable calcium levels (mg L^{-1}).

Figure S2 Clubroot positive survey samples sorted by severity indicating that many sites had low infection severities that were subclinical in the field.

Figure S3 Distribution of samples collected during the clubroot survey 2008-2009 highlighted against the proportion of land in oilseed rape production in the UK during that

time. a) samples negative for clubroot (disease index = 0%), b). samples with a disease index <10%. c). samples with disease indices of 10 - 30%. d). samples with disease indices of >30%.. e). total samples positive for clubroot received during the survey.

Figure S4 Chinese cabbage clubroot infection seedling bioassay test. Plants grown in clubroot free soil (left) and in infested soil (right).

Figure S5 Effect of clubroot on resistant and susceptible oilseed rape varieties. a). Shropshire field trial site showing Kommando plot in the foreground and Mendel in the background, spring 2008. b). Clubroot infected roots of clubroot susceptible oilseed rape Kommando (left) and the resistant variety Mendel (right).

Figure S6 Effect of soil amendments on soil pH at different field trials located in the UK 2007-2010. a). Shropshire 2007-2008, b). Herefordshire 2008-2009, c) Aberdeenshire 2008-2009, d). Warwickshire 2009-2010. Black bars indicate measurements taken in spring, light grey bars indicate measurements taken at harvest. Bars indicate standard error.

Figure S7 Effect of soil amendments on soil extractable calcium at different field trials located in the UK 2007-2010. a). Shropshire 2007-2008, b). Herefordshire 2008-2009, c) Aberdeenshire 2008-2009, d). Warwickshire 2009-2010. Black bars indicate measurements taken in spring, light grey bars indicate measurements taken at harvest. Bars indicate standard error.

Figure S8 Correlation between clubroot severity (%), soil calcium (a) and pH (b) at harvest across all trial sites 2007-2010.

Fig. 1

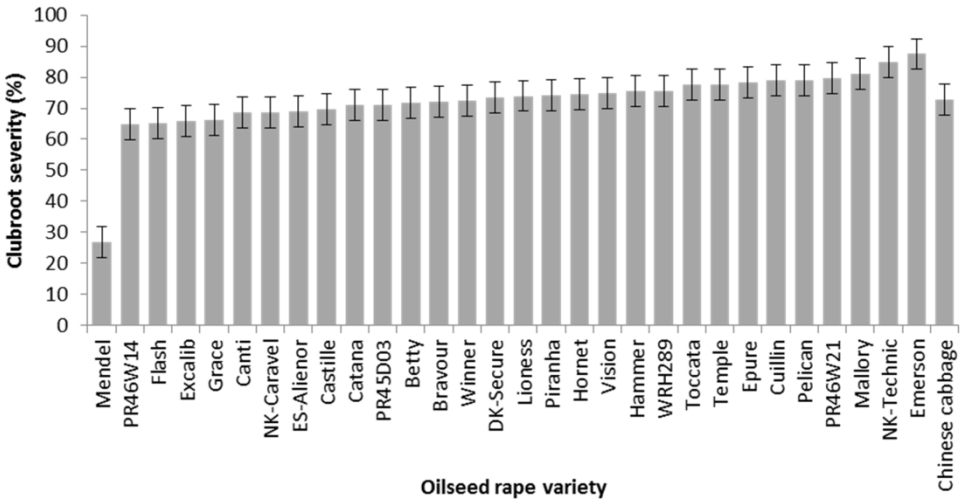
Clubroot severity (%)
100
90
80
70
60
50
40
30
20
10
0
Mendel
PR46W14
Flash
Excalib
Grace
Canti
NK-Caravel
ES-Alienor
Castille
Catana
PR45D03
Betty
Bravour
Winner
DK-Secure
Lioness
Piranha
Hornet
Vision
Hammer
WRH289
Toccata
Temple
Epure
Cuillin
Pelican
PR46W21
Mallory
NK-Technic
Emerson
Chinese cabbage
Oilseed rape variety

Fig. 2.

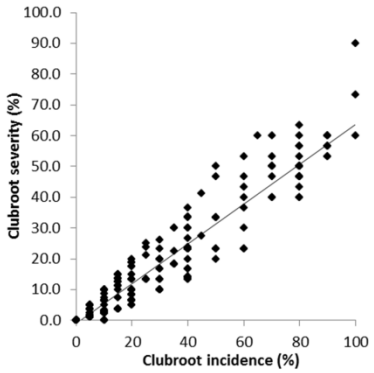
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Clubroot severity (%)
0
20
40
60
80
100
Clubroot incidence (%)

Fig. 3

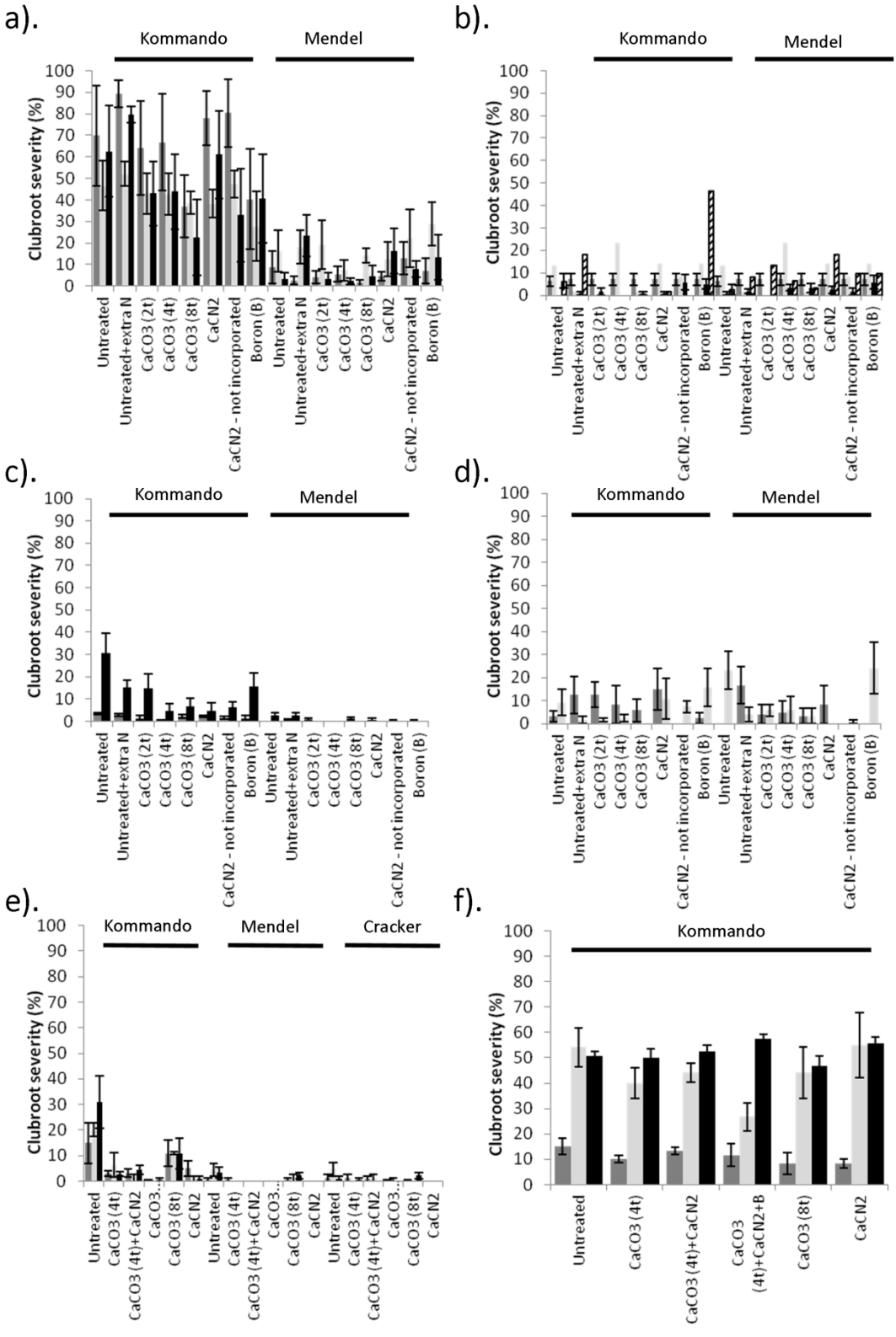
a).
b).
c).
d).
e).
f).
Kommando
Mendel
Cracker
Clubroot severity (%)
Untreated
Untreated+extra N
CaCO3 (2t)
CaCO3 (4t)
CaCO3 (8t)
CaCN2
CaCN2 - not incorporated
Boron (B)
CaCO3 (4t)+CaCN2
CaCO3 (4t)+CaCN2+B

Fig. 4

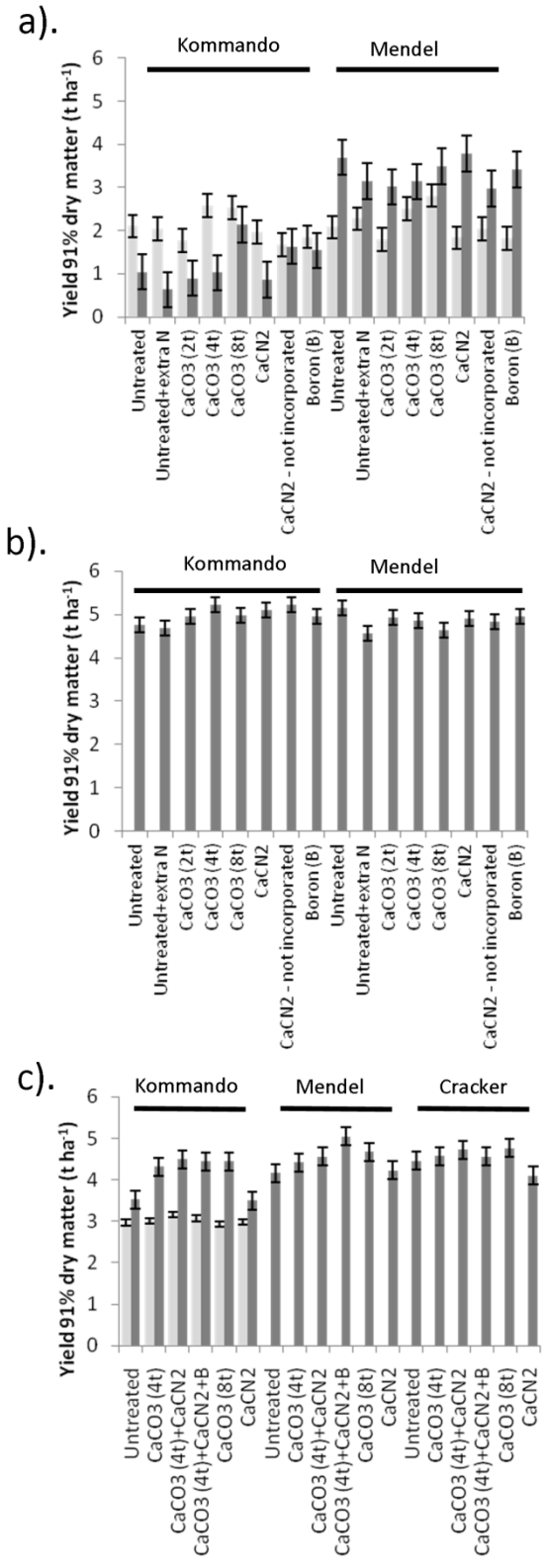
a).
Kommando
Mendel
Yield 91% dry matter (t ha-1)
0
1
2
3
4
5
6
Untreated
Untreated+extra N
CaCO3 (2t)
CaCO3 (4t)
CaCO3 (8t)
CaCN2
CaCN2 - not incorporated
Boron (B)
Untreated
Untreated+extra N
CaCO3 (2t)
CaCO3 (4t)
CaCO3 (8t)
CaCN2
CaCN2 - not incorporated
Boron (B)
b).
Kommando
Mendel
Yield 91% dry matter (t ha-1)
0
1
2
3
4
5
6
Untreated
Untreated+extra N
CaCO3 (2t)
CaCO3 (4t)
CaCO3 (8t)
CaCN2
CaCN2 - not incorporated
Boron (B)
Untreated
Untreated+extra N
CaCO3 (2t)
CaCO3 (4t)
CaCO3 (8t)
CaCN2
CaCN2 - not incorporated
Boron (B)
c).
Kommando
Mendel
Cracker
Yield 91% dry matter (t ha-1)
0
1
2
3
4
5
6
Untreated
CaCO3 (4t)
CaCO3 (4t)+CaCN2
CaCO3 (4t)+CaCN2+B
CaCO3 (8t)
CaCN2
Untreated
CaCO3 (4t)
CaCO3 (4t)+CaCN2
CaCO3 (4t)+CaCN2+B
CaCO3 (8t)
CaCN2
Untreated
CaCO3 (4t)
CaCO3 (4t)+CaCN2
CaCO3 (4t)+CaCN2+B
CaCO3 (8t)
CaCN2

Fig. 5

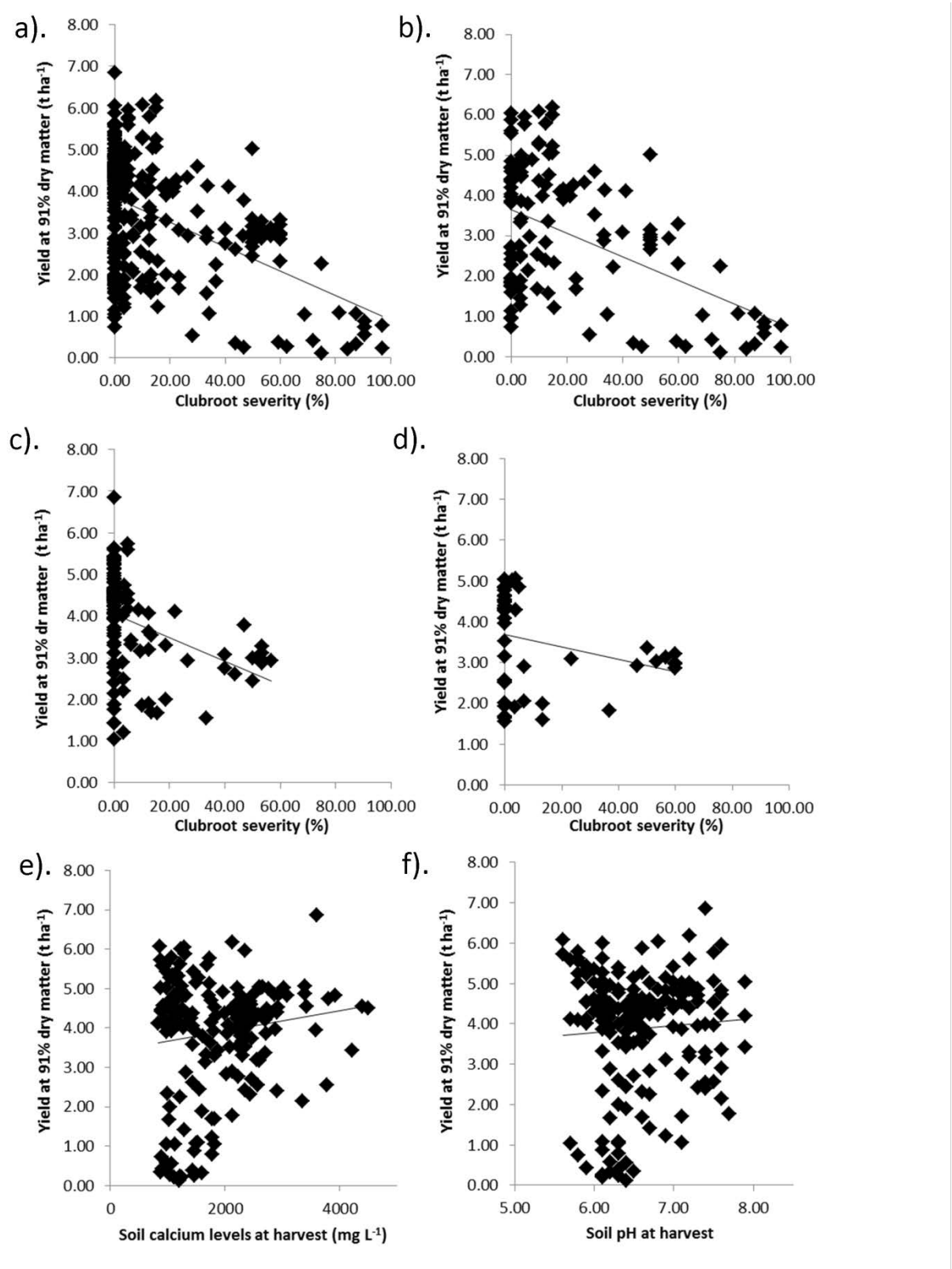
a).
b).
c).
d).
e).
f).
Yield at 91% dry matter (t ha-1)
Yield at 91% dr matter (t ha-1)
Clubroot severity (%)
Soil calcium levels at harvest (mg L-1)
Soil pH at harvest
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
0.00
20.00
40.00
60.00
80.00
100.00
0
2000
4000
5.00
6.00
7.00
8.00

Table S1. Oilseed rape varieties tested in clubroot bioassay.

Code number	Variety	Code number	Variety
1267	Mendel	1907	Flash
1355	Toccata	1930	ES-Alienor
1378	Winner	1947	DK-Secure
1583	Lioness	1953	Vision
1592	Bravour	1955	Hammer
1593	Victory	1956	Dimension
1608	Castille	1963	NK-Caravel
1684	Excalib	1965	NK-Technic
1692	Betty	1970	PR46W21
1710	Grace	1975	Piranha
1780	Hornet	1976	Mallory
1834	Canti	1978	Emerson
1857	Catana	1982	Cuillin
1897	Temple	1983	Pelican
1902	PR45D03	1989	Epure
1904	PR46W14	Chinese cabbage	SB I KILO

Table S2 Field sites characteristics autumn weather and measurements of pre-trial pH, calcium and clubroot severity

Factor / Site	Aberdeen 2007/ 2008	Shropshire 2007/2008	Aberdeen 2008/2009	Herefordshire 2008/2009	Aberdeen 2009/2010	Warwickshire 2009/2010
Soil type	Sandy loam	Silty clay	Sandy loam	Sandy loam	Sandy loam	Medium loam
Cropping history [1]	Winter barley WOSR Spring barley	WOSR Winter wheat WOSR	Winter barley Spring barley No data	Winter wheat Potatoes WOSR	Winter barley WOSR Winter barley	Winter wheat WOSR Winter wheat
Clubroot epidemic	Very low all season	Very severe from early Autumn	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 wet, some early frosts	Late drilled, wet	Wet, cool, but crop grew well	Dry at sowing. Early onset of winter
Av. Soil temp [2]	11.3°C	13.3°C	11.3°C	12.5°C	11.6°C	14.1°C
Rainfall [3]	4.4	74.7	140.8	82.6	335.6	50.4
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^{-1})	1857	1720	2407	999	2127	1720
Clubroot severity pre trial (bioassay)	22.6	35.0	7.1	83.0	24.6	11.3

[1] Most recent crop first, [2] average soil temperature (measured at a minimum depth of 10 cm) from sowing date until the end of October, [3] total rainfall (mm) from sowing date until the end of October.