

Relationship between *Spongospora subterranea* f. sp. *subterranea* soil inoculum level, host resistance and powdery scab on potato tubers in the field

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The relationship between initial soil inoculum level of *Spongospora subterranea* f. sp. *subterranea* (Sss) and the incidence and severity of powdery scab on potato tubers at harvest was investigated. In all experiments soil inoculum level of Sss (sporeballs/g soil) was measured using a quantitative real-time PCR assay. Of 113 commercial potato fields across the UK, soil inoculum was detected in 75%, ranging from 0 to 148 Sss sporeballs/g soil. When arbitrary soil inoculum threshold values of 0, <10 and >10 sporeballs/g soil were set, it was observed that the number of progeny crops developing powdery scab increased with the level of inoculum quantified in the field soil preplanting. In four field trials carried out to investigate the link between the amount of inoculum added to the soil and disease development, disease incidence and severity on progeny tubers was found to be significantly ($P < 0.01$) greater in plots with increasing levels of inoculum incorporated. There was a cultivar effect in all years, with disease incidence and severity scores being significantly greater in cvs Agria and Estima than in Nicola ($P < 0.01$).

Keywords: host resistance, inoculum level, powdery scab, *Spongospora subterranea*

Introduction

Powdery scab of potato, caused by the plasmodiophorid pathogen *Spongospora subterranea* f. sp. *subterranea* (Sss), can cause extensive losses in the British ware crop and in Scottish seed production (Wale, 2000) and is a problem worldwide. The disease is characterized by unsightly pustules covering the tuber surface and reduces quality for the prepack market and, if the symptoms are very severe, renders stocks unsuitable for processing. When mature, the tuber scabs form powdery masses of thick-walled resting structures called sporosori (also known as 'cystosori' or 'sporeballs') which contain large numbers of resting spores and are an important source of inoculum because they may survive in soil for decades, making powdery scab a difficult disease to control (de Boer, 2000).

Powdery scab may be seedborne (Hide, 1981; Read *et al.*, 1995) or soilborne (Letham *et al.*, 1988; Merz *et al.*, 2006) with the result that planting disease-free and uncontaminated seed tubers in infested soil (Letham *et al.*, 1988; Merz *et al.*, 2006) or infested tubers in uninfested soil (Falloon *et al.*, 1996; de Nazareno &

Boschetto, 2002) can lead to the development of disease in progeny tubers. In uninfested soils, transmission of the pathogen from infected seed tubers to progeny does not appear to be straightforward. Burnett (1991) and Keiser *et al.* (2007) found no consistent correlation between the level of seed tuber infection and subsequent disease levels on progeny tubers. Other studies reported lower incidence and severity of disease on progeny tubers than on the seed stock from which they were grown (Braithwaite *et al.*, 1994; de Nazareno & Boschetto, 2002). Furthermore, a number of studies have shown that disease may not develop where temperature and/or soil moisture conditions are unfavourable, even where soils are known to be infested or diseased tubers are planted (Hughes, 1980; Christ & Weidner, 1988; Nakayama *et al.*, 2007).

The biphasic nature of the life cycle of *S. subterranea* ensures that the pathogen is both persistent, with the production of resting spores, but also capable of very rapid multiplication through the formation of secondary zoospores if conditions are suitable. This means that infection is not consistently related to initial inoculum levels, but rather to secondary infection by zoospores if conditions are conducive to their formation. This may explain the contradictory findings of a number of studies investigating the relationship between inoculum and disease. For example, van de Graaf *et al.* (2005), in inoculated pot experiments

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(5–50 sporeballs/g soil), and Merz *et al.* (2006), in field trials correlating multiple spot sites of soil inoculum and disease, both found no evidence of a relationship between soil inoculum concentration and the incidence and severity of disease on tubers. Parker (1984) found that naturally occurring soil inoculum resulted in a disease incidence and severity similar to that obtained in pots to which additional inoculum had been added. Similarly, Christ (1989) compared field plots with natural inoculum levels to plots to which additional inoculum had been added and found no difference in disease levels between the two. In contrast, Qu & Christ (2006) found a significant relationship between soil inoculum level and disease incidence when they tested 17 field soils in the USA and found them to be contaminated with Sss at levels between 0 and 14 400 sporeballs/g soil (determined using a competitive PCR assay). In addition, Shah *et al.* (2005), after adding sporeballs to pots, found that a significant asymptotic relationship existed between inoculum concentration (ranging from 0 to 12 000 sporeballs/g soil) and the severity of disease in tubers.

Nakayama *et al.* (2007) tested 29 potato fields in Japan using a competitive PCR assay and found the highest sporeball density to be 105 sporeballs/g soil. They found no evidence of a significant relationship between sporeball density in the soil and tuber disease severity, but did find a significant positive relationship between the amount of disease on the roots of plants and tuber disease severity. This is in accord with the conclusion of Burnett (1991) that initial soil pathogen concentration was of less importance than the build-up of inoculum during the root multiplication phase.

Therefore, it is proposed that higher initial inoculum levels offer the pathogen a greater opportunity to infect and cause disease, thereby establishing a link between initial inoculum level and disease. However, because of the production of zoospores and secondary infections under favourable conditions, disease development may be high irrespective of initial inoculum levels. The impact on disease of both initial inoculum levels and secondary infection will be dependent upon environmental factors.

The aim of this work was to investigate the relationship between initial soil inoculum levels (sporeballs), measured using quantitative real-time PCR (van de Graaf *et al.*, 2003) and the development of powdery scab. This study comprised four main parts. First, to investigate the relationship between soil and seed inoculum levels and powdery scab development in commercial potato growing fields across the UK. Secondly, the relationship between soil inoculum level and subsequent disease in the absence of seed inoculum was investigated by growing minitubers of a number of cultivars within commercial potato crops. Thirdly, a series of small-scale field trials were established within commercial crops to investigate the interaction between cultivar resistance and soil inoculum level and their effects on powdery scab development.

Finally, four field trials were carried out to investigate the link between soil inoculum level and disease development.

Materials and methods

Soil samples

For each commercial crop (experiment 1) a 4-ha portion of the field from which samples would be taken was identified. A soil sample was taken using a soil-sampling spear from the top 10 cm of soil of at least 100 points in a W-shape across the selected portion of the field to give a total of approximately 1 kg. For the minituber and small-scale field trials (experiments 2 and 3), inoculum levels were based on the 4-ha area in which the commercial crop was planted. For the inoculum-level field trials (experiment 4), soil was collected using the same approach, but the area from which the samples were taken was restricted to that of each main plot.

Seed and progeny tuber sampling and disease assessment

For each seed stock, 50 tubers were visually assessed for powdery scab. In monitored commercial crops, minituber trials and the small-scale trials, 50 progeny tubers were washed and assessed visually for powdery scab incidence. Within the inoculum-level field trials, all progeny tubers were harvested from each plot and assessed visually for powdery scab incidence and severity (based on an increasing severity scale of 0–6; <http://www.spongopora.ethz.ch/LaFretaz/scoringtable.htm>).

Real-time PCR: quantification of soil inoculum

Soil DNA extractions were carried out according to the method of Brierley *et al.* (2009). Individual 1-kg soil samples, collected as described previously, were thoroughly mixed. A 60-g subsample of soil was taken from each 1-kg sample and placed in a Retsch milling bowl (Planetary Ball Mill PM 400) with 120 mL extraction buffer and 12 ball bearings and milled at 15 g for 5 min. Following milling, triplicate 1.5-mL aliquots were taken from each bowl and DNA extractions carried out on each. Bowls were cleaned with 96% ethanol and 0.2 M NaOH between samples to prevent cross-contamination. Duplicate samples from each DNA extraction were analysed using the real-time PCR assay of van de Graaf *et al.* (2003) and inoculum expressed as sporeballs/g soil.

Experiment 1: crop monitoring

In order to ascertain the levels of seed- and soilborne inoculum causing disease in commercial seed and ware potato production, 113 fields were monitored over 3 years (2005–2007). In each case, a soil sample was taken from the field before planting and assessed for Sss contamination as described above. In addition, seed stocks and

progeny tubers were assessed visually for powdery scab. Additional details of monitored crops are given in Lees *et al.* (2010).

Experiment 2: minituber monitoring

To gain information on the effect of soilborne inoculum and cultivar resistance on the development of powdery scab in the absence of seed inoculum, a total of 25 (seven to nine per year) small minituber trials were established in ware crops which were being monitored as described above. At each site, in each of two drills, 10 minitubers of three cultivars were planted and labelled in each year: Maris Piper (powdery scab resistance rating 3 on a 1–9 scale of increasing resistance according to The British Potato Variety Database <http://varieties.potato.org.uk/menu.php>), Saxon (6) and Sante (8) in 2005, Maris Piper (3), Saxon (6) and Romano (5) in 2006, and Maris Piper (3), Sante (8) and Nadine (3) in 2007. At harvest, daughter tubers were lifted and visually assessed for powdery scab incidence (percentage of tubers with symptoms). The results were related to the amount of soilborne contamination as measured by real-time PCR in the soil sample taken from the whole field, i.e. that of the commercial crop in which the trial was located.

Experiment 3: small-scale field trials

In 2007, 18 small-scale field trials were established within commercial ware potato crops across England and Scotland to evaluate the interaction of cultivar resistance and level of soilborne contamination by Sss. At each site, small replicated trials comprising six prepacking varieties with a range of resistance ratings to powdery scab were planted. Trials were placed within the farm crop and comprised three replicate plots of 20 tubers of each variety in a randomized block design. Individual plots were 10 tubers \times 2 drills with 25 cm between tubers. A 1-m gap was left at the ends of the trial area and a blank drill between the farm crop and the trial area where possible. Trials were planted close to the date the field crop was planted and seed was planted at a 15-cm depth. Trial plots received the same crop protection and husbandry treatments as the adjacent crop. At harvest, daughter tubers were lifted and assessed visually for disease.

Experiment 4: soil inoculum field trials

In 3 years (2009, 2010 and 2011) four field trials were set up to investigate the effect of initial soil inoculum level on powdery scab development at final harvest. Trials were carried out at a site at The James Hutton Institute (JHI) in each year and also by SAC at Fingask, Aberdeenshire in 2011. In each of the 3 years the powdery scab susceptible potato cv. Agria (resistance rating 3) and the moderately susceptible cv. Nicola (6) (Merz *et al.*, 2011) were used in the JHI trials. Cultivars Estima (susceptible, rating 3) and Nicola (6) were compared in the 2011 SAC trial. All seed was kept at 4°C in the dark until required.

Trials were located on a field site at JHI on which potatoes had never knowingly been grown and at SAC on a site where no soil inoculum could be detected. The soil from each site was sampled and tested using real-time PCR and found to be negative for the presence of Sss. In each of the trials four main plots (inoculum levels 1–4) were created. Inoculum was created by peeling cv. Agria tubers heavily infected with powdery scab. Peel was air-dried and macerated in a blender, before being mixed with twice its volume of vermiculite (4 L peel: 8 L vermiculite). Two further dilutions from this bulk were made; a 1:10 and a 1:100 bulk to vermiculite. Level-4 main plots received the equivalent of 3.6 L macerated peel per plot, level-3 main plots 0.36 L and level-2 main plots 0.04 L. Vermiculite only was added to level-1 main plots. The inoculum for each level was spread across the main plot area. Inoculum was spread onto prepared ground prior to ridges being drawn up; the creation of ridges and planting helped to incorporate the inoculum through the drill. After planting, a single soil sample was taken from each main plot at the JHI site (consisting of 25 \times 10 g cores bulked) and the level of Sss (sporeballs/g soil) determined by real-time PCR.

Each main plot consisted of four rows of 12 plants, surrounded by guard rows and separated by at least two rows. Two replicates of each cultivar were randomly assigned to plots within each main plot. In 2009, 2010 and 2011 (SAC) all plots were irrigated using trickle tape; 25 mm was applied when soil moisture deficit reached 18 mm. In 2011 (JHI), a split-plot design was created to compare irrigated with unirrigated conditions. However, as no significant differences for disease incidence and severity were found between irrigated and unirrigated treatments, the means of both treatments are presented. At the time of final harvest, all progeny tubers from each plot were assessed for powdery scab incidence and severity (score on a 0–6 scale), and results expressed as the mean incidence and severity score of powdery scab disease per plot.

Statistical analysis

All statistical analyses were carried out using GENSTAT 14th edition (VSN International Ltd.). In experiment 4, the effects of soil inoculum level (d.f. = 3) and cultivar (d.f. = 1) on progeny tuber disease in individual field trials were analysed using ANOVA. If necessary data was transformed prior to analysis.

Results

Experiment 1: crop monitoring

In the 113 commercial potato fields monitored, soil inoculum detected by quantitative real-time PCR ranged from 0 to 148 Sss sporeballs/g soil. Soil inoculum was detected in the majority (75%) of fields, whereas only 25% of seed stocks had powdery scab symptoms. There were only three occasions when no

inoculum was detected in the soil yet considerable levels of disease (>10% incidence) developed, and of these one was planted with contaminated seed. It was much more common for inoculum to be detected in the soil yet for no disease to subsequently be found on the progeny crop. The occurrence of disease was lower in 2005 and 2006 than in 2007. When arbitrary threshold values for soil inoculum of 0, <10 and >10 sporeballs/g soil were set, it was observed that both the number of progeny crops developing powdery scab and the mean incidence of disease in crops (percentage of tubers with powdery scab) increased with the level of inoculum quantified in the field soil before planting (Fig. 1). There was no relationship found between the level of seed inoculum and subsequent disease on progeny tubers. When seed with powdery scab was planted into contaminated soil there was no evidence of an increase in disease (data not shown).

Experiment 2: minituber trials

When minitubers were planted in trials set amidst commercial crops, powdery scab developed predominantly on the susceptible cv. Nadine and, to a lesser extent, on cv. Maris Piper (Fig. 2). Little or no disease was found on the other cultivars. Only five progeny stocks (7%) of the 75 assessed in total (three cultivars \times 25 sites) had powdery scab symptoms despite no inoculum being detected in the soil sample. These five stocks originated from only two field sites.

Experiment 3: small-scale field trials

Eleven of the 18 sites set up to compare cultivar resistance over a range of inoculum levels had no detectable *Sss* soil inoculum. Disease incidence did not exceed 2% at 10 of

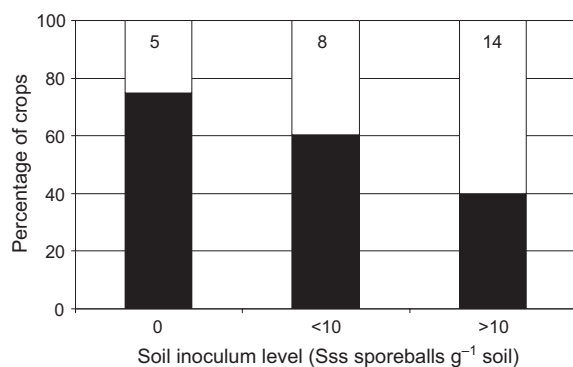


Figure 1 Percentage of 113 potato crops monitored with (□) and without (■) powdery scab on progeny tubers when 0 ($n = 20$ crops), <10 ($n = 73$ crops) and >10 ($n = 20$ crops) *Spongospora subterranea* f. sp. *subterranea* (Sss) sporeballs /g soil were detected in soils before planting. The mean incidence of disease in all crops within the three soil inoculum categories is indicated by the number in each column.

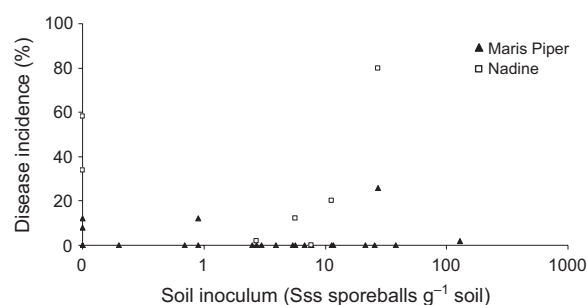


Figure 2 Experiment 2: relationship between powdery scab incidence (%) on progeny tubers of two potato cultivars grown from minitubers and soil inoculum level (*Spongospora subterranea* f. sp. *subterranea* (Sss) sporeballs/g soil) at 25 field sites with varying levels of soil inoculum.

these 11 sites, whilst at one site disease incidence in the more susceptible cultivars Pentland Squire (resistance rating 4) and Lady Christl (3) was 16 and 12%, respectively (Fig. 3), signifying that undetected soil inoculum may have been present.

Very little disease was found on the more resistant cultivars Saxon (resistance rating 6) and Sante (8), or on Maris Piper, despite its low resistance rating (3) at any site, irrespective of soil inoculum level (data not shown). However, King Edward, which has a relatively high disease resistance rating (7), and the susceptible cultivars Lady Christl and Pentland Squire, generally developed higher levels of disease with increasing soil inoculum levels (Fig. 3).

Experiment 4: soil inoculum field trials

Visual assessment of seed stocks revealed that both cultivars had powdery scab symptoms (Agria 10, 14 and 36%; Nicola 14, 44 and 52% incidence) in 2009, 2010 and 2011 (JHI), respectively. Mean powdery scab disease severity did not exceed 0.9 (on a 0–6 scale of increasing disease severity) for either cultivar in any year. The Estima and Nicola stocks used in the 2011 SAC trial were visibly clear of powdery scab symptoms.

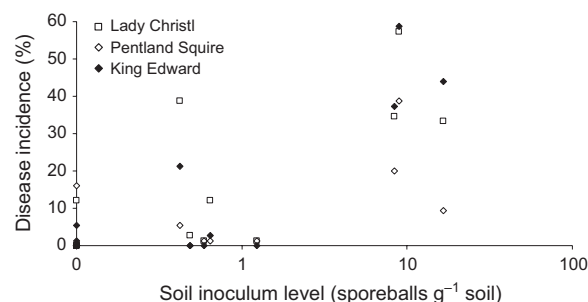


Figure 3 Relationship between powdery scab incidence (%) on progeny tubers of three potato cultivars and soil inoculum level of *Spongospora subterranea* f. sp. *subterranea* across 18 field sites.

The amount of detectable Sss in the four artificially created inoculum levels differed between years, as would be expected as each was individually infested in the year of experimentation. In the JHI trials, detectable Sss was lowest in 2010 and highest in 2011 (Table 1). In all years at JHI, some inoculum was detected in the level-1 (uninfested) main plots after the other main plots had been artificially infested.

In all four trials there was a significant difference (ANOVA, $P < 0.01$) between both the incidence and severity of disease in both cultivars in plots to which inoculum had been added compared to uninoculated plots (Fig. 4). The extent to which the increasing level of inoculum at levels 2 to 4 affected disease varied between years and cultivars. In 2009, there was a significant increase in incidence of disease, but not severity, in both cultivars with increasing level of soil contamination (levels 2 to 4). For Agria, the incidence of disease continued to increase at levels 2 to 4 in 2010 and 2011, but as the incidence was already high at level 2, subsequent increases at levels 3 and 4 were not significant; increases in disease severity at levels 2 to 4 were also not significant. Disease incidence did increase significantly in Nicola in 2010 with increasing inoculum level, but not in 2011 at either JHI or SAC. Disease incidence in Nicola and Estima at SAC in 2011 in uninoculated plots was relatively high in comparison to levels found in other trials. Progeny tubers of Agria from plots to which no inoculum had been added (level 1) had a mean disease severity score of <1 (0–6 scale), in contrast to progeny tubers from level-4 plots with a mean disease severity score of 4 in 2009, 3 in 2010 and 2 in 2011. The difference in symptom coverage between tubers with scores of 1 and 4 is shown in Figure 5.

The more resistant cultivar, Nicola, reached a maximum of 72% incidence of disease but with a mean disease severity score of just over 1. The more susceptible cultivar, Agria, reached a maximum of 99% incidence with a mean disease severity score of up to 3.9. In all years, disease incidence and severity scores were significantly greater in Agria than Nicola ($P < 0.01$; Fig. 4).

Discussion

Previous difficulties in accurately identifying the relative importance of different sources of inoculum and the environmental factors influencing infection and disease development have in part been caused by an inability to

accurately detect and quantify Sss inoculum levels in soil. With the development and validation of a new technique to effectively extract target DNA from soil (Brierley *et al.*, 2009), followed by use of a real-time PCR assay (van de Graaf *et al.*, 2003) to quantify DNA of Sss, it was possible in the present study to carry out extensive disease risk assessments where inoculum levels on seed and in soil are associated with a level of disease risk.

In this study, the maximum level of Sss contamination found in 113 soils sampled from commercial potato fields within the UK was 148 sporeballs/g soil. Merz (1993) surveyed 78 soils from potato producing areas of Switzerland and reported that soils highly contaminated with Sss had inoculum densities >500 sporeballs/g soil as determined using a baiting technique in which a correlation was made between the intensity of root infection and the sporeball inoculum density in nutrient solution (Merz, 1989). The large variation in sporeball levels found between individual studies is likely to be associated in part with the different methodologies used for quantification.

Using a reproducible method to quantify sporeballs within soil and surveying commercial potato growing fields in the UK, the experimental work in the present study was based on relatively low levels of Sss inoculum. It should, however, be noted that the patchy distribution of inoculum through the ridge, and the trace inoculum in level-2 and -3 plots, may have impaired the relative quantification of the inoculum levels. Inoculum levels <1 sporeball/g soil may be detected but are not quantifiable (Brierley *et al.*, 2009). The results from experiment 4 should be interpreted in terms of the relative amount of inoculum added to plots within each year, rather than absolute values of sporeballs/g soil.

The seasons over which the field trials (experiment 4) were carried out were conducive to powdery scab development, as illustrated by the near 100% incidence of disease on cv. Agria in each year, in conjunction with the fact that each trial was irrigated. Under favourable environmental conditions it might have been expected that infection could build up sufficiently through secondary production of zoosporeangia to cause high levels of disease in all the main plots to which inoculum had been added, irrespective of the initial starting level of primary inoculum (sporeballs). There is some evidence of this occurring at the SAC site in 2011, but to a lesser extent in the JHI trials. However, the results from all years clearly demonstrate that the level of primary soil inoculum did affect the level of powdery scab.

It has been demonstrated that resistance to powdery scab exists in some cultivars in the UK (Gans *et al.*, 1987) and worldwide (Genet *et al.*, 1996; Merz *et al.*, 2011). However, despite the availability of disease-resistant cultivars, genetic resistance to Sss currently plays a minor role in disease control as cultivars are usually selected by growers for characteristics other than their ability to resist powdery scab (Harrison *et al.*, 1997). In 2011, of the five top cultivars planted in Great Britain by area, which together represented 38% of the total planted area

Table 1 *Spongospora subterranea* f. sp. *subterranea* (sporeballs/g soil) quantified in main plots at The James Hutton Institute (JHI) inoculated with varying levels of sporeballs in three trial years

	Soil inoculum (sporeballs/g soil)			
	Level 1	Level 2	Level 3	Level 4
2009	1.2	0.1	3.7	57.2
2010	0.6	0.7	0.6	5.7
2011	1.3	17.2	20.3	214.5

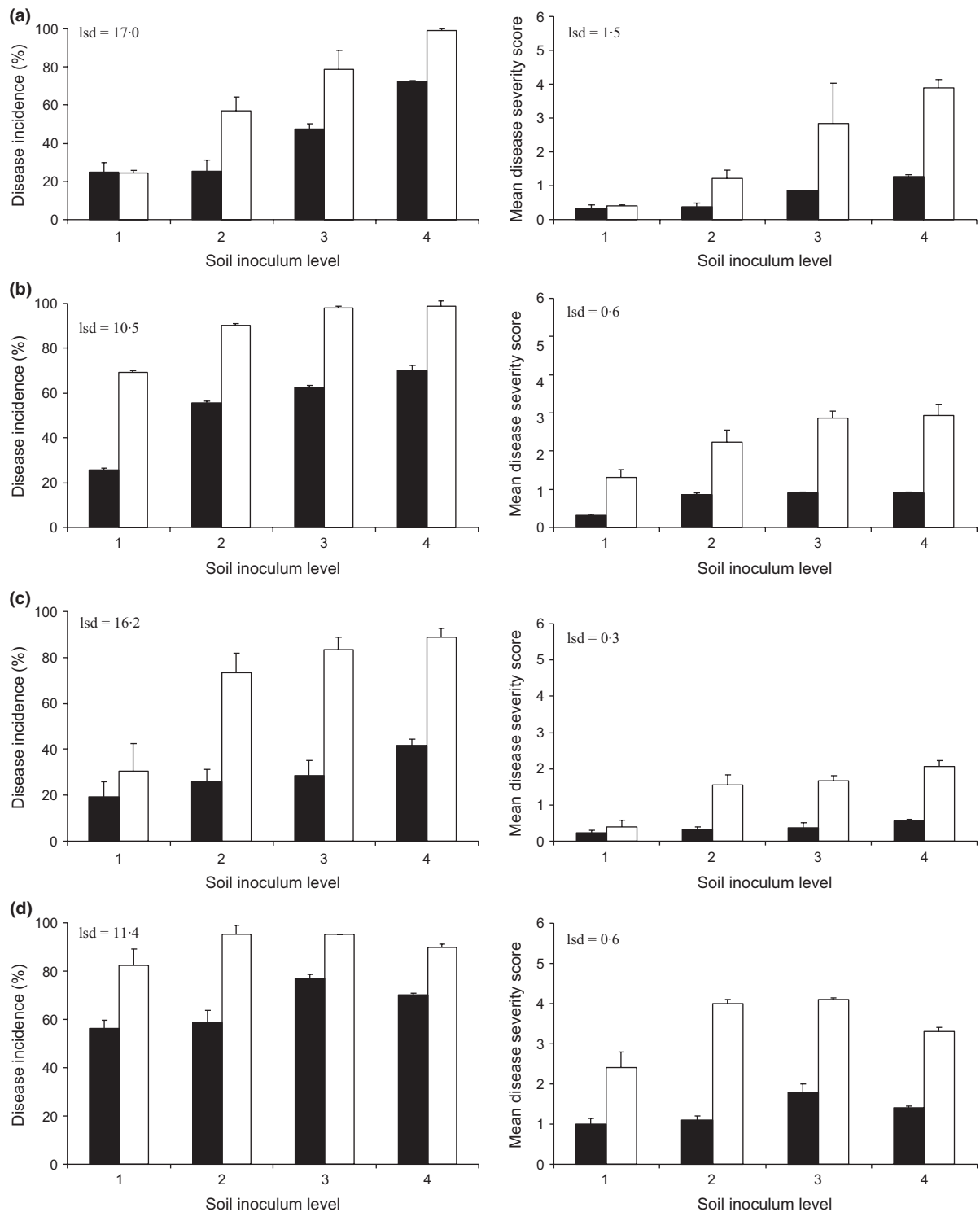


Figure 4 Effect of soil inoculum level (1–4 scale of increasing contamination) of *Spongospora subterranea* f. sp. *subterranea* (Sss) on powdery scab incidence and severity in two cultivars, Nicola (■) and Agria (□) in field trials carried out in (a) 2009, (b) 2010 and (c) 2011 (JHI); and (d) in Nicola (■) and Estima (□) in a trial carried out in 2011 (SAC). Data are means of two replicate plots + SE in 2009, 2010 and 2011 (SAC) and four replicate plots + SE in 2011 (JHI).

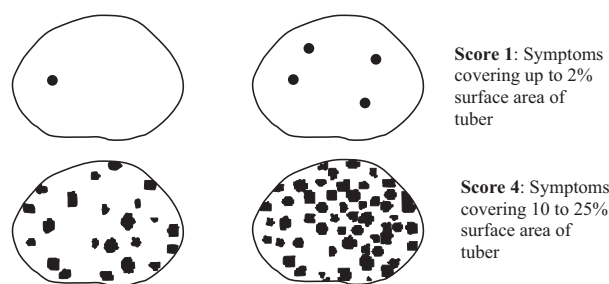


Figure 5 Disease severity scores, illustrating difference in surface area covered by powdery scab symptoms on potato tubers with a score of 1 compared with those with a score of 4.

of 126 328 ha (Potato Council provisional planting figures by variety 2011, <http://www.potato.org.uk>), three (Maris Piper, Estima, Lady Rosetta) had resistance ratings of 3 on a 1–9 scale of increasing resistance, one (Maris Peer) a rating of 4 and one (Maris Peer) a rating of 6, according to The British Potato Variety Database <http://varieties.potato.org.uk/menu.php>. However, of all the methods assessed for reducing the incidence and severity of powdery scab, it is widely recognized that host resistance is the most effective and sustainable approach, when used in combination with other disease management practices in an integrated way. Burgess & Wale (1994, 1996) showed cultivar resistance to be an important factor influencing the severity of powdery scab.

In all of the field trials carried out in the study presented here, disease resistance was found to be an effective method of controlling powdery scab when comparing cultivars Agria, Estima and Nicola. However, some discrepancies between disease resistance rating and progeny disease in the small-scale field trials (experiment 3) were observed. Difficulties in establishing consistent susceptibility rankings have been reported resulting from a number of factors, including differential performance in pot and field experiments (Gans & Vaughan, 2000) and the emergence of between-year differences (Torres *et al.*, 1995; Gans & Vaughan, 2000; Lees, 2000) linked to weather conditions and the patchy distribution of the pathogen in soil (Lees, 2000) or genotype \times environment interactions (Gans & Vaughan, 2000). Timing of tuber development varies between cultivars and may also have an effect on disease unless environmental conditions are continuously optimal for infection for all cultivars during tuber development. However, recent trials conducted across Europe showed good consistency of resistance ratings between trials (Merz *et al.*, 2011), but these trials did not include King Edward or Maris Piper.

Although arbitrary disease risk categories have been set for powdery scab soil inoculum levels within this study, they must be considered in a wider context. For example, low-risk soils where no inoculum has been detected will only be truly low-risk if the planted seed is free from Sss contamination. Additionally, in seasons when conditions are not generally conducive to disease development then powdery scab can still develop in crops grown in the presence of high levels of inoculum, or where

a microclimate suitable for disease exists, i.e. areas prone to waterlogging. This study illustrates that quantifying soil inoculum levels prior to planting a potato crop can be used as a tool in disease management. Depending upon the levels found, field and cultivar selection can be used to limit the risks of powdery scab on progeny tubers.

Acknowledgements

Funding of this work by The Potato Council is gratefully acknowledged. The authors thank Greenvale AP, MBM Produce Ltd and The Higgins Group for assistance in crop monitoring and supply of seed and soil samples.

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