Project Report

Epidemiology, autecology and control of *Spongospora subterranea*, cause of potato powdery scab

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Stuart Wale *SAC*

Pieter van de Graaf & Alison Lees *SCRI*

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Preface

Powdery scab causes extensive losses in the British ware crop and is even more damaging to Scottish seed production. In wet years, estimated annual losses in Scotland can be as high as £7m. The disease is caused by *Spongospora subterranea* which is also the vector of potato mop-top virus (PMTV), one of the causes of spraing.

This project was undertaken as a collaboration between Scottish Agricultural College Aberdeen and the Scottish Crop Research Institute. It was jointly funded by the BPC and the Scottish Executive Environment and Rural Affairs Department. Its aim was to develop an integrated management strategy for powdery scab by gaining a better understanding of the epidemiology of *S. subterranea*, and by evaluating a range of chemical, biological and cultural treatments for their effectiveness against powdery scab.

The work has shown that the viability of *S. subterranea* resting structures (sporeballs) in the soil decreased over time, but more rapidly in soil held at 20ºC than at 4ºC. Soil moisture had no effect on sporeball survival over two years. Inoculum level in the soil or on seed bore a poor relationship to disease development. Environmental factors, such as temperature, soil type and soil moisture were much more important, though these affected powdery scab and root gall development differently.

The single most effective control measure was found to be varietal resistance. Growing under fleece reduced powdery scab in susceptible varieties. The most effective chemical measure was soil treatment with Fluazinam.

The molecular diagnostic methods developed in the project are currently being used as part of a project to improve decision making for the management of potato diseases.

Summary

This project investigated the biology and control of powdery scab of potatoes caused by *Spongospora subterranea*. Powdery scab occurs widely in northern Europe and is a major disease of potatoes. The quality and quantity of both ware and seed potatoes in Britain are badly affected by the disease but it is a particular problem in northern Britain where soil conditions are conducive to disease development. There are no effective control methods currently available.

As an obligate parasite, the pathogen is difficult to study. The research programme in this project was aimed at developing an integrated management strategy for powdery scab. To achieve this, the epidemiology of *S. subterranea* was studied, and chemical, biological and cultural treatments were evaluated for their effectiveness against powdery scab.

To enable accurate quantification of *S. subterranea* DNA in samples of different origin, a real-time PCR assay specific to this pathogen was developed at SCRI. It was shown that this assay could reliably detect and quantify DNA from sporeballs, zoospores and plasmodia/zoosporangia of *S. subterranea*.

The tomato bait plant technique was used as a tool for the study of zoospore release in time and by combining this technique with selective PCR, it was shown that sporeball germination and the infection of tomato roots took place within one week of inoculation of the bait plant system with sporeballs of *S. subterranea*. A clear peak in zoospore release was found after three weeks when secondary zoospores became abundant. The limits for the detection of *S. subterranea* DNA in tomato roots using a real-time PCR assay were found to be very low, but quantification at high inoculum levels was inaccurate.

Viability of sporeballs of *S. subterranea* in soil decreased over time. After two years, almost no viable sporeballs were detected in clay soil kept at 20°C, but at 4°C sporeball viability declined more slowly and remained relatively high. No significant effect of soil moisture level on sporeball survival was found. The success rate of detection of sporeballs in soil was strongly dependent on soil type and soil chemicals co-extracted with DNA were found to inhibit the PCR reaction. In particular, loamy and sandy soils yielded many false negative reactions. Detection was improved by flooding air-dried soil samples with water for three days and extracting DNA from the soil and floodwater together.

Both the incidence and severity of potato powdery scab were found to be influenced by a range of environmental factors, including temperature, soil type and soil moisture regime when tested under controlled conditions. Soil inoculum level did not have a significant effect on infection and disease development and even low levels of inoculum were regularly found to result in severe disease symptoms. No relationship was observed between the occurrence of powdery scab on tubers and galls on roots of potato variety ‘Estima’. Both symptoms occurred independently and even when they did occur together their severity was usually not correlated. This was partly explained by the fact that the effect of environmental factors, in particular temperature, on powdery scab and root gall development was shown to be different.

A GB-wide trial investigating the relationship between the level of seed-borne disease and that developing on the progeny was carried out by SAC. Evidence from this field trial and a five year survey in England & Wales confirms that there is no simple or consistent relationship between powdery scab inoculum on seed and disease developing on the progeny crop. It appears that disease risk is much greater in the north and west of GB and this may relate to relative conduciveness of environmental conditions rather than inoculum level.

A range of chemical, biological and cultural treatments were evaluated against powdery scab under controlled conditions at SCRI. Several treatments were found to be more effective against soil borne inoculum of *S. subterranea* than zinc. Fluazinam and mancozeb were the most effective chemicals tested against powdery scab and root galling, but phytotoxic side effects were occasionally observed. The application of prawn shell waste or freeze dried powder of *Brassica juncea* also greatly reduced powdery scab incidence, but had no controlling effect on root gall symptoms.

A series of field trials were carried out by SAC to test potential chemical control options. These were tested in situations where soil or seed were the main sources of inoculum. As in previous evaluations of chemical control trials, no chemical was fully effective and results were extremely variable. Severity of tuber infection was more frequently reduced than incidence. Fluazinam incorporated into soil proved the most effective chemical control option, supporting earlier studies. Variability in trial results may be explained by differences in inoculum.
Applying zinc in irrigation water and the use of calcium cyanamate as a fertiliser treatment failed to achieve control of powdery scab.

A series of trials evaluated how effective integration of control measures would be on the incidence and severity of powdery scab. Variety had the greatest impact on powdery scab. Simply by changing from a susceptible variety to a moderately resistant one, the incidence and severity of disease fell significantly. Matching variety to disease risk is a clear option for growers to reduce powdery scab risk. There was a reduction of powdery scab under fleece with susceptible varieties. This effect was either due to a less favourable environment for infection and disease development under the fleece or a reduction in the wetting of the ridges by shedding water into furrows. Other factors tested individually gave little or no reduction in powdery scab. Although, the results of these trials do not unambiguously confirm it, it seems logical that to effect the greatest control of powdery scab, control measures should be integrated.

Genetic variation within *S. subterranea* from different sources in Scotland was investigated using the AFLP technique, but the results were difficult to interpret since no methods were found to obtain enough clean DNA of the pathogen.

Tubers were sampled from certified Scottish seed tuber stocks by SASA and tested at SCRI for the presence of *S. subterranea* DNA in tuber washings and skin peelings. The incidence of *S. subterranea* was high in all stocks tested (Estima and Hermes), but high DNA levels were found only in the washings from Estima tubers (regardless of the presence of powdery scab symptoms) and in the skin of Estima tubers with powdery scab.

This project has extended our knowledge of the biology of *Spongospora subterranea* and thereby permitted greater insight into infection processes and its epidemiology. Further development of PCR diagnostic methods have been made, but there remains a need for further development of the technique, particularly for the detection in soil, and interpretation of the results before it can be put into commercial use. Apart from utilisation of varietal resistance to powdery scab, other control measures offer little contribution. Integration of control measures is likely to be the most effective way for growers to reduce the impact of the disease. As the most effective chemical control option, it is recommended that off-label approval be sought for fluazinam. A survey of seed stocks confirmed the wide-spread occurrence of the pathogen, even in a more resistant variety.

Introduction and background

Aims, background and objectives

The overall aim of the project was to develop an integrated management strategy for powdery scab, which would reduce the impact of the disease on the cultivation of both seed and ware potatoes.

Powdery scab of potatoes is a widespread problem caused by *Spongospora subterranea*. The pathogen forms resting structures (sporeballs) that are very persistent and can survive in soil for many years. Powdery scab causes extensive losses in the British ware crop and is even more damaging to Scottish seed production. In wet years, estimated annual losses in Scotland can be as high as £7m, and the disease forms a major problem for seed growers. *S. subterranea* is also the vector of potato mop-top virus (PMTV), one of the causes of spraing.

Apart from potato and related species, the host range of *S. subterranea* includes other crop plants, such as oilseed rape, sugar beet and spinach, and a large number of common weed species, including chickweed, poppy, nettle and fat-hen. However, the role of alternative hosts on disease incidence in potato remains unknown. In most hosts, *S. subterranea* forms only zoosporangia and occasionally galls in the roots, but in potato, cankers and scabs can be formed on the tubers.

Inoculum of *S. subterranea* can be either soil- or seed-borne but the relative importance of these sources is unclear. Powdery scab is especially prevalent in cool and wet climates, but the exact effect of environmental factors, such as temperature, moisture and soil type, is not fully understood.

There are no adequate control measures for powdery scab available to farmers, and this situation is aggravated by the widespread cultivation of susceptible varieties. New control measures are urgently needed, but in order to make optimisation of the control of powdery scab possible, it is necessary to first collect more information on the biology of *S. subterranea*.

The objectives of the project were:

- To determine the relationship between *S. subterranea* inoculum and disease development

- To evaluate disinfectants, fungicides, novel chemicals and biofumigants as pre-plant soil and tuber treatments against powdery scab

- To evaluate agronomic measures that would reduce the incidence of powdery scab

- To study the population structure of *S. subterranea* and determine its variability

- To determine the amount and distribution of *S. subterranea* inoculum present on seed tubers

Real-time PCR assay

The quantification of *S. subterranea* in different sample types was essential for the detailed studies into the epidemiology and control of powdery scab and root galling described in this report. An ELISA-test for quantifying *S. subterranea* was available, but mainly for the detection of sporeballs. Quantification of *S. subterranea* DNA was also possible using a competitive PCR assay (Bell *et al*., 1999), but a more rapid and accurate method was needed for processing large numbers of samples. Real-time PCR using specific primers and a TaqMan® probe is a quick and reliable method with which many samples can be tested simultaneously and this method has been used with success for the detection and quantification of a range of potato pathogens. Thus, a sensitive and quantitative real-time PCR assay specific to *S. subterranea* was developed for use as a reliable tool for the detection and quantification of this pathogen.

Assay development and specificity

The internal transcribed spacer regions of *S. subterranea* were accessed from databases on the Internet and used to design forward and reverse primers and a TaqMan® probe for use in a real-time quantitative PCR assay. The fluorogenic probe was labelled at the 5' end with the fluorescent reporter dye FAM and modified at the 3' end with the quencher dye TAMRA.

Real-time quantitative PCR was performed in 96-well reaction plates with optical caps using an ABI Prism 7700 Sequence Detection System. For each sample, 1 µl template DNA was added to 24 µl reaction mix consisting of 12.5 µl TaqMan® Universal PCR Master Mix, 9.5 µl sterile HPLC water, 0.75 µl each of the forward and reverse primers (10 µM), and 0.5 µl of the TaqMan® probe (5 µM). The universal thermal cycle protocol recommended by Applied Biosystems was used for PCR amplification: 50°C for 2 min, followed by 95°C for 10 min, then 45 cycles at 95°C for 15 s and 60°C for 1 min, which was repeated 45 times.

A range of standards containing different amounts of *S. subterranea* DNA was included in the real-time PCR assay. DNA was extracted from a known number of sporeballs following the method of Bell *et al*. (1999) and diluted with TE buffer to obtain a dilution series of DNA equivalent to 100000, 10000, 1000, 100, 25 and 10 sporeballs per ml. The C_t value, which is the number of PCR cycles needed to reach a minimum level of reporter fluorescence associated with an exponential increase in PCR product, was calculated for each unknown sample by the software and automatically compared with the C_t values of the standard series. By comparison with the standard curve of the C_t values against the logarithm of the standard amount of sporeball DNA, the amount of *S. subterranea* DNA in each unknown sample was expressed in sporeball equivalents (from now on referred to as 'units') based on the C_t value of the sample.

DNA was extracted from sporeballs from potato tubers with powdery scab collected by SAC from 15 different locations in Northern Britain (see Table 9.1 in "Pathogen genetic variation (AFLP)"). The DNA extracts were tested for amplification in the real-time PCR assay using the primer/probe set designed for the detection of *S. subterranea*. The specificity of the primers and TaqMan® probe was tested in a separate assay using genomic DNA from a range of potato pathogens and soil micro-organisms. A non-template control with 1 µl TE buffer instead of DNA was always included and all samples were tested in duplicate.

The amounts of DNA of *S. subterranea* used for the standard dilution series in the real-time PCR assay were equivalent to 100, 10, 1.0, 0.1, 0.025 and 0.01 sporeballs and the standard series was amplified consistently in the real-time PCR assay. Only DNA equivalent to 0.01 sporeballs was not consistently amplified. The average C_t value obtained when an equivalent of 0.025 sporeballs was amplified in the real-time PCR assay was 37 cycles, compared to an average C_t value of 24 with DNA equivalent to 100 sporeballs (1×10^5 sporeballs per ml TE buffer). As expected, the non-template control had a C_t value > 45 cycles. The correlation coefficient of the standard curves used for calculating the amounts of DNA in the unknown samples was always greater than 0.94.

The real-time PCR assay was able to detect DNA of *S. subterranea* from the 15 British sources listed in Table 9.1. DNA of the other micro-organisms tested was not amplified in the real-time PCR assay and always had C_t values > 45 cycles.

Detection of sporeballs

DNA was extracted from sporeballs in water and soil. Extraction buffer (20 ml) was added to different numbers (0, 2, 6, 10, 20, 60, 100, 200 and 1000 per sample) of sporeballs in SDW. For the soil extractions, silty clay soil from Aberdeenshire, Scotland was obtained from SAC, air dried, passed through a 1 centimetre mesh sieve and stored at room temperature. Sterile distilled water (SDW) containing varying numbers of sporeballs (0, 1, 3, 5, 10, 30, 50, 100 and 500 per 10 g sample) was added to samples of the dried clay soil which were then air dried again before DNA extraction. DNA was extracted from sporeballs in water and soil using the method described by Bell *et al*. (1999). In all cases, there were five replicates per treatment.

The real-time PCR assay could reliably detect and quantify sporeballs suspended in water. The average amount of DNA detectable from these samples was comparable to the numbers added to the original sample (Table 4.1). Only at concentrations lower than 1 sporeball per ml was the discrimination between inoculum levels less successful. The majority of samples at these low concentrations tested negative, while detection was much more consistent at concentrations > 1 sporeball per ml.

The detection rate of sporeballs in clay soil was high for most inoculum levels, possibly due to natural contamination of the clay soil used. Quantification of sporeballs in soil was less accurate than of sporeballs in water, but significant inhibition took place in only two of the eight inoculum levels tested (5 and 10 sporeballs per ml) (Table 4.1).

The real-time PCR assay developed for *S. subterranea* detected and quantified the pathogen in water and soil. Specificity of the primers and probe was confirmed by a lack of amplification of DNA from 18 other potato pathogens and soil fungi. DNA samples of *S. subterranea* from 15 different locations in Britain were all amplified as expected. The real-time PCR assay was very sensitive with consistent amplification of standard DNA quantities equivalent to 0.025 sporeballs (in 1 µl) and occasional amplification of lower DNA quantities. These results are similar to the minimum detection levels for conventional PCR and ELISA.

The real-time PCR assay was able to detect and quantify *S. subterranea* both in artificially and naturally contaminated soil samples. Using the protocol of Bell *et al.* (1999), the detection limit should be 2 sporeballs per g soil (10 g soil in 20 ml buffer). In our assay, DNA of *S. subterranea* was detected at concentrations lower than 2 sporeballs per g soil and was also detected in the non-inoculated control. This was probably due to natural contamination of the clay soil. The detection and quantification of sporeballs in clay soil was less consistent than in water possibly due to binding of DNA to soil particles or inhibition of the PCR by soil chemicals. In addition to the extraction method, the detection of low levels of inoculum in soil is dependent on the sampling technique and the likelihood of including sporeballs in the small sample used for the DNA extraction. According to Bell *et al*. (1999), conventional PCR was able to detect ≥ 5 sporeballs per g soil consistently, but these workers did not attempt to quantify concentrations < 10 sporeballs per g soil. In contrast, ELISA can only detect and reliably quantify very high levels of sporeballs in soil (> 2000 sporeballs per g) (Walsh *et al*., 1996). Due to the natural contamination of the soil used in the experiments described here, the exact detection limit of sporeballs in soil using the real-time PCR assay is difficult to establish, but is unlikely to differ much from the detection limit for conventional PCR.

TABLE 4.1 DETECTION AND QUANTIFICATION OF DNA OF *SPONGOSPORA SUBTERRANEA* AFTER DIRECT EXTRACTION FROM SPOREBALLS IN WATER AND SOIL (N = 5). THE DNA WAS QUANTIFIED BY REAL-TIME PCR ASSAY.

Amount of inoculum added (sporeballs per ml liquid or g soil)	Direct extraction from sporeballs in water		Direct extraction from sporeballs in clay soil	
	Percentage positive samples	Average amount of DNA detected (units* per ml) ± S.E.	Percentage positive samples	Average amount of DNA detected (units per g) ± S.E.
0	0	0 ± 0	80	0.5 ± 0.2
0.1	20	0.03 ± 0.03	80	0.8 ± 0.3
0.3	40	0.2 ± 0.1	40	0.8 ± 0.5
0.5	40	0.1 ± 0.1	80	1.4 ± 0.6
1	60	1.0 ± 0.7	60	2.2 ± 1.8
3	100	3.4 ± 1.5	80	2.3 ± 0.7
5	80	4.6 ± 1.4	60	1.3 ± 0.8
10	100	14.6 ± 5.6	100	3.8 ± 1.7
50	100	41.2 ± 15.8	100	37.0 ± 11.3

* One unit equals the amount of DNA extracted from a single spore ball of average size.

Real-time PCR assay - main conclusions:

- **The primer/probe designed to detect *S. subterranea* DNA is effective. Sensitive, selective quantification of *S. subterranea* DNA by real-time PCR assay is now possible.**
- **The real-time PCR assay can be used in experiments to detect *S. subterranea* DNA from sporeballs in water and soil.**
- **Quantification of *S. subterranea* DNA in soil samples can be inaccurate if soil chemicals inhibit the PCR reaction.**

Tomato bait plant technique

Tomato bait plants are widely used to detect the presence of *S. subterranea* in soil samples. The bait plant roots are usually assessed for infection visually using a microscope, but a combination of the bait plant technique with PCR would be more reliable. Conventional and real-time PCR assays were combined with the tomato bait plant technique to investigate the release of zoospores from sporeballs in time and the limits of sporeball detection by bait plants respectively.

Zoospore release in time

Tomato plants cv. Moneymaker were used as bait plants in trays with nutrient solution (Merz, 1989). Each tray contained 300 ml nutrient solution and four bait plants. Sandy loam soil was inoculated with sporeballs in concentrations of 0, 0.1, 0.5, 1.5 or 3 sporeballs per g, and samples of the soil (40 g per tray) were added to the nutrient solution with ten replicates (trays) per treatment. Over a four-week period, one plant was removed from each tray and a sample of nutrient solution taken every seven days. The tomato root samples were cut into small pieces and ground in 600 µl of Reagent 1 of a Nucleon® PhytoPure plant DNA extraction kit. The DNA extraction kit protocol was then followed.

For DNA extraction from zoospores, the nutrient solution samples were filtered using a sterile syringe and 5 µm syringe filter and frozen overnight to kill the zoospores. After defrosting, samples were centrifuged at 13400 *g* for 10 min, the supernatant was removed and the remaining pellet subjected to the Nucleon® PhytoPure DNA extraction kit protocol, using only one third of the amounts of the chemicals prescribed for 0.1 g plant tissue samples.

Conventional PCR amplification of the DNA extracted from the bait plant roots and from the zoospores was carried out using *S. subterranea* specific primers as described by Bell *et al.* (1999). PCR products were visualised using 2% (v/v) agrose gels. The number of replicates with a visibly positive reaction was counted for each treatment, and the results for each week tested using chi-square or Fisher's Exact tests.

S. subterranea was first detected one week after addition of the soil to the nutrient solution (Figure 5.1). However, this was in the control treatment to which no sporeballs had been added, suggesting that the soil was naturally contaminated with the pathogen. After two weeks, zoospores were detected in the nutrient solution (Figure 5.2), and the infection of bait plant roots rose to high levels in most of the treatments (Figure 5.1). Figure 5.2 shows that the course of zoospore release in time was identical for all treatments, and no significant differences in the percentage of positive samples were found between the different inoculum levels ($\alpha = 0.05$). A clear peak in zoospore presence, reaching 100% detection, was obvious in the third week, after which numbers rapidly declined again (Figure 5.2).

The course of bait plant infection in time was also very similar for the different treatments, and only after four weeks were there significantly more infected plants at the two highest inoculum levels than at the three lower levels ($\alpha = 0.05$). The non-inoculated soil did not significantly differ from the ones inoculated with 0.1 or 0.5 sporeballs per g soil at any time ($\alpha = 0.05$).

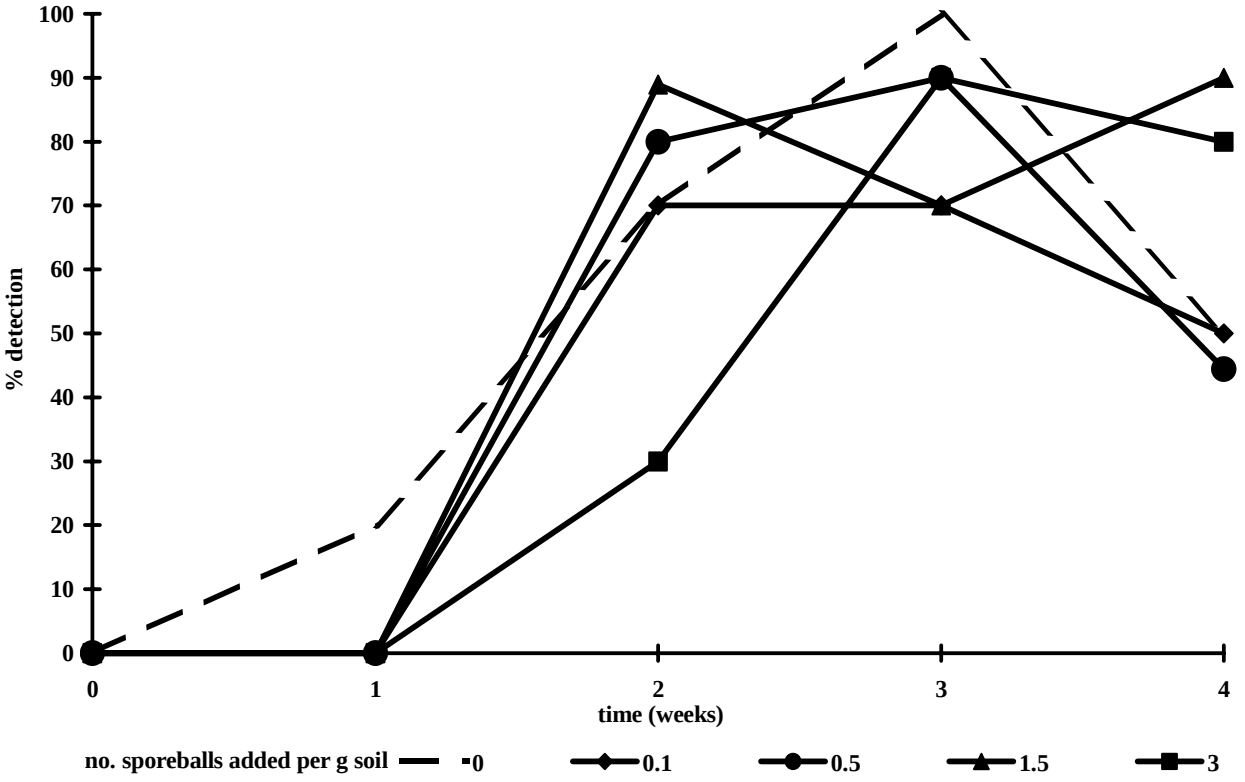


FIGURE 5.1 PERCENTAGE TOMATO BAIT PLANT ROOT SAMPLES IN WHICH *SPONGOSPORA SUBTERRANEA* WAS DETECTED USING PCR, AT DIFFERENT POINTS IN TIME AFTER INOCULATION WITH SOIL WITH DIFFERENT AMOUNTS OF SPOREBALLS.

The results of this experiment indicate that zoospore release and root infection may take place within one week after *S. subterranea* sporeballs are added to the system, which agrees with observations on bait plant infection by Fornier (1997) and is close to the nine days reported for potato root infection (Würzer, 1964). The peak in zoospores numbers after three weeks could be explained by the release of secondary zoospores from zoosporangia in the bait plant roots. In most cases, root infection detection levels were higher than zoospore detection levels, probably due to the mobility of the zoospores and the method of sampling. However, DNA extraction from both sample types was successful, and this method of detection is more reliable than visual examination of stained bait plant roots. Visual examination of root infection can lead to confusion between zoosporangia of *S. subterranea* and infection by other soil organisms. This is avoided by using PCR with specific primers. Few significant differences were found between the different inoculum levels, but this may have been due to the amount of sporeballs naturally present in the soil.

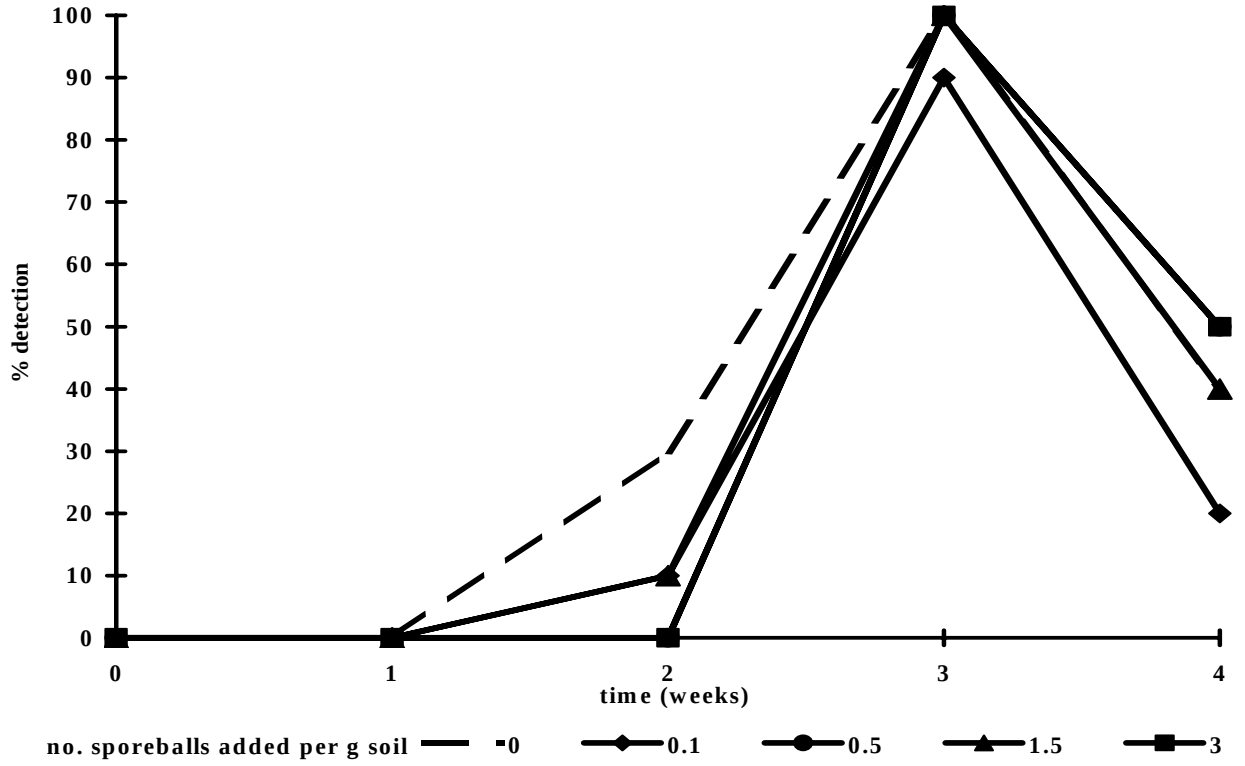


FIGURE 5.2 PERCENTAGE NUTRIENT SOLUTION SAMPLES IN WHICH *SPONGOSPORA SUBTERRANEA* WAS DETECTED USING PCR, AT DIFFERENT POINTS IN TIME AFTER INOCULATION WITH SOIL WITH DIFFERENT AMOUNTS OF SPOREBALLS (N= 10).

Sporeball detection limits

The effectiveness of the bait plant method for the detection of different numbers of sporeballs was tested by combining this method with the real-time PCR assay described earlier. Individual tomato seedlings were planted in 60 ml plastic pots wrapped in aluminium foil, which contained 50 ml of nutrient solution. The tomato seedlings were placed through small holes in the aluminium foil with their root system suspended in the nutrient solution and then incubated in a growth room with a 16 h light regime at a constant temperature of 15°C. Different numbers of sporeballs (0, 5, 15, 25, 50, 150, 250, 500 or 2500 sporeballs per 50 ml) were added to the nutrient solution. One bait plant was used per replicate, with five replicates per treatment. Following two weeks of baiting, the tomato roots were dried, weighed and used for DNA extraction as described above. The nutrient solution remaining in each pot was filtered using a sterile syringe and a 5 µm syringe filter and frozen to kill the zoospores. After defrosting, samples were centrifuged at 13400 *g* for 10 min, the supernatant was removed and DNA from the zoospores was extracted as described above.

Plasmodia and zoosporangia of *S. subterranea* formed in the roots of tomato plants during the bait test were readily detected by the real-time PCR assay (Table 5.1), but PCR amplification of DNA extracted from bait plants inoculated with low inoculum levels was not consistent. The

majority of DNA samples taken from bait plants inoculated with less than 0.3 sporeballs per ml (15 sporeballs per plant) did not produce amplification products. However, *S. subterranea* was detected in almost all plant samples inoculated with > 1 sporeball per ml nutrient solution.

The average quantity (units) of *S. subterranea* DNA detected by the real-time PCR assay in inoculated plants was always higher than the quantity present in the initial inoculum, showing that the pathogen had multiplied within the host tissues. For example, to create an inoculum concentration of 5 sporeballs per ml of nutrient solution, 250 sporeballs were initially added to each plant as inoculum. The average dry weight of the plant roots was 10 mg, and thus the average quantity of DNA detected per bait plant at this inoculum level (5 sporeballs per ml) was 18556 units, almost 75 times the 250 units originally added.

The real-time PCR assay was also able to detect zoospores of *S. subterranea* in the bait plant nutrient solution at all levels of added inoculum. However, the amounts of DNA detected were very low and the results were less consistent than for the bait plant roots (Table 5.1). In the bait plant test, the quantity of *S. subterranea* DNA detected as plasmodia and zoosporangia in the plant roots and as zoospores in the nutrient solution was greater in samples treated with inoculum concentrations of 5 or 10 sporeballs per ml than at a concentration of 50 sporeballs per ml. However, due to the large variation within the treatments, this effect was not statistically significant.

TABLE 5.1 DETECTION AND QUANTIFICATION OF DNA OF *SPONGOSPORA SUBTERRANEA* AFTER EXTRACTION FROM PLASMODIA AND ZOOSPORANGIA IN TOMATO ROOTS AND ZOOSPORES IN NUTRIENT SOLUTION AFTER A BAIT PLANT TEST WITH DIFFERENT LEVELS OF ADDED INOCULUM (N = 5). THE DNA WAS QUANTIFIED BY REAL-TIME PCR ASSAY.

Amount of inoculum added (sporeballs per ml nutrient solution)	Extraction from plasmodia and zoosporangia in bait plant roots		Extraction from zoospores in bait plant nutrient solution	
	Percentage positive samples	Average amount of DNA detected (units* per mg dry weight) ± S.E.	Percentage positive samples	Average amount of DNA detected (10^{-3} units per ml) ± S.E.
0	0	0 ± 0	0	0 ± 0
0.1	40	1.3 ± 0.8	20	0.6 ± 0.6
0.3	60	1.5 ± 1.2	40	1.9 ± 1.2
0.5	80	4.8 ± 1.5	60	2.1 ± 0.9
1	60	8.1 ± 5.1	20	1.4 ± 1.4
3	80	88.5 ± 31.3	60	7.7 ± 4.5
5	100	1855.6 ± 607.7	100	118.8 ± 105.9
10	100	1683.2 ± 671.8	100	114.0 ± 51.7
50	100	1564.4 ± 822.8	60	30.3 ± 24.9

* One unit equals the amount of DNA extracted from a single sporeball of average size.

Bait plants are generally thought to provide more sensitive detection than methods that involve direct DNA extraction from the soil. Detection of *S. subterranea* at concentrations as low as 0.1 sporeballs (cystosori) per ml nutrient solution has been reported. However, detection and quantification has always relied on examination of stained bait plant roots by microscope, a laborious and unreliable process. With this technique, stages before the formation of zoosporangia are usually not detected and no distinction can be made between zoosporangia of *S. subterranea* and those formed by other root pathogens. By combining the bait plant technique with the real-time PCR assay, initial inoculum concentrations $\geq$ 0.5 sporeballs per ml were reliably detected and quantified, and regularly even lower concentrations were detected.

The average amount of DNA detected in the bait plant root samples by the real-time PCR assay was always more than the initial amount added due to multiplication of the pathogen in the host. At 5 sporeballs per ml, the bait plant roots appeared to have been fully colonised by *S. subterranea* since no increase in amount of pathogen DNA was detected at higher inoculum levels. Thus it appears that *S. subterranea* can only be quantified reliably by the bait plant method if inoculum levels in the sample are very low.

Zoospores were successfully detected using the real-time PCR assay at all levels of added inoculum, but the results were not as consistent as those for the bait plant roots. This could be due to the fragile nature of the zoospores or to the fact that the majority of zoospores were already attached to the host roots at the time of sampling. As described in the first experiment, at 15°C most secondary zoospores are not released until three weeks after inoculation, but some of the DNA detected in the real-time PCR assay could have come from secondary zoospores released from early matured zoosporangia in the bait plant roots.

Tomato bait plant technique - main conclusions:

- **Combination of the tomato bait plant test with a PCR assay is a reliable method for the detection of *S. subterranea*.**

- **Conventional and real-time PCR assays can successfully detect *S. subterranea* DNA from zoosporangia/plasmodia in roots and zoospores in water.**

- **Quantification of *S. subterranea* using the bait plant method is not reliable at high inoculum levels.**

Inoculum in soil and on tuber

Overall objective: To determine the relationship between inoculum and disease development

Viability of sporeballs in soil

Experiments under controlled conditions

Objective: To determine the relationship between (soil-borne) inoculum and disease development by determining the viability of cystosori in soil and how it is affected over time by environmental factors. This involved testing a range of soils from sites throughout Britain using controlled experiments in the glasshouse (SCRI).

Milestone 1: Establish glasshouse system for testing viability and productivity and measuring inoculum levels in soil – *achieved*

Milestone 2: Preliminary estimate on inoculum viability in soil (provided it declines enough to be measurable within 30 months) – *achieved for clay soil*

Milestone 4: Preliminary assessments for field work – *achieved*

The aim of this experiment was to study the effect of different controlled environmental conditions on the survival of sporeballs of *S. subterranea* in soil. The following experimental factors were tested:

soil type
soil moisture level
temperature

Three types of soil (silty clay, sandy loam and loamy sand) were collected by SAC in different areas in Scotland. Large quantities of soil were air dried and sieved. Since moisture is an important experimental factor, the soil moisture characteristic curves were determined for each of the three soils using water column and pressure plate techniques. The resulting curves are given in Figure 6.1.

On basis of the moisture curves, water was added to the soils to bring them to three different moisture levels (-15, -1 and 0 bar). The soils were spiked with 50 *S. subterranea* sporeballs per g, while controls without artificial inoculation were also included in the experimental set-up to account for natural contamination with the pathogen. The soils were distributed into 60 ml plastic pots and incubated at 4°C or 20°C.

Samples of the different soils were taken at the start of the experiment in July 2000 (t = 0) and DNA was extracted using the method of Bell *et al.* (1999). DNA of *S. subterranea* was quantified using the real-time PCR assay described earlier.

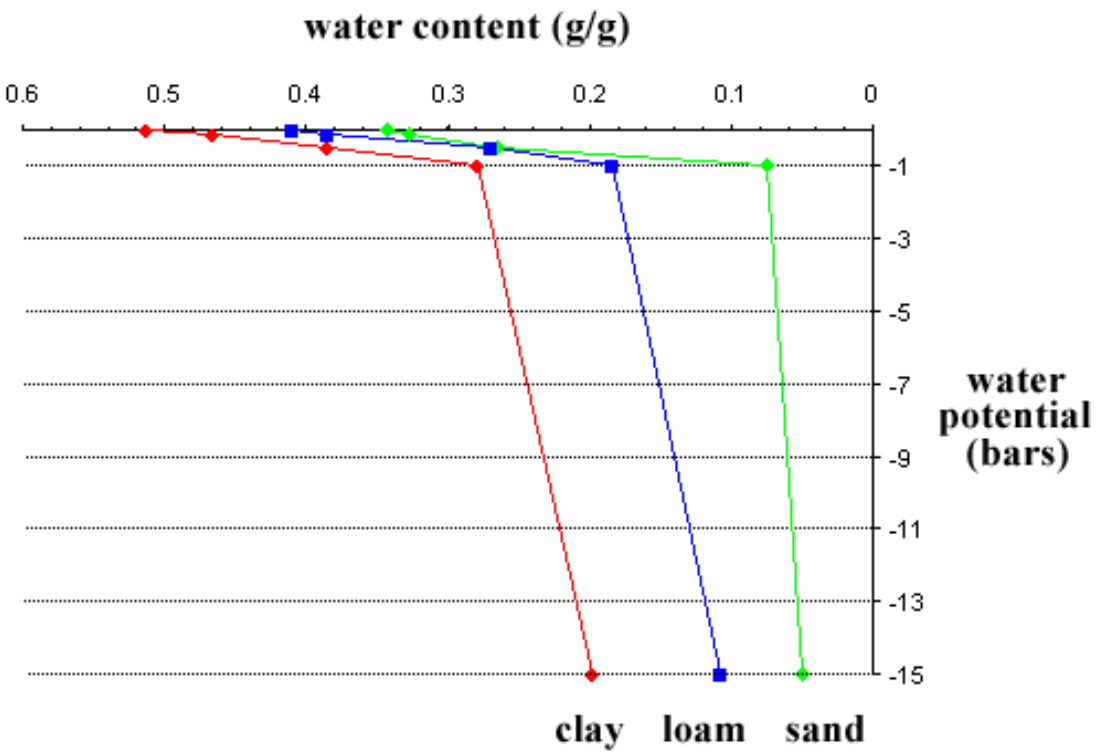


FIGURE 6.1 SOIL MOISTURE CHARACTERISTIC CURVES FOR THREE DIFFERENT TYPES OF SOIL USED FOR EXPERIMENTS AT SCRI.

Table 6.1 gives the percentage of positive soil samples. As could be expected, most control samples were negative and did not contain *S. subterranea* DNA at detectable levels. However, many of the inoculated samples were also negative, especially the loamy sand and sandy loam samples. Only in the silty clay could the presence of *S. subterranea* be detected. Given the number of sporeballs added to the soils before incubation (50 per g soil) and the method used for extraction of DNA, spiked soil samples should all test positive, since the amount of DNA in them should be equivalent to at least 16 sporeballs. Although many samples tested negative, those that did test positive at t = 0 yielded on average the amounts of *S. subterranea* DNA expected (Table 6.1).

TABLE 6.1 DETECTION AND QUANTIFICATION OF *SPONGOSPORA SUBTERRANEA* IN INOCULATED AND NON-INOCULATED SOIL SAMPLES AFTER DIRECT DNA EXTRACTION AT T=0 USING A REAL-TIME PCR ASSAY (N = 8).

soil type	soil moisture level	non-inoculated		inoculated	
		percentage positive samples	amount of DNA detected (units per g soil)*	percentage positive samples	amount of DNA detected (units per g soil)*
loamy sand	Dry	0	-	0	-
	Damp	0	-	0	-
	Wet	0	-	0	-
sandy loam	Dry	0	-	0	-
	Damp	0	-	0	-
	Wet	0	-	0	-
silty clay	Dry	0	-	50	28.9
	Damp	13	15.9	0	-
	Wet	0	-	50	17.8

* positive samples only.

During the incubation period, the containers were weighed regularly and water added where necessary to keep the moisture at the required levels. After 25 months of incubation under different conditions, further samples of the clay soil were tested in August 2002. Table 6.2 shows that at 4°C, the number of positive samples was slightly lower than at t=0 for most moisture levels. However, the average amount of *S. subterranea* DNA detected was similar. These results suggest (not significant) that the viability of sporeballs decreases quicker at higher temperatures, which could be explained by a higher germination rate at 20°C compared with 4°C. There was no clear effect of moisture regime on viability.

TABLE 6.2 DETECTION AND QUANTIFICATION OF *SPONGOSPORA SUBTERRANEA* IN INOCULATED AND NON-INOCULATED CLAY SOIL SAMPLES USING A REAL-TIME PCR ASSAY AFTER INCUBATION AT DIFFERENT TEMPERATURES FOR 25 MONTHS (N=8).

soil type	Soil moist-ure Level	4°C				20°C			
		Non-inoculated		inoculated		non-inoculated		inoculated	
		% positive samples	amount of DNA detected (units per g soil)*	% positive samples	amount of DNA detected (units per g soil)*	% positive samples	amount of DNA detected (units per g soil)*	% positive samples	amount of DNA detected (units per g soil)*
silty clay	Dry	13	11.7	38	17.8	0	-	0	-
	Damp	0	-	13	26.7	0	-	0	-
	Wet	0	-	38	36.9	0	-	13	59.7

* positive samples only

The large number of negative samples, the low amounts of DNA detected in the positive samples from soil, and the differences between the three soil types in detection rate at t=0 could be explained by the presence of soil chemicals such as humic acids from organic matter, which may inhibit PCR reactions. The three soil types used were analysed by SAC, but no clear link between the real-time PCR assay results and soil organic matter content was found. The silty clay soil had the highest organic matter content and the loamy sand contained the least. Another inhibitory factor could be the presence of heavy metals. Magnesium, copper and zinc levels did not differ greatly between the soil types while levels of other metals were not determined.

To determine whether the soil extracts inhibited the PCR reaction and thus the detection of *S. subterranea*, samples were tested that contained 1.0 µl of standard DNA (equivalent to 1, 10, 100, 1000 or 10000 sporeballs) and 1.0 µl of soil extract (instead of 1.0 µl water) in a real-time PCR assay. Five samples per soil type were used. One negative control (extraction buffer only) was also included. The amount of DNA detected in these samples was then compared to the standard dilution series without soil extract. In Table 6.3, the results of this test are summarised and it shows that all soils inhibited the PCR to a high level. In samples from sandy loam, inhibition was very high at 95% on average, while in silty clay detection was the least inhibited at an average inhibition rate of 71%. Only one sample (from silty clay) showed values which were close to the standard. Surprisingly, the negative control sample also inhibited amplification at 79%.

These findings correspond with the differences found in number of positive samples between the soil types. Silty clay had a higher percentage of positive samples than the two lighter soils. It is difficult to determine which chemical in the extracts from the loamy soils might be responsible for the inhibitory effect observed, but the results mean that the method of DNA extraction will have to be adapted so that more of the soil chemicals are removed from the DNA samples.

TABLE 6.3 EFFECT OF EXTRACTS FROM DIFFERENT SOILS ON AMPLIFICATION OF *SPONGOSPORA SUBTERRANEA* DNA USING A REAL-TIME PCR ASSAY (N=5).

Standard Number of DNA added (sporeballs)	Loamy sand		Sandy loam		Silty clay		Extraction buffer only	
	Average amount Detected	% detect-ion	average amount detected	% detect-ion	average amount detected	% detect-ion	amount detected	% detect-ion
1	0.46	46	0	0	0.53	53	0	0
10	0.75	8	0.13	1	0.99	10	0.74	7
100	12	12	8.7	9	28	28	15	15
1000	160	16	74	7	242	24	335	34
10000	1741	17	928	9	2931	29	4686	47
Average		20		5		29		21

It was investigated whether detection could be improved by using a MoBio® UltraClean Soil DNA Isolation Kit, which includes a special solution for removing humic acid PCR inhibitors. The kit was tested using different amounts of a naturally infested loam soil (0.25, 0.5, 0.75 or 1.0 g). Also included were samples to which 500 *S. subterranea* sporeballs were added. The DNA extracts were then used in a real-time PCR assay. However, the results were disappointing and only with samples of 0.25 g soil did some detection of *S. subterranea* occur. Spiked soil did not yield better results than unspiked soil and only the positive controls (500 sporeballs without soil) were detected consistently. The test was repeated with 0.25 g samples of the three soil types mentioned above in which the concentration of sporeballs was 0, 1, 5, 15 or 50 sporeballs per g soil. Again only the positive control without soil gave consistent positive results in a real-time PCR assay. Thus, it appears that the inhibiting effect of soil chemicals on PCR is not removed by the use of a MoBio UltraClean Soil DNA Isolation Kit. The fact that only small samples of soil could be used with this kit was also a disadvantage. This meant that other solutions for the problem of PCR inhibition in samples of DNA from soil needed to be found.

Viability of sporeballs in soil (controlled conditions) - main conclusions:

- **The amount of *S. subterranea* DNA detected in soil samples is often lower than expected. This is probably due to inhibition of PCR by co-extracted soil chemicals.**

- **Soil type has an effect on the detection of *S. subterranea* in soil. Substances in loamy and sandy soils in particular strongly inhibit the PCR reaction.**

- **The viability of sporeballs of *S. subterranea* in clay soil decreases in time, but the speed of the decline depends on temperature.**

Field experiments

Objective: To determine the relationship between (seed-borne) inoculum and disease development. This involved planting a uniformly infected stock at different experimental sites throughout Britain (SAC).

Milestone 3: Establish field experiments to tests of standard stocks – *achieved*

Milestone 4: Preliminary assessments for field work – *achieved*

Experiments were combined with those on the effect of disease threshold and environmental factors on infection of the growing crop, see section B ii of this chapter.

Disease threshold and environmental factors

Experiments under controlled conditions

Objective: To determine the relationship between (soil-borne) inoculum and disease development by assessing the effect of environmental factors on infection of the growing crop by Spongospora, and the final disease incidence and severity. This was done by conducting pot experiments under controlled environment conditions. A dilution series of *Spongospora* inoculum was introduced into soil under a range of environmental conditions, and the resulting levels of infection/disease on a susceptible cultivar assessed. The optimal environmental conditions for inoculum multiplication, host infection and the development of disease on different host structures was measured visually, and using PCR assay by regular sampling and assessment at tuber initiation and maturity (SCRI).

Milestone 1: Establish glasshouse system for testing viability and productivity and measuring inoculum levels in soil – *achieved*

Milestone 2: Preliminary estimate on inoculum viability in soil – *achieved*

Milestone 4: Preliminary assessments for field work - *achieved*

The aim of this experiment was to investigate the effect of soil inoculum level and environmental factors on the infection of potato by *S. subterranea* and the development of disease symptoms. The following experimental factors were tested:

- *S. subterranea* inoculum level in soil
- temperature
- soil moisture regime
- soil type
- plant growth stage

Three types of soil (loamy sand, sandy loam and silty clay) were air dried, sieved and mixed with four different levels of *S. subterranea* sporeball inoculum (0, 5, 15 or 50 sporeballs per

gram soil). Plastic pots (3 l) were filled with the soils and were kept under two different moisture regimes, constant (damp, -1 bar) or fluctuating (flooded to saturation then dried for ten days, then flooded again etc.). Tubers of potato cv. 'Estima' were planted in the pots, which were incubated in a growth room at 12ºC. Pots with sandy loam soil were also incubated at 9 and 17ºC. At 17ºC, the first plants were lifted at the tuber initiation stage two months after planting. At 12ºC, this occurred three months after planting, and at 9°C, four months after planting. Mature plants were lifted when foliage had died.

A large number of tubers kept in soil at fluctuating moisture did not germinate, especially in loamy sand and silty clay, but in the other treatments the majority of tubers produced plants. Non-germination was usually due to *Fusarium* dry rot suggesting that the *S. subterranea* inoculum might have been contaminated with this pathogen.

After harvest, all tubers were washed and visually inspected for disease symptoms and scored on basis of percentage tuber area covered with powdery scab. The scale used was published by Merz (2000) in the Proceedings of the First European Powdery Scab Workshop (p.70) and runs from 1 (no symptoms) to 7 (>75% of tuber surface covered in powdery scab). Only mature powdery scab symptoms were taken into consideration. All tubers were peeled and the peelings frozen for DNA extraction and quantification by real-time PCR assay.

All roots were also washed and visually inspected for the presence of root galls. They were scored on the basis of the number and size of the galls for which the following scale was used:

0 no galls
1 one or two galls
2 several galls, mostly small (< 2 mm in diameter)
3 many galls, some > 2 mm in diameter
4 most major roots with galls, some or all > 4 mm in diameter

The roots were frozen for DNA extraction and quantification by real-time PCR assay.

DNA was extracted from the tuber peelings and roots using a Nucleon PhytoPure plant DNA extraction kit. The dry weight of samples was noted and the sample size was reduced if the weight was more than 0.1 g. Real-time PCR assays were performed as described above. Scores were adjusted in relation to the original weight of the sample used for extraction.
None of the data were normally distributed, so non-parametric tests had to be used for statistical analysis. Data were tested using Mann-Whitney U-tests or Chi-square tests with $\alpha = 0.05$.

Effect of soil inoculum level

Interestingly, no clear relationship was found between the number of sporeballs added to the soil and the level of either powdery scab symptoms or root galling. As Figure 6.2 shows, high levels of tuber and root infection and disease symptoms were found in soils with all three levels of added inoculum, and despite the large differences in the number of sporeballs added, few significant differences were found between the treatments with 5, 15 and 50 sporeballs per g soil.

No effect of inoculum level on the percentage of plants with infected tubers was found, but the percentage of plants with infected roots did depend on soil inoculum level. Soil inoculum level also appeared to have an effect on the percentage of mature plants with latently infected tubers.

In the control, 40% of the plants that had infected tubers (about 20% of all control plants) did not show powdery scab. In soils inoculated with 5, 15 and 50 sporeballs per g, the percentage of plants with latent infections was 19, 14 and 7% respectively.

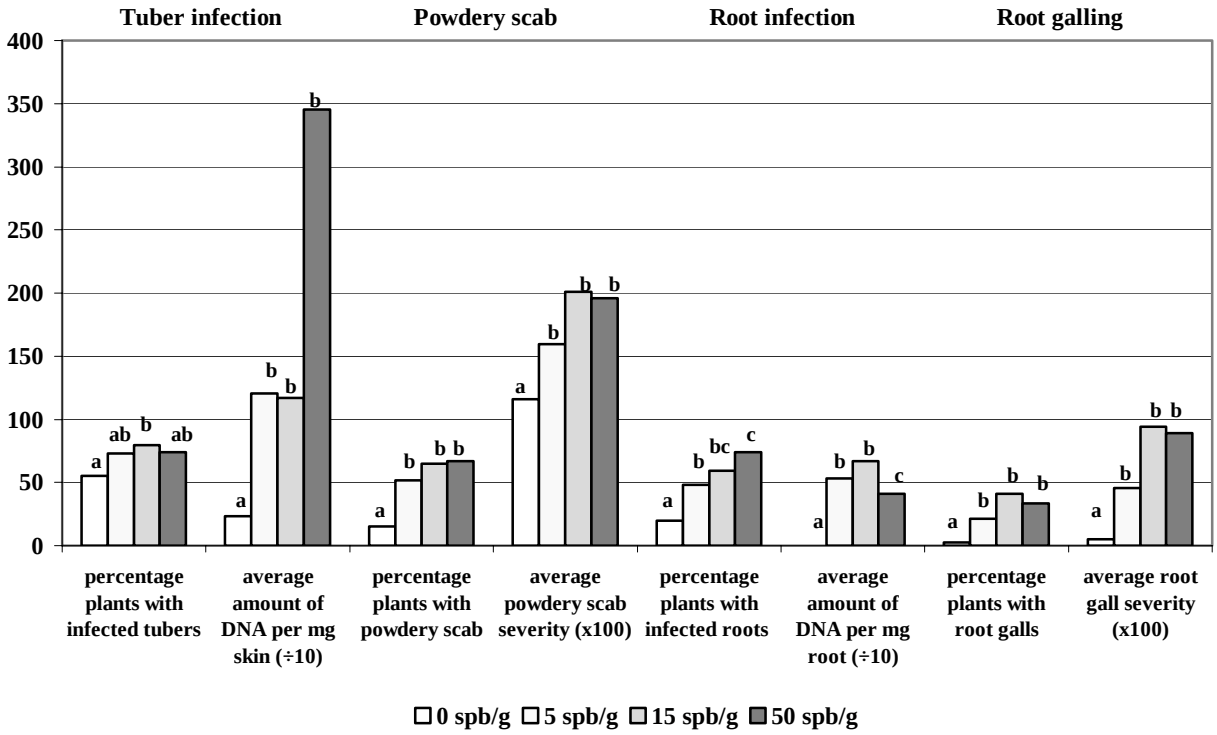


FIGURE 6.2 EFFECT OF SOIL INOCULUM LEVEL (SPOREBALLS PER GRAM SOIL) ON TUBER INFECTION, POWDERY SCAB, ROOT INFECTION AND ROOT GALLING CAUSED BY *SPONGOSPORA SUBTERRANEA* IN MATURE POTATO PLANTS CV. 'ESTIMA'. THE RESULTS FOR DIFFERENT TEMPERATURES, SOIL MOISTURE REGIMES AND SOIL TYPES ARE COMBINED. DIFFERENCES BETWEEN GROUPED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($A = 0.05$).

A slight effect of inoculum on powdery scab incidence was also observed, but none of these inoculum effects were statistically significant.

Some low levels of tuber and root symptoms were found in the non-inoculated controls. However, in most cases, the incidence and severity of powdery scab and root galling at maturity were significantly higher in the inoculated soils than in the controls (Figure 6.2).

Effect of temperature

Tuber infection rates in inoculated damp sandy loam soil were high at all three temperatures tested at an early stage, but powdery scab symptoms were mostly seen at maturity. The incidence, and particularly the severity of tuber infection were higher at lower temperatures, but this effect was not significant (Figure 6.3). Powdery scab incidence and severity at maturity were highest at 12°C. A large number of infected tubers did not develop powdery scab symptoms, especially at 9°C. Figure 6.3 shows that the effect of temperature on root galling was distinctly different from that on powdery scab. Whereas no root galls were found at 9°C and only a few at 12°C, they were common and severe at 17°C at both growth stages examined.

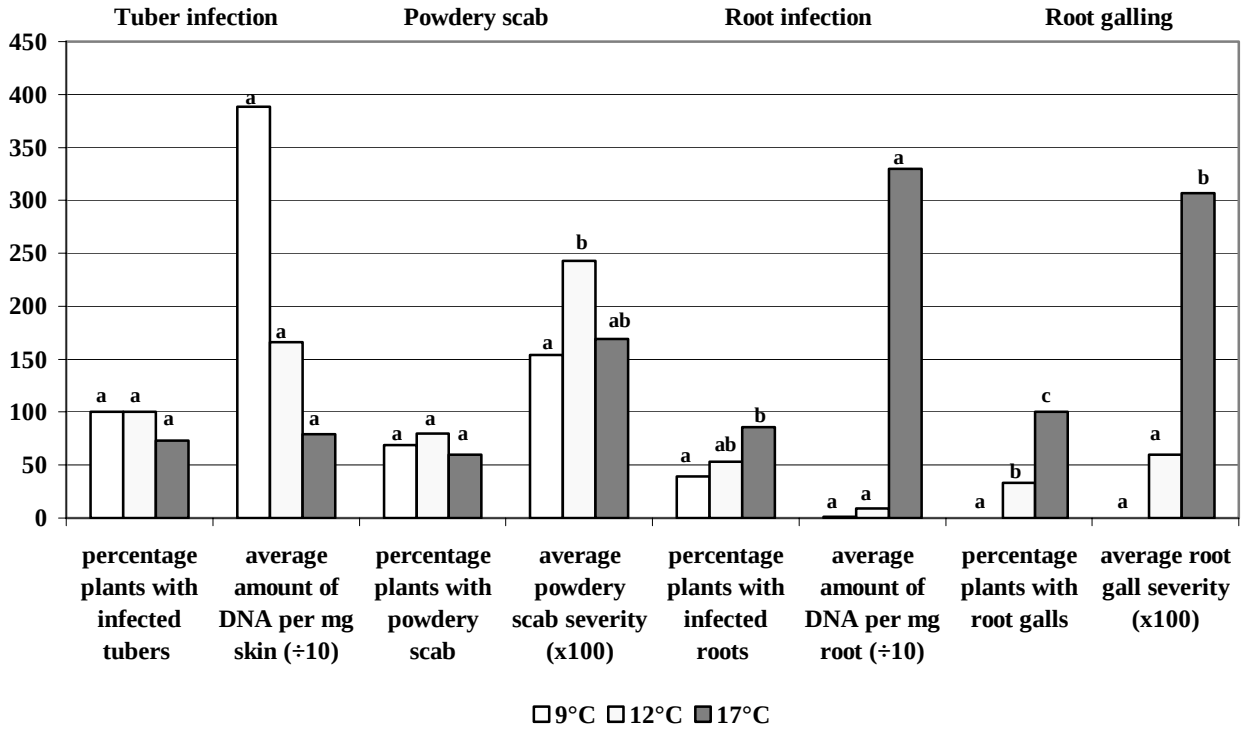


FIGURE 6.3 EFFECT OF TEMPERATURE ON TUBER INFECTION, POWDERY SCAB, ROOT INFECTION AND ROOT GALLING CAUSED BY *SPONGOSPORA SUBTERRANEA* IN MATURE POTATO PLANTS CV. 'ESTIMA' GROWN IN DAMP SANDY LOAM SOIL. THE RESULTS FOR DIFFERENT INOCULUM LEVELS ARE COMBINED. DIFFERENCES BETWEEN GROUPED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

Effect of soil moisture regime

At maturity, tuber infection and powdery scab symptoms were significantly more severe in inoculated soil kept at constant dampness than with fluctuating wetness (Figure 6.4). Root infection and galling were also more common and severe at a constant moisture level, but these differences were not significant. An interaction was found between soil moisture regime and temperature. At 9°C, almost no powdery scab symptoms were observed in soil with fluctuating moisture, and only at 12°C did powdery scab levels at fluctuating moisture reach as high as those in damp soil. No interaction between soil moisture and temperature was observed for tuber infection, root infection or root galling.

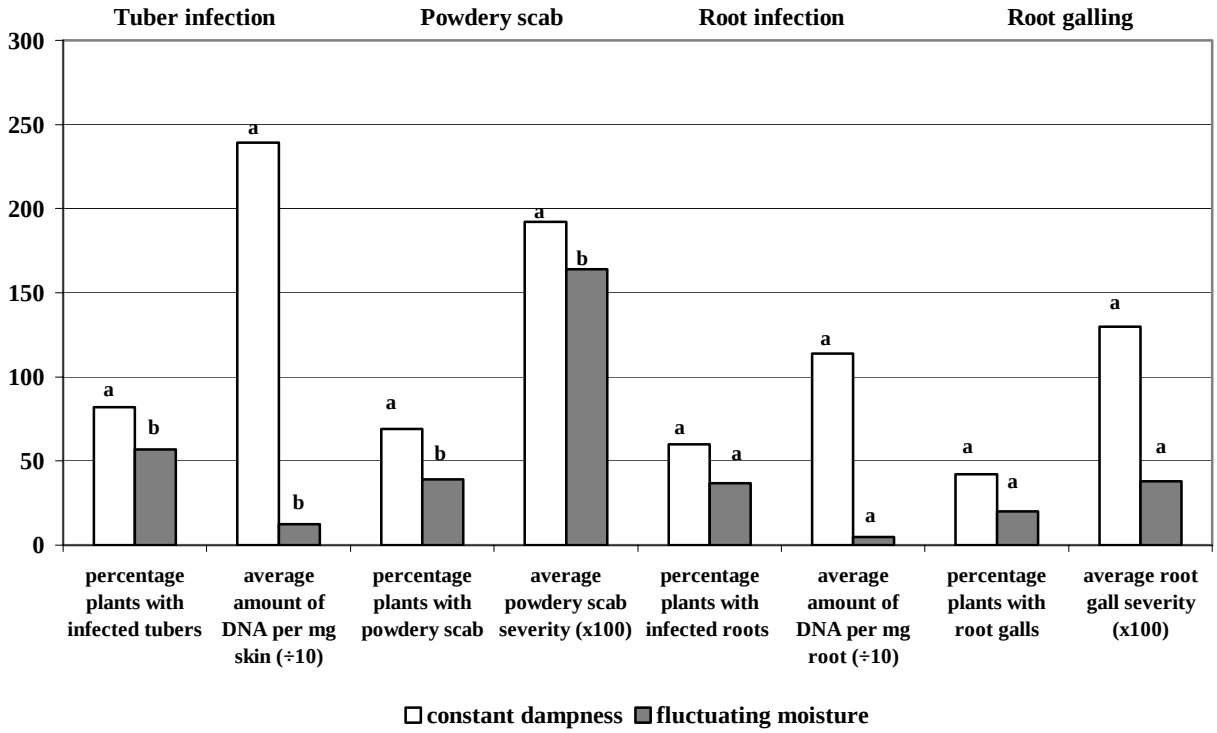


FIGURE 6.4 EFFECT OF SOIL MOISTURE REGIME ON TUBER INFECTION, POWDERY SCAB, ROOT INFECTION AND ROOT GALLING CAUSED BY *SPONGOSPORA SUBTERRANEA* IN MATURE POTATO PLANTS CV. 'ESTIMA' GROWN IN SANDY LOAM SOIL. THE RESULTS FOR DIFFERENT INOCULUM LEVELS AND TEMPERATURES ARE COMBINED. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

Effect of soil type

Figure 6.5 shows that tuber infection and powdery scab symptoms were more severe in lighter soils. At tuber initiation, powdery scab occurred only in loamy sand and sandy loam. The amount of DNA extracted from tuber skins was highest for loamy sand at both growth stages, but powdery scab at maturity was no more severe than in sandy loam. Tuber infection and powdery scab incidence and severity were relatively low in silty clay soil but the differences with the two other soil types were generally not significant. Because the effect of soil type was tested at 12°C, very few root galls were found in the two lighter soils and almost no galls were formed by plants growing in silty clay soil. Only in loamy sand soil were powdery scab symptoms observed in some of the non-inoculated treatments.

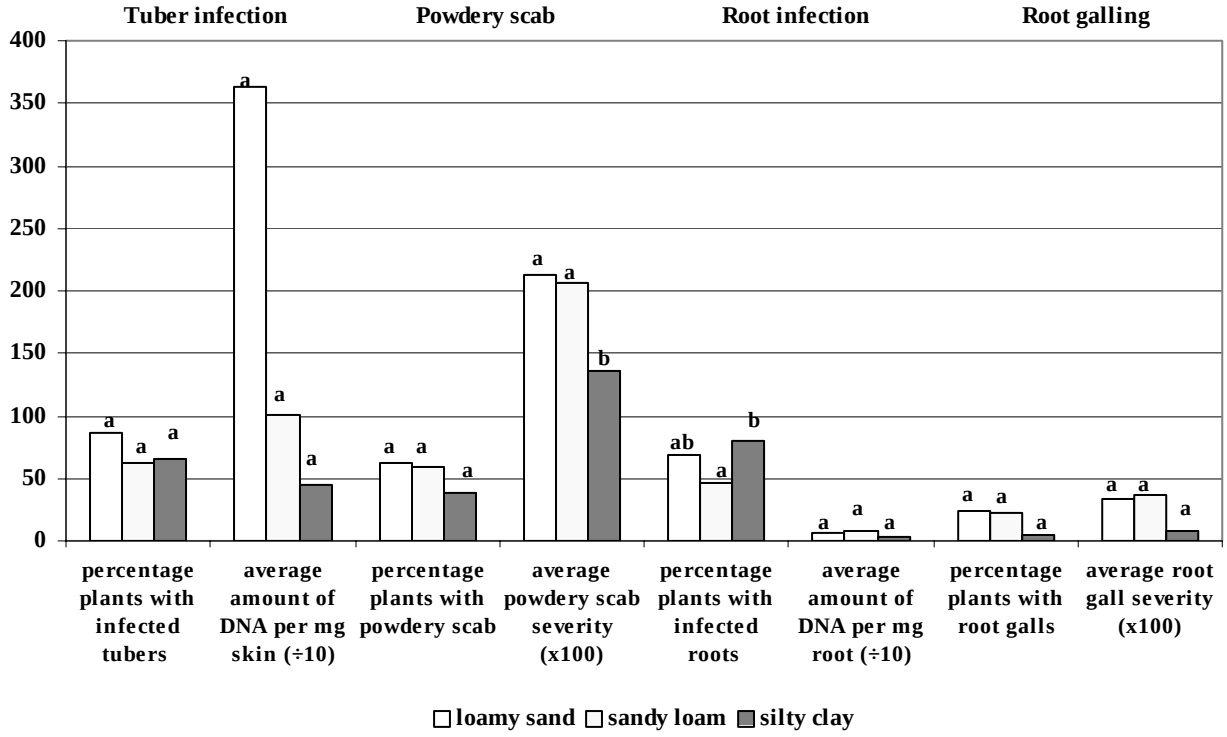


FIGURE 6.5 EFFECT OF SOIL TYPE ON TUBER INFECTION, POWDERY SCAB, ROOT INFECTION AND ROOT GALLING CAUSED BY *SPONGOSPORA SUBTERRANEA* IN MATURE POTATO PLANTS CV. 'ESTIMA' GROWN AT 12°C. THE RESULTS FOR DIFFERENT INOCULUM LEVELS AND SOIL MOISTURE REGIMES ARE COMBINED. DIFFERENCES BETWEEN GROUPED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

Effect of growth stage

The majority of the plants in inoculated soil were infected by *S. subterranea* at an early stage. DNA of the pathogen was detected in around 70% of inoculated plants at tuber initiation. This percentage did not increase during growth, although the average amount of DNA detected in both tubers and roots was higher at maturity as were the incidence and severity of powdery scab and root galling (Figure 6.6).

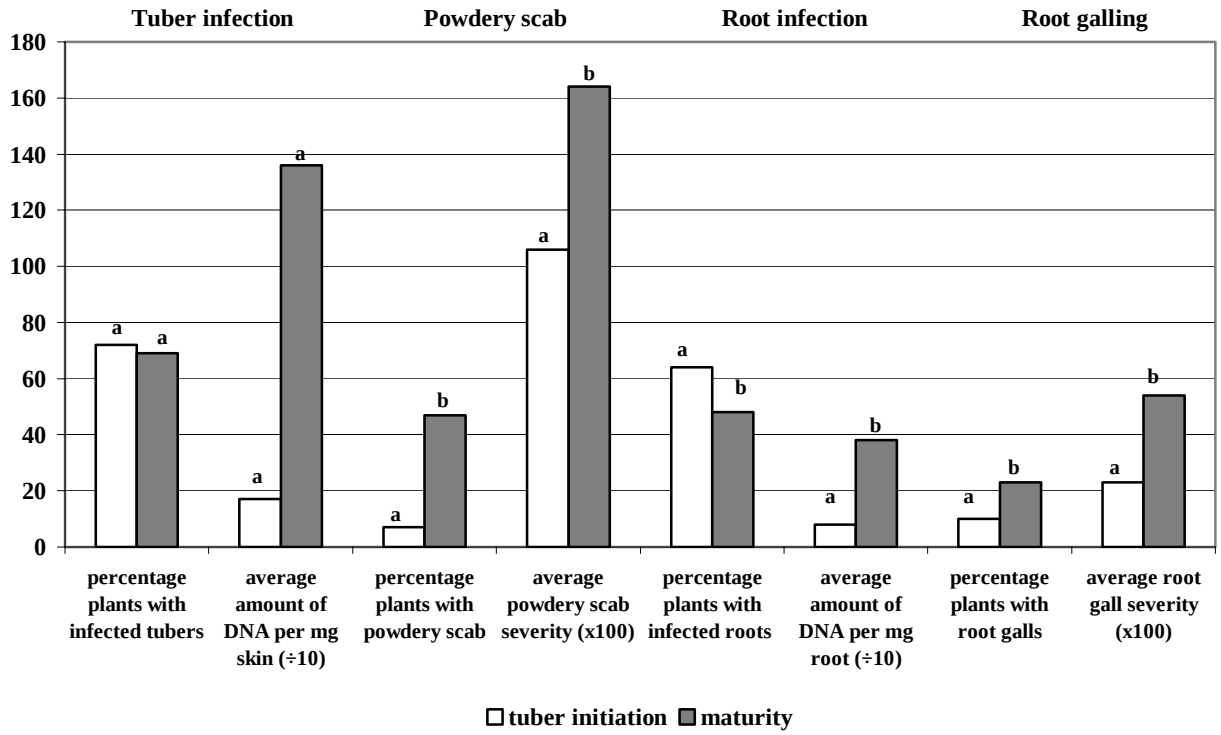


FIGURE 6.6 TUBER INFECTION, POWDERY SCAB, ROOT INFECTION AND ROOT GALLING CAUSED BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' AT TWO DIFFERENT GROWTH STAGES. THE RESULTS FOR DIFFERENT LEVELS OF ADDED INOCULUM, TEMPERATURES, SOIL TYPES AND SOIL MOISTURE REGIMES ARE COMBINED. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT (A = 0.05).

Latent infections

Latent infections of tubers by *S. subterranea* were found to be common. At tuber initiation, only 8% of plants with infected tubers showed powdery scab symptoms (Figure 6.7) and even at maturity, only 55% of plants with infected tubers showed powdery scab symptoms. On the other hand, some samples in which *S. subterranea* was not detected in the tuber skin showed obvious powdery scab symptoms (Figure 6.7 & 6.8). These false negatives could be due to the fact that some infected areas were probably missed during sampling of tuber peeling for extraction. When powdery scab symptoms were present, the average amount of *S. subterranea* DNA present in the tuber skin was significantly higher than when there were no symptoms (Figure 6.8).

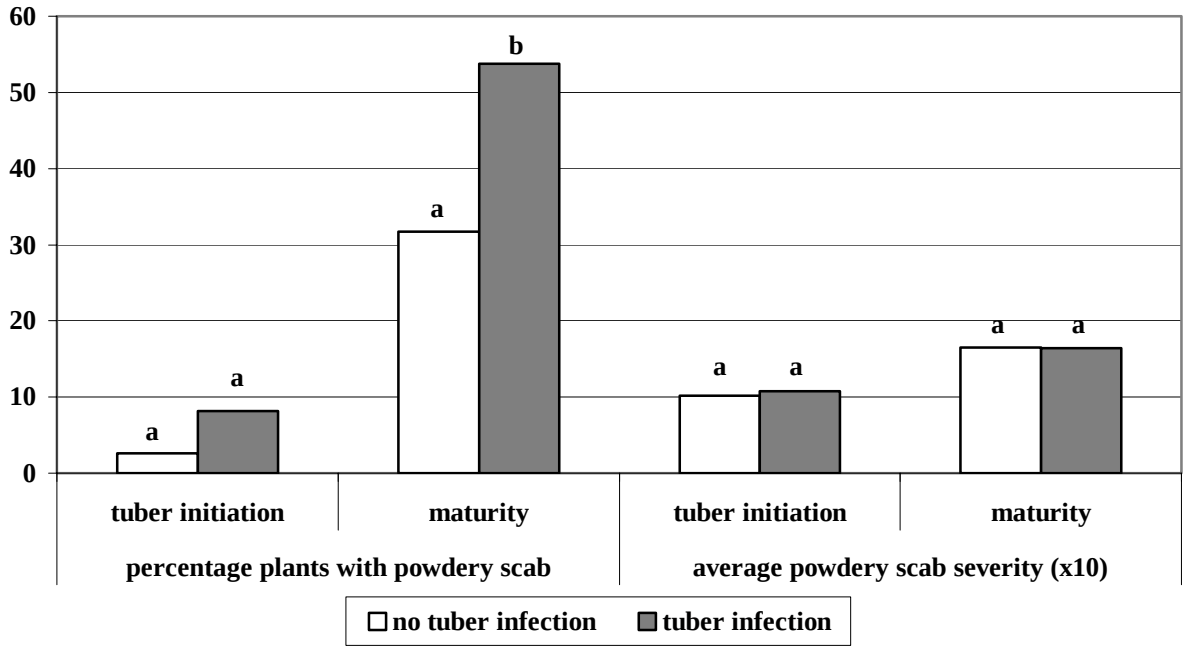


FIGURE 6.7 EFFECT OF THE OCCURRENCE OF TUBER INFECTION BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' ON THE OCCURRENCE AND SEVERITY OF POWDERY SCAB CAUSED BY THE SAME PATHOGEN AT TWO DIFFERENT GROWTH STAGES. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

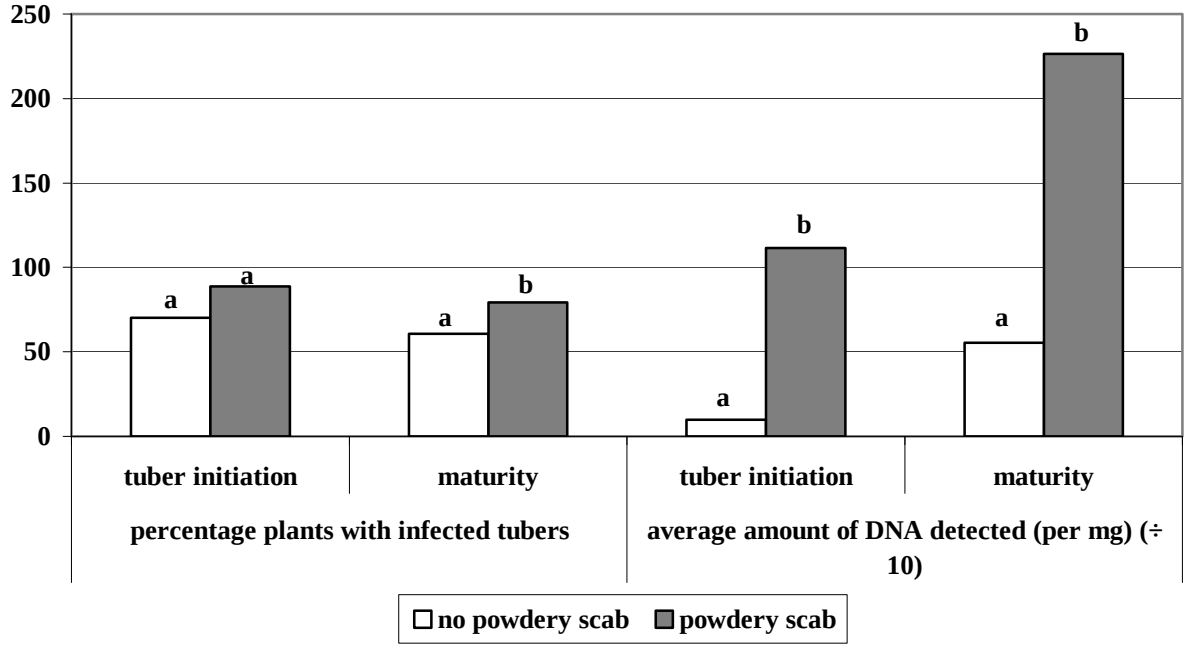


FIGURE 6.8 EFFECT OF THE OCCURRENCE OF POWDERY SCAB CAUSED BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' ON THE DETECTION OF TUBER INFECTION CAUSED BY THE SAME PATHOGEN AT TWO DIFFERENT GROWTH STAGES. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

Root galling was found to be significantly more common and severe in roots in which DNA of *S. subterranea* was detected than in roots in which no DNA of the pathogen was found (Figure 6.9). However, almost 70% of plants in which *S. subterranea* DNA was detected in the roots did not develop root galls at maturity and thus had latent root infections. This was partly due to the fact that few root galls developed at temperatures lower than 17°C. As with the tuber skin samples, there were a number of samples (10% at maturity) that tested negative for *S. subterranea* DNA in the roots even though they clearly showed root galls (Figure 6.9 & 6.10). Again, the sampling technique was probably the cause of these negative samples since only a small part of the root system could be tested, especially in mature plants. The presence of root galls greatly increased the average amount of *S. subterranea* DNA detected in the root samples (Figure 6.10).

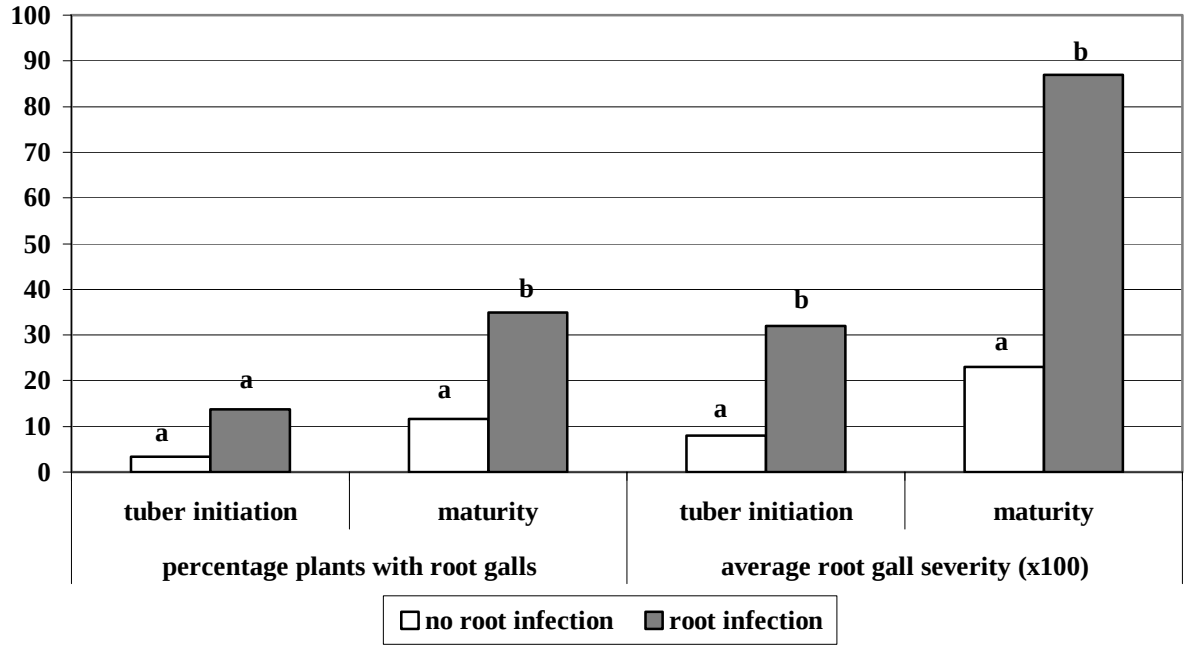


FIGURE 6.9 EFFECT OF THE OCCURRENCE OF ROOT INFECTION BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' ON THE OCCURRENCE AND SEVERITY OF ROOT GALLS CAUSED BY THE SAME PATHOGEN AT TWO DIFFERENT GROWTH STAGES. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

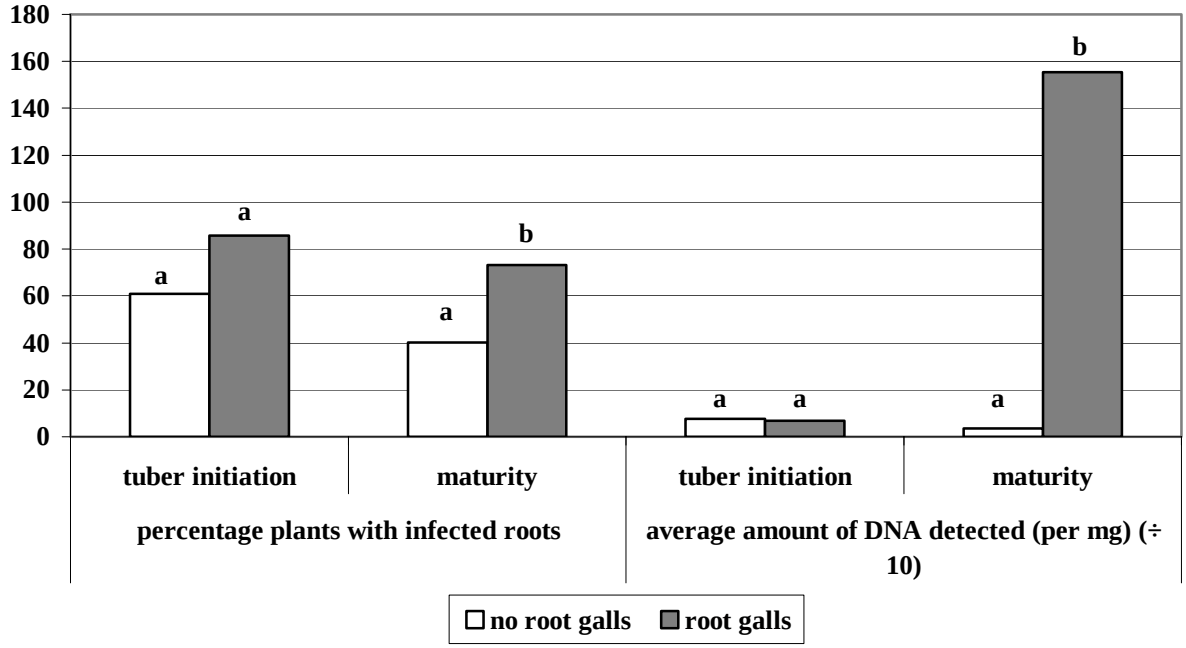


FIGURE 6.10 EFFECT OF THE OCCURRENCE OF ROOT GALLS CAUSED BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' ON THE DETECTION OF ROOT INFECTION CAUSED BY THE SAME PATHOGEN AT TWO DIFFERENT GROWTH STAGES. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

Relationship between tuber and root symptoms

Some researchers have suggested that the susceptibility of potato plants to root galling by *S. subterranea* in pot tests could be used as an indicator for their susceptibility to powdery scab based on the assumption that disease development in potato roots and tubers is related. However, in addition to differences in the effect of temperature on the development of root and tuber symptoms as discussed above, the current experiment has also shown that there is no clear relationship between the occurrence of powdery scab and root galling, at least in cultivar 'Estima'.

Figure 6.11 shows that both at tuber initiation and maturity, the majority of plants with roots infected by *S. subterranea* also had infected tubers. The percentage of infected tubers was significantly higher in plants with infected roots than in plants without root infection. The same was true for the relationship between plants with infected tubers and root galling. However, the pathogen was detected in the tubers of many plants without infected roots or root gall symptoms (Figures 6.11 & 6.12). At maturity, significantly more plants showed powdery scab symptoms when infected roots and/or root galls were present than when these were absent. Nevertheless, powdery scab was observed in almost 40% of the plants in which no root infection was detected (Figure 6.11) and in a similar percentage of plants that had not formed root galls (Figure 6.12).

At both growth stages, the majority of plants with infected tubers also had infected roots, but there were plants without tuber infection in which *S. subterranea* was detected in the roots although these were significantly less common than plants with both tuber and root infection (Figure 6.13). The presence of tuber infection had no significant effect on the development of root galls (Figure 6.13), but if powdery scab symptoms were present this did increase the incidence of both root infection and root galling in mature plants significantly (Figure 6.14). At both growth stages, the majority plants with powdery scab did not form root galls (78 and 65% respectively.).

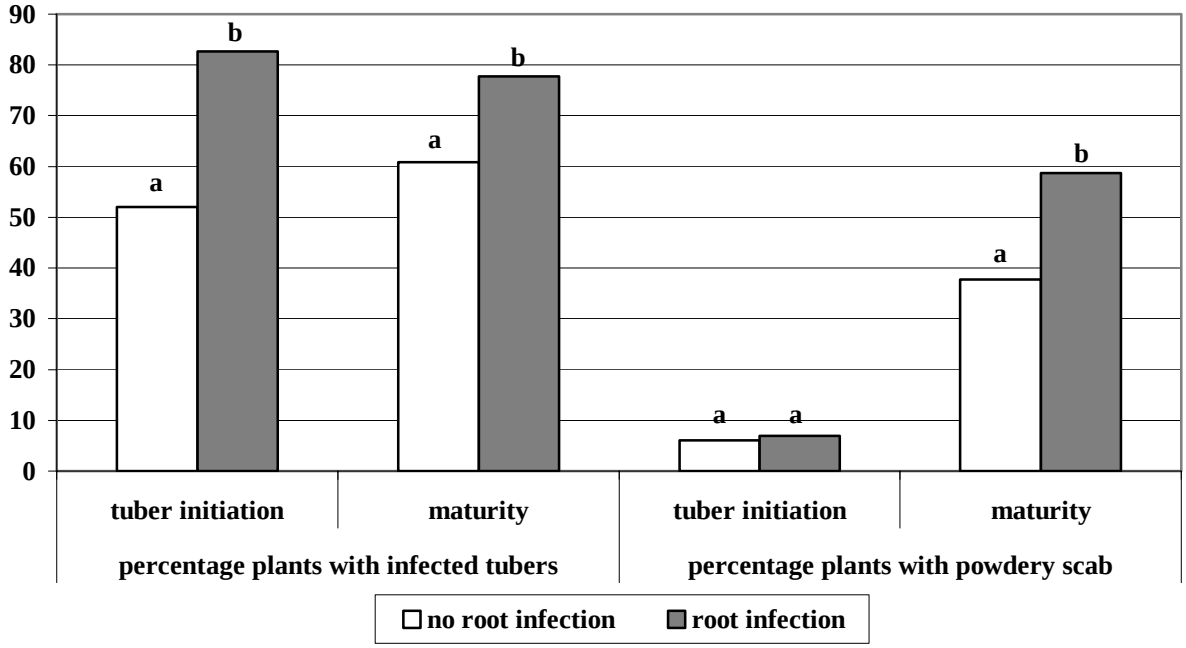


FIGURE 6.11 EFFECT OF THE OCCURRENCE OF ROOT INFECTION BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' ON THE OCCURRENCE OF TUBER INFECTION AND POWDERY SCAB CAUSED BY THE SAME PATHOGEN AT TWO DIFFERENT GROWTH STAGES. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

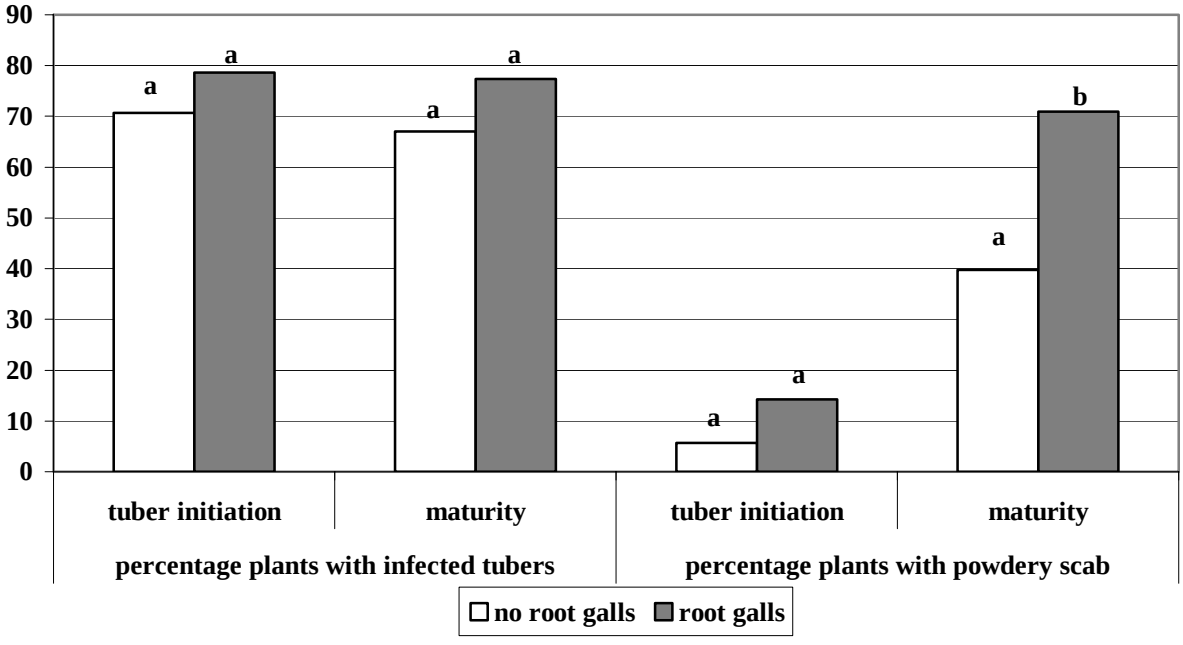


FIGURE 6.12 EFFECT OF THE OCCURRENCE OF ROOT GALLS CAUSED BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' ON THE INCIDENCE OF TUBER INFECTION AND POWDERY SCAB CAUSED BY THE SAME PATHOGEN AT TWO DIFFERENT GROWTH STAGES. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

Some plants without powdery scab did develop root galls (8 and 12% respectively) and the average root gall severity did not differ significantly between plants with and without powdery scab (Figure 6.14).

These results show that there is no clear link between tuber and root symptoms. Even for those plants that did develop both disease symptoms, mostly at 17°C, there was no relationship between root gall severity and powdery scab severity. Thus, it seems unlikely that the formation of root galls can be used as an indicator of susceptibility to powdery scab, despite the fact that both symptoms are caused by the same organism. The development of a root gall test will only be successful when carried out under the right environmental conditions, but even then the fact remains that the biology of root and tuber infection are distinctly different. Potato roots remain susceptible to infection throughout the growing season while tubers are usually only infected by *S. subterranea* at the tuber initiation stage.

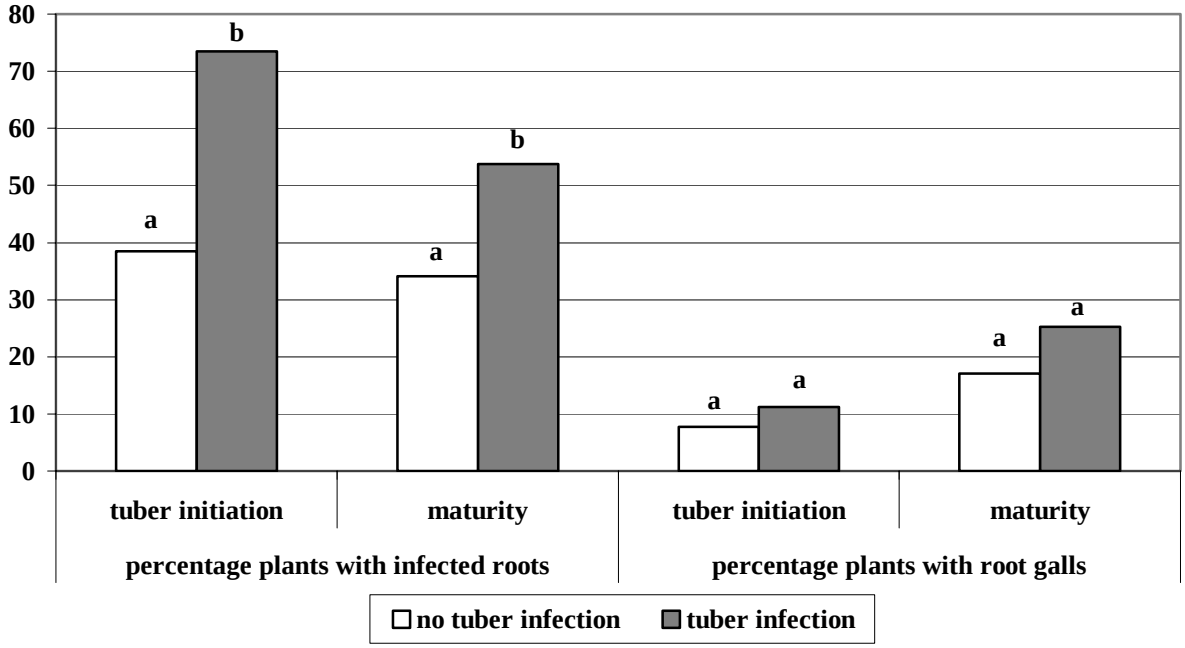


FIGURE 6.13 EFFECT OF THE OCCURRENCE OF ROOT INFECTION BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' ON THE OCCURRENCE OF ROOT INFECTION AND GALLING CAUSED BY THE SAME PATHOGEN AT TWO DIFFERENT GROWTH STAGES. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT (A = 0.05).

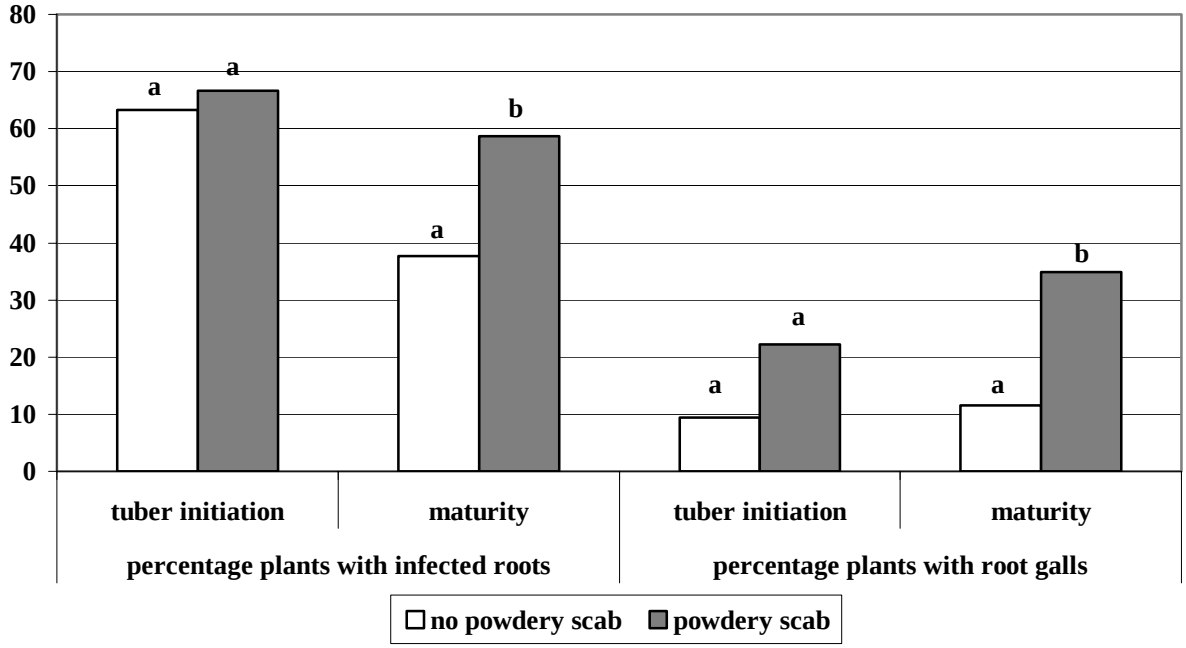


FIGURE 6.14 EFFECT OF THE OCCURRENCE OF POWDERY SCAB CAUSED BY *SPONGOSPORA SUBTERRANEA* IN POTATO PLANTS CV. 'ESTIMA' ON THE OCCURRENCE OF ROOT INFECTION AND GALLING CAUSED BY THE SAME PATHOGEN AT TWO DIFFERENT GROWTH STAGES. DIFFERENCES BETWEEN TWINNED COLUMNS WITH DIFFERENT LETTERS ARE SIGNIFICANT ($\alpha = 0.05$).

Discussion

Important new information on the biology of *S. subterranea*, which contributes to a better understanding of the pathogen and thus hopefully to more effective prevention and control measures for powdery scab in the near future, has been illustrated. The distinctly different effect of temperature on the development of root galls and powdery scab was previously unknown. The optimum temperature for powdery scab symptoms (12°C) agrees with reports in literature, but the finding that disease levels can be high even at temperatures as low as 9°C was unexpected. Also of importance was the discovery of latent infections, first mentioned by Diriwächter & Parbery (1991), which appear to be associated particularly with low temperatures and low inoculum levels. Whether these latent infections can develop into powdery scab lesions or even mature sporeballs under storage conditions is as yet unknown. The possibility of latently infected seed tubers also needs to be considered. More research needs to be carried out into the importance of latent infections in the disease cycle of *S. subterranea*.

The results confirmed that tuber initiation is a critical phase for infection, as previously reported by Burnett (1991) and Diriwächter & Parbery (1991). Similarly, the lack of a significant effect of soil inoculum level on disease also agrees with earlier findings; Burnett (1991) indicated that the initial inoculum level in soil is not important if the right environmental conditions for further disease development are present. Once infection has been established, secondary zoospores released from zoosporangia greatly reduce the importance of soil inoculum. In our trials, infection levels were high for most treatments and it appears that low concentrations of inoculum are enough to cause serious levels of disease. The incidence of infection and disease in non-inoculated soils could have been the result of natural contamination of the soil or of the seed tubers used.

The results on soil moisture regime showed that the moisture level of the soil does not need to be high to obtain high infection and disease levels. The low disease levels in soils with

fluctuating wetness could have been due to the compactness of the soils as a result of the moisture regime and the reduction in plant vigour in these treatments due to the dry periods. In some countries, powdery scab is especially a problem in sandy and loamy soils, and the findings described above agree with these field observations. The pore size of clay soil is small and the resulting low oxygen levels could have played a role in preventing high levels of infection and disease. Data from soil analysis show that this could not have been an effect of pH.

Disease threshold and environmental factors (controlled conditions) - main conclusions:

- **Both the incidence and severity of potato powdery scab are influenced by a range of environmental factors, including temperature, soil type and soil moisture regime.**

- **Soil inoculum level does not have a significant influence on infection and disease development and even low levels of inoculum can result in severe disease symptoms.**

- **Latent tuber infections appear to be common, but more research needs to be conducted into their importance in the spread and occurrence of powdery scab.**

- **There is no relationship between the occurrence of powdery scab on tubers and galls on roots. Both symptoms may occur independently and even when they do occur together their severity is usually not correlated.**

- **The influence of environmental factors, especially temperature, is different for powdery scab and root gall development.**

Field experiments

Objective: To determine the relationship between (seed-borne) inoculum and disease development by assessing the effect of environmental factors on infection of the growing potato crop by *Spongospora*, and the final disease incidence and severity (SAC).

Milestone 3: Establish field experiments to tests of standard stocks - *achieved*

Milestone 4: Preliminary assessments for field work - *achieved*

Methodology

A stock of variety Estima (SE1) with powdery scab infection was graded by hand to produce the levels of infection described in the Table 6.5. below. Sets of tubers were dispatched to 11 sites around GB for planting in a randomised block design with two replicates within commercial ware crops. Each replicate plot comprised 50 tubers as 2 drills x 25 tubers. The field sites selected were chosen in the belief that they were free from soil-borne inoculum of *S. subterranea*. All fields were irrigated. Tubers were harvested from plots within trials and assessed for incidence and severity of powdery scab infection. Site locations are given in Table 6.6.

TABLE 6.5 LEVELS OF VISIBLE TUBER INFECTION BY *SPONGOSPORA SUBTERRANEA* AFTER HAND GRADING A STOCK OF ESTIMA SE1

Treatment no.	
1	No visible infection
2	10% tubers infected with <1/8 area infected
3	20% tubers infected with <1/8 area infected
4	50% tubers infected with <1/8 area infected
5	20% tubers infected, 4% with >1/8 area infected
6	50% tubers infected, 10% with >1/8 area infected

TABLE 6. 6 TRIAL SITE LOCATIONS

	Location	**Co-operating Agronomist**
1	Great Dunmow, Essex	David Hudson, Solanum
2	Chichester, W Sussex	Di Pitts, Best eating potatoes
3	Duxford, Cambridgeshire	David Hudson, Solanum
4	Woodbridge, Suffolk	Graham Tomalin, Greenvale AP
5	Rodington, Shrewsbury	Jeremy Gascoigne, Scott & Newman
6	Great Massingham, Kings Lynn, Norfolk	Tom Dixon, MBM
7	Berwick, Northumberland	D Rankin, E S Black
8	Scunthorpe, Humberside	Graeme Byers, Higgins
9	Ramsey, Cambridgeshire	Dennis Walsh, QV Foods
10	SCRI, Dundee	Stuart Wale, SAC
11	Holme Lacey, Hereford	David Hudson, Solanum

(NB. Farm addresses have not been listed)

TABLE 6.7 SOIL ANALYSES FOR TRIAL SITE LOCATIONS

Site	pH	P	K	Mg	S	Cu	Mn	Bo	Zn
1	8.0	15.4 (H)	286 (H)	185 (M)	14.2 (H)	1.5 (L)	1.5 (L)	1.5 (H)	2.0 (M)
2	6.5	12.0 (M)	150 (M)	105 (M)	9.8 (M)	2.0 (M)	4.2 (M)	1.0 (M)	1.3 (L)
3	8.0	13.6 (M)	174 (M)	196 (M)	8.7 (M)	1.9 (M)	1.6 (L)	1.9 (H)	1.4 (L)
4	7.5	110 (EH)	306 (H)	86 (M)	10.7 (H)	3.7 (M)	1.0 (VL)	1.0 (M)	6.2 (M)
5	6.6	9.0 (M)	157 (M)	113 (M)	10.3 (H)	4.6 (M)	3.5 (M)	1.1 (H)	6.3 (M)
6	8.0	95.7 (EH)	343 (H)	103 (M)	6.5 (M)	2.5 (M)	0.9 (VL)	1.0 (M)	4.5 (M)
7	6.0	3.2 (L)	83.0 (M)	191 (M)	7.9 (M)	5.7 (M)	3.6 (M)	1.1 (H)	4.7 (M)
8	8.2	18.7 (H)	159 (M)	277 (H)	11.3 (H)	3.2 (M)	1.1 (VL)	1.4 (H)	4.9 (M)
9	8.1	9.1 (M)	225 (H)	379 (H)	47.1 (H)	2.2 (M)	1.3 (VL)	5.5 (EH)	4.0 (M)
10*	5.9	101	160	137	12	14.9	89	0.5	12.0
11	5.4	5.9 (M)	110 (M)	237 (H)	17.8 (H)	3.0 (M)	5.5 (M)	0.9 (M)	1.9 (M)

* analysed by Phosyn, all others analysed by SAC

Results

Powdery scab incidence was greatest at the northern and western sites, Dundee, Northumberland, Shrewsbury and Hereford (Table 6.8). The incidence was moderately high at the West Sussex site but this was subject to severe waterlogging and not lifted until January. The results should be treated with caution. At the Dundee site there was no effect of inoculum level on incidence or severity. At Northumberland, the greatest inoculum level (treatment 6) resulted in a much greater incidence and severity. At Shrewsbury and Hereford, there was a trend to more disease with the greatest inoculum.

At other sites, the incidence and severity of powdery scab were low, with no clear relationship to the level of inoculum (Table 6.8).

The impact of inoculum is most clearly indicated where % marketable tubers are considered. At the Northumberland site, the highest inoculum level reduced marketable tubers markedly. At Dundee, Hereford and Shrewsbury, the effect of inoculum was not apparent.

These results support the findings in Scotland (Burnett, 1991) and elsewhere (e.g. Australia – de Boer, personal communication) that there is generally a poor relationship between visible disease on seed and the resultant infection of progeny. They also suggest that northern and western areas of GB are most prone to powdery scab and that low levels of the disease on seed do not markedly affect infection of the progeny in eastern and southern England.

TABLE 6.8 PERCENTAGE INCIDENCE OF POWDERY SCAB

	Site											
Treatment	**1**	**2+**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Mean**
1	3	23	6	1	13	3	15	2	10	35	18	10.6
2	3	14	8	6.2	9	5	17	2	3	30	13	9.6
3	1	16	2	5	11	8	10	0	4	44	10	9.5
4	2	25	7	6	19	6	13	0	5	42	20	12.0
5	2	9	8	7	15	1	27	2	6	25	11	10.4
6	12	22	12	4	21	6	52	4	10	20	25	16.6
SED	3.7	-	5.2	3.0	7.8	1.5*	15.5	2.4	4.5	11.8	10.6	-

Percentage severity of powdery scab

	Site											
Treatment	**1**	**2+**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Mean**
1	0.06	0.4	0.12	0.01	0.31	0.05	1.44	0.03	0.25	1.71	0.64	0.46
2	0.04	0.2	0.16	0.06	0.23	0.05	0.84	0.05	0.03	1.2	0.92	0.35
3	0.02	0.2	0.02	0.07	0.31	0.09	0.36	0	0.09	1.94	0.53	0.34
4	0.06	0.4	0.12	0.29	0.62	0.07	0.29	0	0.1	2.79	0.78	0.51
5	0.05	0.1	0.09	0.09	0.43	0.01	1.44	0.02	0.2	1.91	1.15	0.54
6	0.38	0.4	0.27	0.11	0.53	0.07	6.75	0.27	0.16	1.13	2.31	1.2
SED	0.05**	-	0.10	0.15	0.22	0.03	1.6	0.16	0.13	0.93	1.11	

Percentage marketable tubers (bag market – 5% severity threshold)

	Site											
Treatment	**1**	**2+**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Mean**
1	100	100	100	100	99	100	92	100	97	89	94	87.1
2	100	100	99	100	99	100	95	100	100	91	94	87.8
3	100	100	100	100	100	100	98	100	99	87	97	88.1
4	100	99	100	98	96	100	99	100	99	83	95	87
5	100	100	100	100	97	100	91	100	99	88	93	86.8
6	99	99	99	99	99	100	73	98	100	93	90	85.1

* ** Significant at $p<0.05$ and $p<0.01$ respectively

+ The West Sussex site was subject to waterlogging and was harvested late. The results are not included in the mean figures

Discussion

From this experiment, there is little evidence that a consistent or simple relationship exists between the level of inoculum on seed and subsequent disease developing on the progeny crop. At most sites in the field trial, as much disease developed on the visually disease-free treatment as on those with visible infection. Assuming the fields into which the seed was planted were uncontaminated, this suggests that the visually disease-free tubers were carrying sufficient inoculum as sporeballs to initiate disease. These data confirm the view that environmental conditions are the major driver of disease development.

Of the six treatments tested in the field trial, the level of powdery scab in treatment six was well above the maximum SEERAD certification standard. The increase in disease at four sites with treatment six and little difference in disease between other treatments suggests that, as a base-line for limiting disease development in the subsequent crop, the SEERAD standard is sufficient. There is no evidence from the field trial that tightening the standard will reduce the risk of powdery scab in the subsequent crop under similar environmental conditions. It seems likely that only a complete absence of disease or tuber contamination might achieve this.

In the field trial, there was greater incidence and severity of disease in the northern and western sites, with those in the south and east of England showing only low levels of disease. This result occurred despite most of England experiencing above average rainfall in 2000. It is possible that the climatic conditions at tuber initiation, the most critical phase for infection, in the south and east of England are generally less conducive for infection that in the north or west of GB. It is also possible that soils in the north and west are wetter for longer or cooler at the critical time or perhaps that soils in the south and east are more suppressive. Powdery scab does occur (sometimes severely) in parts of England but is not considered a problem in other parts.

Where soil is already contaminated, it seems unlikely that a low level of further contamination would influence the risk of disease, if the view that environmental conditions are the major driver of disease development is accepted. The exception to this might be concerns that infected seed could contaminate ‘clean’ land such as in the potato growing areas on the English/ Welsh border.

Where environmental conditions are less conducive to powdery scab development, disease can occur where a high level of inoculum is present. Thus, it is a reasonable aim of seed production to minimise disease development and a reasonable aim of purchasers of seed to buy seed with limited levels of powdery scab.

Disease threshold and environmental factors (field trials) - main conclusions:

- **There is little evidence that a consistent or simple relationship exists between the level of inoculum on seed and subsequent disease developing on the progeny crop.**
- **Environmental conditions are the major driver of disease development.**
- **There is no evidence from the field trial that tightening the SEERAD standard will reduce the risk of powdery scab in a subsequent crop.**

- **Where soil is already contaminated, it seems unlikely that a low level of further contamination would influence the risk of disease, if the view that environmental conditions are the major driver of disease development is accepted.**

- **It is a reasonable aim of seed production to minimise disease development and a reasonable aim of purchasers of seed to buy seed with limited levels of powdery scab.**

Improving sporeball detection in soil

The aim of this experiment was to improve methods for the detection of *S. subterranea* sporeballs in soil. As described above, the original protocol gave inconsistent results and needed improvement to increase sensitivity.

The first aspect addressed was the cycling conditions used for the amplification of *S. subterranea* DNA in conventional PCR. The original program was developed by Bell *et al* (1999) for the *S. subterranea* specific primers designed at SCRI and consisted of the following cycles:

95°C for 2 min
repeated 35 times:
- **95°C for 20 sec**
- **64°C for 25 sec**
- **72°C for 50 sec**

72°C for 10 min
4°C soaking

This program was compared with a longer program using the same primers:

95°C for 2 min
repeated 35 times:
- **95°C for 45 sec**
- **64°C for 60 sec**
- **72°C for 90 sec**

72°C for 5 min
4°C soaking

It was shown that the longer program yielded more, and brighter, bands than the shorter program. However, there were still negative results for some samples which should have tested positive, so products from the first PCR round with SCRI primers were used for a second PCR round with primers adapted from those developed by Qu *et al*. (1998) at University College Dublin (UCD), which are internal to the SCRI primers. The performance of the UCD primers was compared using two PCR programs similar to the ones above except for the annealing temperature which was 55°C instead of 64°C. However, unlike with the SCRI primers, a longer program did not greatly improve DNA amplification with the UCD primers. Eventually, the following nested PCR protocol was developed for the improvement of amplification and thus detection using conventional PCR:

1. Dilute the DNA extracts 1:2 and add the double amount to the PCR reaction mix.
2. Amplify the DNA using the SCRI primers with the longer PCR program.
3. Use the products of this first PCR round undiluted in a second amplification round using the UCD primers with the shorter PCR program.

This protocol gave the most and the brightest bands, although still did not amplify all samples containing *S. subterranea* DNA. Therefore, the nested PCR was used on DNA extracted from samples of three different soils, which were treated in different ways before extraction. Samples of each soil type were inoculated with 50 *S. subterranea* sporeballs per g, but unspiked samples were also tested. The following treatments were evaluated:

1. **air drying**: half of the samples were air dried in open plastic trays at 18°C for 24 hours (to condition sporeballs for germination) whilst the others were not dried and were incubated at 18°C in the dark in sealed plastic pots.
2. **flooding**: half of the air dried samples and half of the non-dried samples were then flooded with SDW (10 ml per 10 g soil) for three days (to trigger the release of zoospores), while the other samples were used for extraction without further treatment.
3. **filtering**: half of the *flooded* samples were used for extraction without further preparation (soil and flood water together), while in the other half, only the supernatant was used after filtering through a 5 μm filter (detection of zoospores only).

Overall, there were 36 different treatments (soil type x inoculum x drying x flooding/filtering = 3 x 2 x 2 x 3). There were three separate extraction rounds in which each treatment was replicated once. All samples were frozen overnight to kill zoospores. After defrosting, all samples containing soil were used for DNA extraction following the method published by Bell *et al*. (1999) while DNA from the zoospore solutions was extracted using a DNA extraction kit.The results of this experiment are shown in Table 6.4. Air drying of the soil did not result in better detection and dried (37% detection) and undried (35%) samples yielded similar results. However, for proper quantification of DNA in soil samples, drying is an essential step. Detection of *S. subterranea* in soil samples was considerably improved by flooding. Overall, the average detection rate of samples, which were not flooded, was almost zero, while in flooded samples the average was 46% (regardless of drying). The effect of using only filtered floodwater depended on the soil type. In loam 1, filtering improved detection, but in loam 2 it inhibited it. In clay, filtering did not have an effect on the detection rate, so on average the use of filtered solution with zoospores did not contribute to better detection. Since filtering is an extra step which involves more time and money than extraction from soil and water together, it seems better to leave this step out. Thus, the best method for treating soil samples before DNA extraction to detect *S. subterranea* is:

1. Air dry the soil for at least 24 h at 18°C.
2. Weigh 10 g samples of dried soil.
3. Flood the samples with 10 ml SDW each for 3 days at 18°C in the dark.
4. Extract DNA from the soil and floodwater together.

Table 6.4 also shows that the detection rate depended on soil type and that the loam soils yielded less positive samples than the clay. This observation supports the idea that lighter

soils are more inhibitive to the PCR reaction than heavier soils as suggested on basis of the findings described above with the real-time PCR assay.

Together with the use of nested PCR the above protocol should increase the detection of *S. subterranea* in soil samples compared with extraction of DNA from untreated soil and amplification with one round of PCR. The improved sample treatment before DNA extraction should also help to increase the detection rate of *S. subterranea* in soil using a real-time PCR assay.

TABLE 6.4 NUMBER OF SAMPLES IN WHICH *SPONGOSPORA SUBTERRANEA* WAS DETECTED FOR DIFFERENT TYPES OF SOIL TREATED IN DIFFERENT WAYS BEFORE DNA EXTRACTION USING A CONVENTIONAL PCR ASSAY.

soil type	inoculum added	treatment						
		air dried			not dried			**total**
		not flooded	flooded, not filtered	flooded, filtered	not flooded	flooded, not filtered	flooded, filtered	
loam 1	no	0	0	2	0	0	1	3
	yes	0	1	2	0	1	1	5
	total	**0**	**1**	**4**	**0**	**1**	**2**	**8**
								33%
loam 2	no	0	0	0	0	3	0	5
	yes	0	3	0	0	2	0	5
	total	**0**	**3**	**0**	**0**	**5**	**0**	**10**
								42%
clay	no	0	3	2	0	2	3	10
	yes	0	3	2	1	1	3	10
	total	**0**	**6**	**4**	**1**	**3**	**6**	**20**
								83%
all soils	no	0	3	6	0	5	4	18
	yes	0	7	4	1	4	4	20
	total	**0**	**10**	**10**	**1**	**9**	**8**	**38**
								53%

Improving sporeball detection in soil - main conclusions:

- **The detection of *S. subterranea* in soil samples using a PCR assay can be improved by flooding dried soil samples for three days and by then extracting DNA from soil and floodwater together.**

- **The detection of *S. subterranea* in soil samples using a conventional PCR assay can be improved further by using nested PCR.**

Chemical control

Experiments under controlled conditions

Objective: To evaluate disinfectants, fungicides, novel chemicals and biofumigants as pre-plant soil and tuber treatments by carrying out pot experiment with these compounds incorporated into the soil following the addition of *Spongospora* inoculum. The viability of soil inoculum will then be assessed over time (at two growth stages) (SCRI).

Milestone 5: Assess glasshouse trials for relative efficacy of different chemicals, identify promising new candidate chemicals for field trials - *achieved*

A large experiment to evaluate fungicides, novel chemicals and other products as soil treatments and pre-plant tuber treatments under controlled conditions was carried out at SCRI. The experiment consisted of two parts:

1. Evaluation of the effectiveness of single products against infectivity and survival of sporeballs of *S. subterranea* in soil and on seed.
2. Evaluation of the effectiveness of combinations of products against infectivity and survival of sporeballs of *S. subterranea* in soil.

Soil applied

Single products

In 2001, a range of products was tested against soil borne inoculum of *S. subterranea* (Table 7.1). Sandy loam soil was obtained from SAC, air dried and sieved (1 cm) to remove large stones. Inoculum of sporeballs was prepared by scraping powdery scabs from potato tubers provided by SAC. The scrapings were ground and sieved through a 53 micron sieve and suspended in SDW. The soil was mixed with half the amount of water needed to bring it to a moisture level of around –1 bar. *S. subterranea* inoculum of 50 sporeballs per gram soil was then added to half of the remaining water, which was then thoroughly mixed with the already damp soil by hand. The non-inoculated control was mixed with water without sporeballs. The appropriate treatment was mixed into the inoculated soil using the remaining water. No treatment was applied to the non-inoculated and inoculated controls. The soils were distributed into three litre plastic pots and incubated in controlled environment growth rooms at 17ºC with 16 h daylight. One potato tuber cv. Estima (VTSC grade, 25–35 mm) was planted per pot within two days after mixing the soil and treatment. There were five replicates per treatment.

The moisture content in the pots was checked regularly and water added where necessary to maintain constant dampness. At two different points in time (tuber initiation and maturity/senescence), plants were lifted and roots and tubers checked for powdery scab symptoms and rated as described previously.

TABLE 7.1 SOIL APPLIED TREATMENTS TESTED ON EFFICACY AGAINST DISEASE CAUSED BY SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN THE 2001 TRIAL.

Treatment type	Treatment name	Application rate per litre of soil
Fungicides	fluazinam (Shirlan; 500 g/l)[1]	0.03 ml
	mancozeb (Mancozeb 80; 455 g/l)[2]	0.33 ml
	elemental sulphur (Sulfer 95)[3]	1.25 g
	zinc oxide (80%)[4]	0.15 g Zn
Growth stimulants/ biocontrol products	prawn shell waste[5]	3% w/w
	Biomex (contains *Trichoderma* spp.)[6]	0.01 ml
	StimaGro (contains *Streptomyces* spp.)[7]	2.5 mg
Biofumigants	freeze dried powder of the following crops[8]:	
	rape, *Brassica napus* 'Synergy'	0.1% v/v
	rape, *Brassica napus* 'Bristol'	0.1% v/v
	rape, *Brassica napus* 'Broad Leaf Essex'	0.1% v/v
	cabbage, *Brassica oleracea* 'Thousand Headed Kale'	0.1% v/v
	mustard, *Brassica campestris* 'Debut'	0.1% v/v
	mustard, *Brassica juncea*	0.1% v/v
	mustard, *Sinapis alba*	0.1% v/v
	radish, *Raphanus sativus*	0.1% v/v

The products tested were supplied/produced by: 1 Zeneca; 2 Agrichem; 3 New-Trition; 4 Sigma; 5 Claymore shellfish; 6 OMEX; 7 Kemira Agro; 8 Elaine Booth, SAC.

No powdery scab was observed in plants lifted at tuber initiation. At this stage, root galls were present in most of the treatments, but were generally less common and severe than in the inoculated control (Figure 7.1). Only fluazinam, mancozeb and sulphur prevented root gall development at tuber initiation completely. Apart from these three treatments, freeze dried powder of *B. juncea*, *B. napus* 'Bristol', *B. napus* 'Broad Leaf Essex' and *B. napus* 'Synergy' were the only treatments which significantly reduced root gall severity at tuber initiation compared to the non-treated inoculated control ($\alpha = 0.05$).

In mature plants, root galls were more widespread than in plants at tuber initiation stage and only the fluazinam treatment prevented root galling at maturity completely (Figure 7.2). Many of the biological and biofumigant treatments increased root gall severity compared with the non-treated inoculated control. It is possible that the efficacy of these treatments declined in time and that root infection took place when *S. subterranea* was no longer affected by their biological activity.

Fluazinam, mancozeb, sulphur, zinc oxide and powder of *Sinapis alba* all significantly reduced the incidence of root galling, but only fluazinam and mancozeb had a significant effect on root gall severity.

Powdery scab occurred commonly in many of the mature plants. Four out of five of the non-treated inoculated control plants had tubers with powdery scab and the average disease severity in these plants was 2.0 (up to 5% of tuber surface covered with powdery scab). Some of the biological and biofumigant treatments induced the development of powdery scab symptoms compared with the control (Figure 7.3), although not as strongly as root gall development. Both fluazinam and mancozeb were very effective and no tubers with powdery scab were found in these treatments. The same was true for the treatment with prawn shell waste, which not only prevented powdery scab development but also appeared to promote plant growth compared with the other treatments.

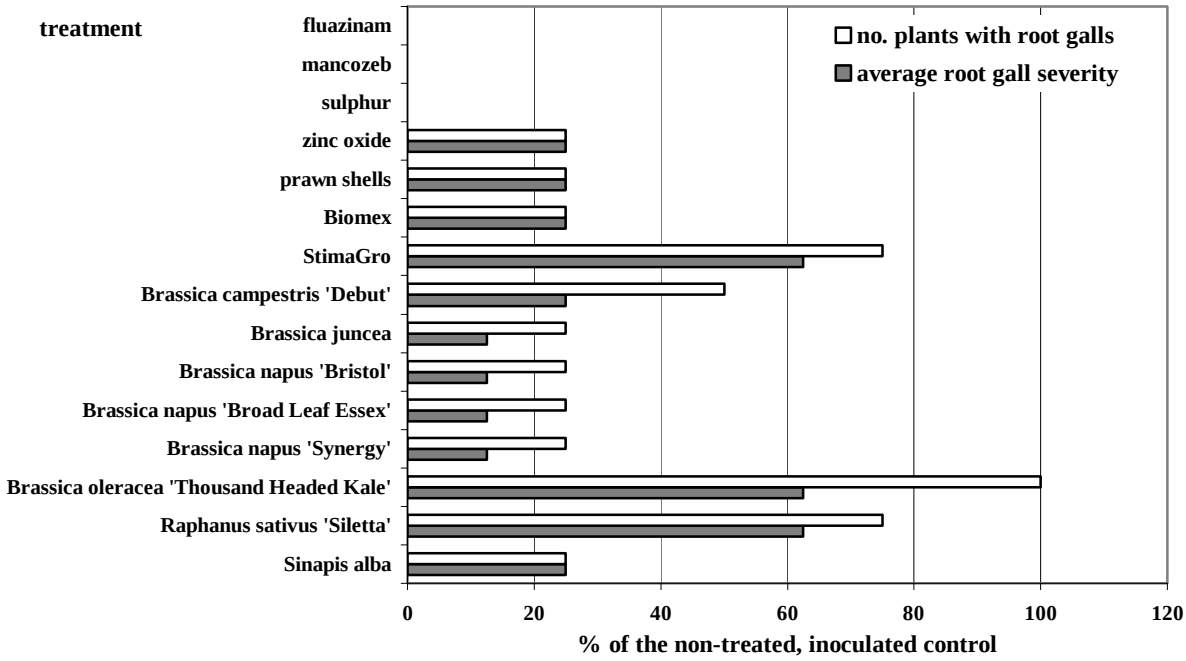


FIGURE 7.1 EFFICACY OF DIFFERENT SOIL APPLIED TREATMENTS IN REDUCING ROOT GALLING CAUSED BY SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN POTATO CV. 'ESTIMA' AT TUBER INITIATION IN THE 2001 TRIAL, EXPRESSED AS A PERCENTAGE OF THE NON-TREATED, INOCULATED CONTROL (N = 5).

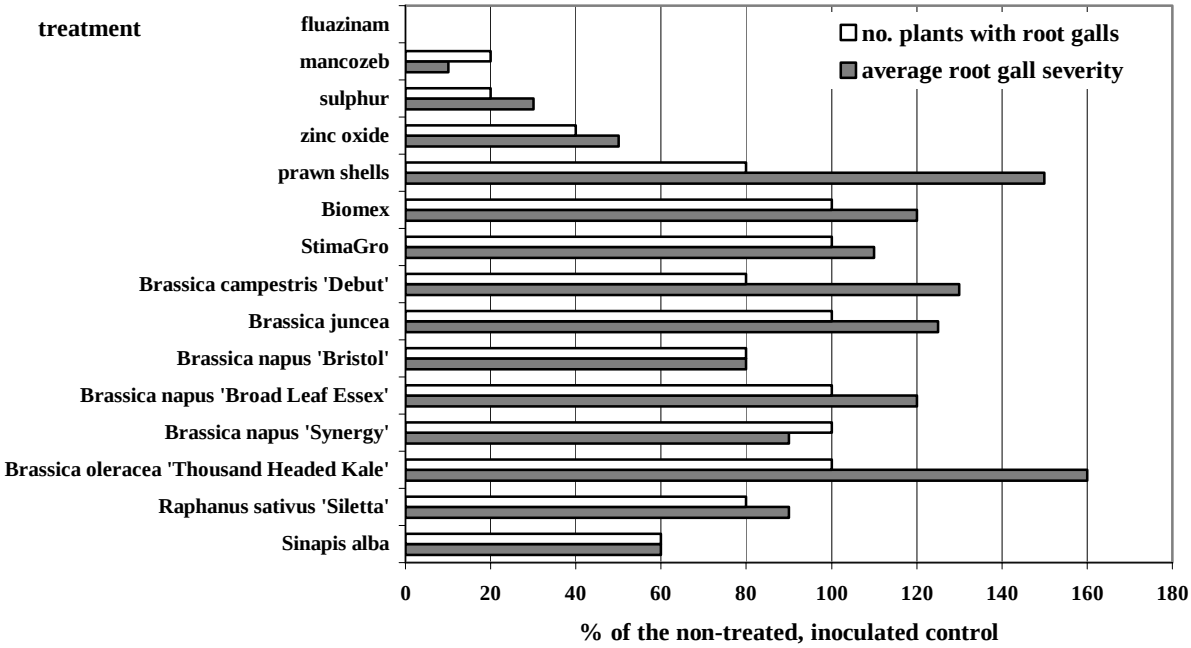


FIGURE 7.2 EFFICACY OF DIFFERENT SOIL APPLIED TREATMENTS IN REDUCING ROOT GALLING CAUSED BY SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN POTATO CV. 'ESTIMA' AT MATURITY IN THE 2001 TRIAL, EXPRESSED AS A PERCENTAGE OF THE NON-TREATED, INOCULATED CONTROL (N = 5).

The biological products Biomex and StimaGro had no controlling effect on powdery scab at all, while only one of the biofumigant treatments, powder of *Brassica juncea*, caused a significant reduction in powdery scab ($\alpha = 0.05$). Treatments with sulphur, zinc oxide and powder of *Raphanus sativus* also considerably reduced powdery scab incidence and severity but these effects were not statistically significant ($\alpha = 0.05$).

Several products identified in this trial showed potential as treatments against powdery scab. Fluazinam and mancozeb were overall the most effective products tested, but there were some indications that they might be slightly phytotoxic in the rates applied. Delayed emergence and reduced growth was observed in a few plants treated with these products. Sulphur and zinc oxide, which are currently the most commonly applied treatments in the field against powdery scab, reduced disease incidence and severity at maturity by 50 to 80% and thus remain reasonably effective. The use of prawn shell waste showed promising results as far as powdery scab symptoms were concerned but did not prevent root galling. The same is true for amendment of the soil with freeze dried powder of *Brassica juncea* or *Raphanus sativus*. All the other biocontrol or biofumigant treatments had no or little controlling effect on disease caused by *S. subterranea* and in some cases even promoted symptom development. It was decided to use the most effective products in a second trial in which products were combined and applied at different points in time.

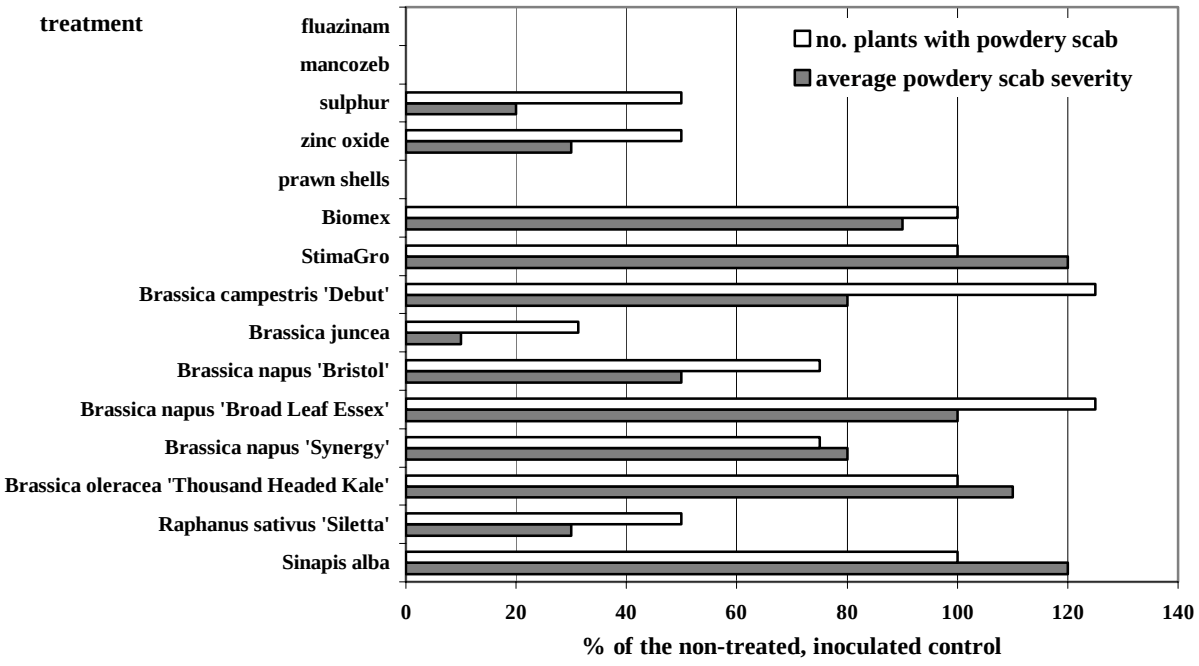


FIGURE 7.3 EFFICACY OF DIFFERENT SOIL APPLIED TREATMENTS IN REDUCING POWDERY SCAB CAUSED BY SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN POTATO CV. 'ESTIMA' AT MATURITY IN THE 2001 TRIAL, EXPRESSED AS A PERCENTAGE OF THE NON-TREATED, INOCULATED CONTROL (N = 5).

Timing and combination of applications

Some of the soil applied single products that showed promising results in the first trial were tested again in 2002 (Table 7.2). Prawn shell waste and freeze dried powder of *B. juncea* were also tested in combination with fluazinam (at two different rates) and zinc oxide. The two non-chemical treatments were mixed with the soil at the start of the experiment, while the chemicals were applied just after tuber initiation as a drench. Dazomet (Basamid G), a biofumigant with the same mode of action as *Brassica* powder, was also included in the trial.

The set-up of this experiment was the same as described above for the 2001 trial, but plants were lifted at maturity only.

Soil for the dazomet treatments underwent an extra preparation step. The sandy loam soil for these treatments was mixed with the inoculum of 50 sporeballs per gram soil in the amount of water needed to bring the soil to a moisture level of around –1 bar. Dazomet was then added and thoroughly mixed with the damp soil by hand. Due to an extra preparation step, separate controls were used for the dazomet treatment. The inoculated control was mixed only with inoculum in water and the non-inoculated control was mixed with water without sporeballs. The soil was left at room temperature in a large aerated room. After five weeks, samples of the soil were tested for phytotoxicity by wetting them and incubating them with cress seeds in air tight containers. The cress seeds in all samples germinated and grew normally indicating that the break down products of dazomet were no longer present in high concentrations. The remaining soil was then distributed into three litre plastic pots and incubated as described above for the other treatments.

TABLE 7.2 SOIL APPLIED TREATMENTS AND COMBINATIONS OF TREATMENTS TESTED ON EFFICACY AGAINST SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN THE 2002 TRIAL.

Treatment type	Treatment name	Application rate per litre of soil
Fungicides	fluazinam (Shirlan; 500 g/l)	0.015 ml
	fluazinam (Shirlan; 500 g/l)	0.03 ml
	mancozeb (Dithane NT, 75%)	0.20 mg
	zinc oxide (80%)	0.15 g Zn
Growth stimulants/ biocontrol products	prawn shell waste	3% w/w
Biofumigants	dazomet (Basamid G, 98%)	0.4 g
	freeze dried powder of the following crops:	
	mustard, *Brassica juncea*	0.1% v/v
	radish, *Raphanus sativus*	0.1% v/v
Combinations	prawn shell waste + zinc oxide	3% w/w + 0.15 g Zn
	prawn shell waste + fluazinam	3% w/w + 0.015 ml
	prawn shell waste + fluazinam	3% w/w + 0.03 ml
	Brassica juncea powder + zinc oxide	0.1% v/v + 0.15 g Zn
	Brassica juncea powder + fluazinam	0.1% v/v + 0.015 ml
	Brassica juncea powder + fluazinam	0.1% v/v + 0.03 ml

Root galling was most effectively controlled by fluazinam (both rates) and mancozeb (Figure 7.4). Dazomet (Basamid) also significantly reduced root gall incidence and severity compared with the inoculated control, but none of the other treatments had a significant effect on root symptoms caused by *S. subterranea* ($\alpha = 0.05$). Root gall symptoms were severe in the treatments with prawn shells and freeze dried powders of *B. juncea* and *R. sativus*. Application of zinc oxide or fluazinam (7.5 g/l) after tuber initiation significantly reduced root gall incidence and severity in the case of plants treated with prawn shell waste. However, none of the additional treatments significantly reduced root galling when applied in combination with *B. juncea* powder ($\alpha = 0.05$).

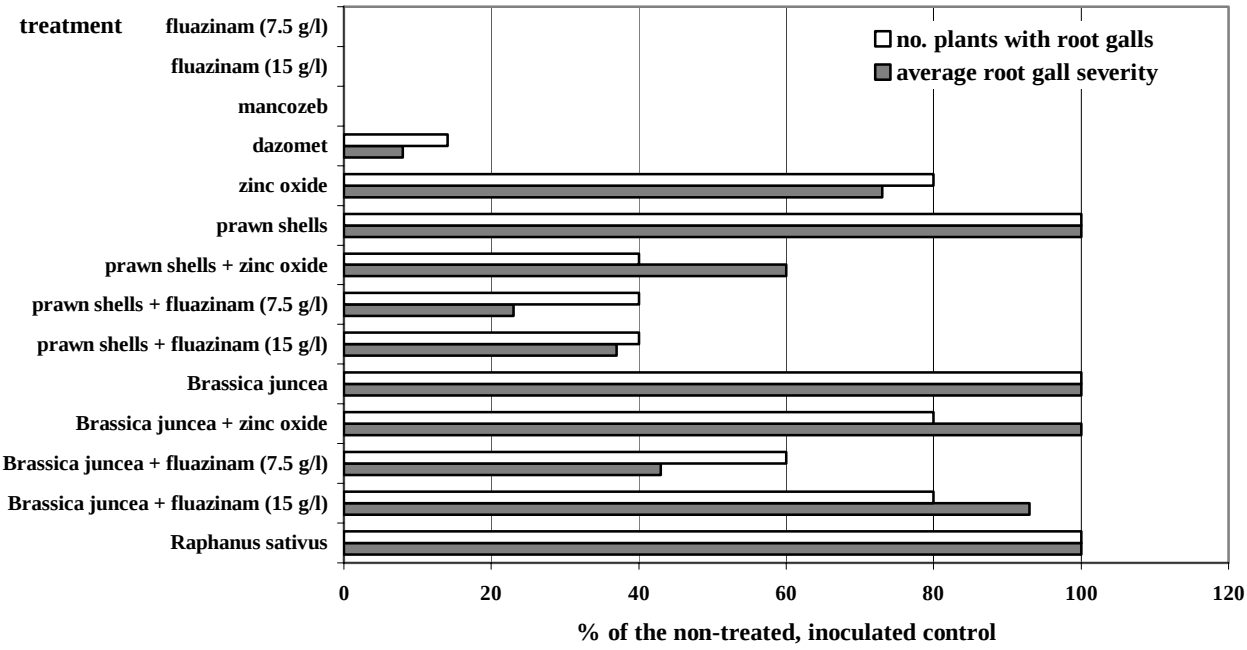


FIGURE 7.4 EFFICACY OF DIFFERENT SOIL APPLIED TREATMENTS IN REDUCING ROOT GALLING CAUSED BY SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN POTATO CV. 'ESTIMA' AT MATURITY IN THE 2002 TRIAL, EXPRESSED AS A PERCENTAGE OF THE NON-TREATED, INOCULATED CONTROL (N = 5).

Powdery scab was fully controlled by fluazinam and mancozeb (Figure 7.5). Treatments with prawn shell waste fully eliminated powdery scab as well, except in combination with fluazinam at 15 g/l where there was nevertheless a significant reduction in powdery scab severity compared with the inoculated control. Powdery scab incidence and severity were also significantly reduced by *B. juncea* powder alone and in combination with fluazinam at 7.5 g/l ($\alpha = 0.05$), but in combination with zinc oxide or fluazinam at a higher rate this powder was not as effective. The application of zinc oxide or fluazinam after tuber initiation had no significant additional effect on powdery scab incidence or severity when combined with either prawn shell waste or *B. juncea* powder. Such applications seemed in some cases to increase disease rather than prevent it, possibly due to an adverse effect on beneficial micro-organisms or plant growth. Dazomet reduced both powdery scab incidence and severity, but these effects were not significant due to a low level of powdery scab in the corresponding inoculated control ($\alpha = 0.05$). The relatively high level of powdery scab observed in the dazomet treated plants could have been due to contaminated seed tubers. Zinc oxide and freeze dried powder of *R. sativus* were the least effective treatments in the 2002 trial, although both still reduced powdery scab incidence and severity compared with the inoculated control (not significant) (Figure 7.5).

Strong phytotoxic effects were observed in the dazomet treatments, suggesting that not all break down products had disappeared at the time of planting. A slight growth reduction was observed in plants treated with fluazinam at both rates applied.

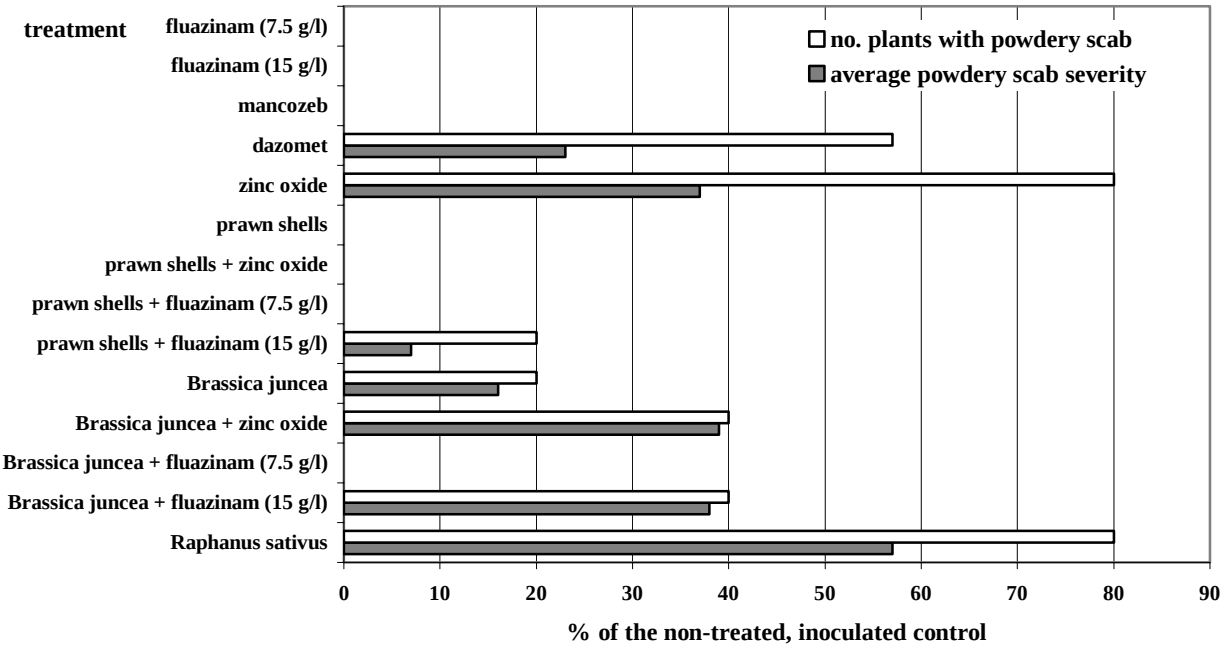


FIGURE 7.5 EFFICACY OF DIFFERENT SOIL APPLIED TREATMENTS IN REDUCING POWDERY SCAB CAUSED BY SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN POTATO CV. 'ESTIMA' AT MATURITY IN THE 2002 TRIAL, EXPRESSED AS A PERCENTAGE OF THE NON-TREATED, INOCULATED CONTROL (N = 5).

Comparison of results from two trials

The results for the treatments repeated in the 2002 trial were largely similar to those for the same treatments in the 2001 trial and no significant differences between the years were found for any treatment ($\alpha = 0.05$). Fluazinam was overall the most effective treatment and prevented the development of both root galls and powdery scab completely in both years. In the 2001 trial, root galls were observed in some of the plants treated with mancozeb but in 2002 no root galling was found (Figure 7.6). This can be explained by the fact that in 2001 another formulation of mancozeb (Mancozeb 80) was used than in 2002 (Dithane NT). It appears that Dithane NT is slightly more effective against root galling caused by *S. subterranea* than Mancozeb 80, although both formulations completely prevented the development of powdery scab symptoms. Also, Dithane NT did not show the phytotoxic effects observed in plants treated with Mancozeb 80.

Both prawn shell waste and freeze dried powder of *B. juncea* had almost the same effect on root galling and powdery scab in 2001 as in 2002. However, zinc oxide and *R. sativus* powder were slightly less effective against both types of disease symptoms in the 2001 trial compared with the 2002 trial (Figures 7.6 & 7.7). This could be due to the fact the disease pressure was higher in the second trial, as the average powdery scab severity score for the non-treated, inoculated control in 2001 and 2002 show (2.0 and 2.7 respectively). This could have been due to a higher amount of inoculum on the seed used in 2002 compared with the seed in 2001. The average root gall score in the inoculated control was the same in both years (2.0).

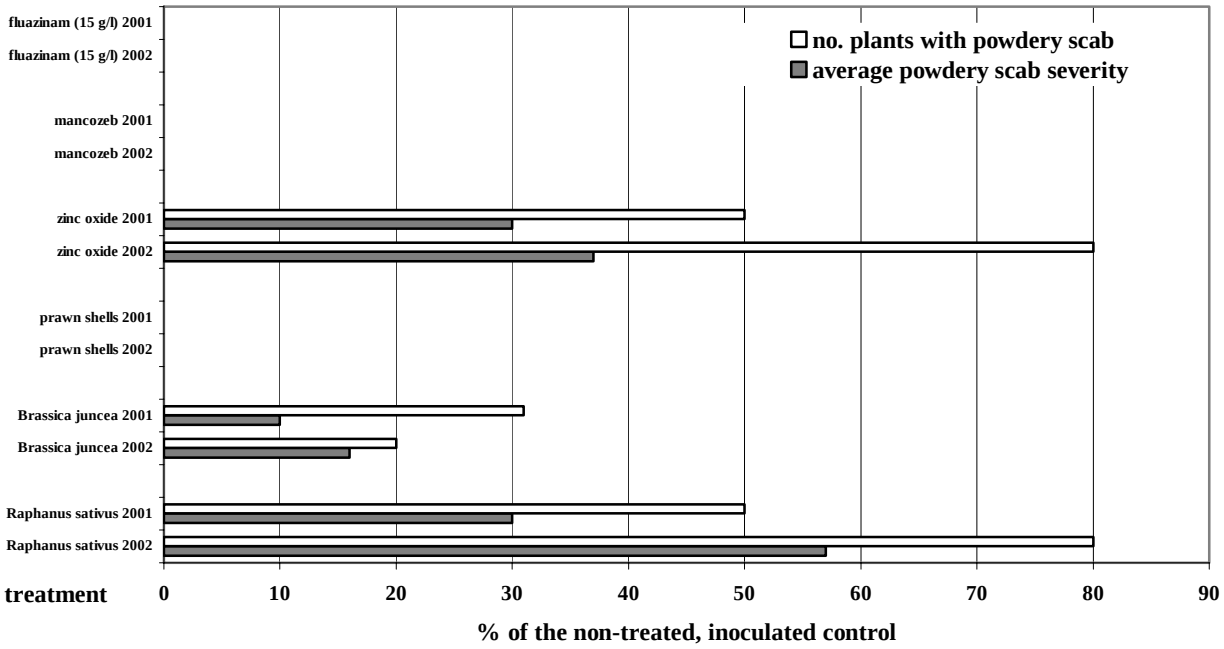


FIGURE 7.6 COMPARISON OF THE EFFICACY OF DIFFERENT SOIL APPLIED TREATMENTS IN REDUCING ROOT GALLING CAUSED BY SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN POTATO CV. 'ESTIMA' AT MATURITY IN TWO DIFFERENT TRIALS, EXPRESSED AS A PERCENTAGE OF THE NON-TREATED, INOCULATED CONTROL (N = 5).

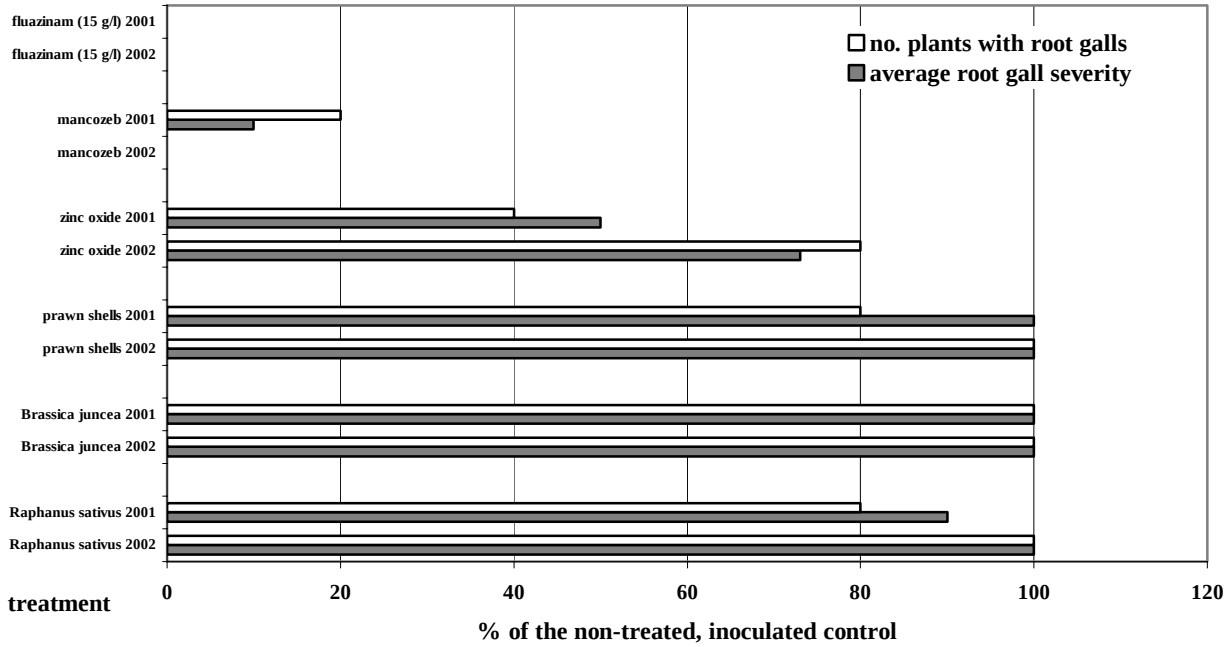


FIGURE 7.7 COMPARISON OF THE EFFICACY OF DIFFERENT SOIL APPLIED TREATMENTS IN REDUCING POWDERY SCAB CAUSED BY SOIL BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN POTATO CV. 'ESTIMA' AT MATURITY IN TWO DIFFERENT TRIALS, EXPRESSED AS A PERCENTAGE OF THE NON-TREATED, INOCULATED CONTROL (N = 5).

Tuber applied

Two products were tested against tuber borne inoculum of *S. subterranea* (Table 7.3).

TABLE 7.3 TUBER APPLIED TREATMENTS TESTED ON EFFICACY AGAINST TUBER BORNE INOCULUM OF *SPONGOSPORA SUBTERRANEA* IN THE 2001 TRIAL.

Treatment type	Treatment name	Application
Fungicide	fluazinam (Shirlan; 500 g/l)[1]	0.3 ml product/kg tubers
Disinfectant	peroxyacetic acid (Jet 5, 3%)[2]	5 min dip

The products tested were supplied/produced by: 1 Syngenta; 2 Certis

In case of these pre-plant tuber treatments, 'Estima' tubers were inoculated with sporeballs by dipping them in a sporeball solution (50,000 per ml), air drying them and then treating them with the product to be tested. The tubers were shortly dipped in fluazinam and air dried or dipped in peroxyacetic acid for 5 min and air dried. Control tubers were not treated. All tubers were planted in non-inoculated sandy loam soil and placed in growth rooms under the same conditions as described above for the soil applied treatments. Plants were lifted at tuber initiation and maturity and assessed as described above.

No powdery scab or root galls were found at tuber initiation in the plants grown from seed tubers treated with fluazinam or peroxyacetic acid. Some plants in the inoculated control showed light root galling but no powdery scab was present. Even at maturity, root galling (incidence 40%, average severity 0.6) and powdery scab (incidence 20%, average severity 1.1) symptoms were very limited in the inoculated control. One out of five plants in the fluazinam treatment showed symptoms similar to the positive control. Root galls were not observed in the peroxyacetic acid treatments but one plant showed tubers with light powdery scab. The low disease incidence in the inoculated control meant that no significant effects of any of the two treatments could be demonstrated. It could be that the amount of inoculum applied to the tubers was too low to show treatment effects.

Chemical control (controlled conditions) - main conclusions:

- **Several treatments have been identified which are more effective against soil borne inoculum of *S. subterranea* than zinc.**
- **Fluazinam and mancozeb are the most effective chemicals for the control of disease caused by *S. subterranea*. However, phytotoxic side effects may occur.**
- **Application of prawn shell waste to the soil before planting can eliminate powdery scab but is not effective against root galling. Root gall incidence is reduced by applying a chemical treatment after tuber initiation.**
- ***Brassica juncea* is the most effective biofumigant crop tested and application of freeze dried powder of this species to the soil before planting may significantly reduce powdery scab incidence and severity.**

Field experiments

Objective: To evaluate disinfectants, fungicides, novel chemicals and biofumigants as pre-plant soil and tuber treatments by setting up replicated field plot experiments to examine their effectiveness in the field in the situations where soil contamination is present and/or tuber infection/contamination is present (SAC).

Milestone 6: Assess field trials for relative efficacy of different chemicals, and produce interpreted grower report on results - *achieved*

Methodology

In each of three years, two trials were carried out in Aberdeenshire to evaluate potential chemical control measures. The source of inoculum in one of the two trials was predominantly seed-borne, and the other was soil- or soil- and seed-borne. The trials were a randomised block design with five replicates. Plot sizes were usually 6.25m x 4 drills. A summary of the sites is given in Table 7.4.

TABLE 7.4 DETAILS OF FIELD TRIAL SITES FOR THE EVALUATION OF CHEMICAL CONTROL MEASURES

Year	Site	Source of inoculum	Variety	Date planted	Date haulm destruction	Date harvested
2000	Tillycorthie	Soil	Estima*	11 May	7 & 11 August	2 October
2000	Lochgreens	Seed	Estima*	5 June	14 & 18 September	5 October
2001	Tillycorthie	Soil	Estima	28/29 May	13 & 21 September	13 October
2001	South Fornet	Seed	Estima*	21 May	Not known	27 October
2002	Tillycorthie	Soil	Estima*	31 May	4 & 12 September	7 November
2002	West Fingask	Seed	Estima	17 May	4 & 12 September	31 October

* with seed infection

In each trial crop safety was determined by measuring emergence at regular intervals, crop vigour and tuber yield and tuber number. After harvest, tuber disease incidence and severity was estimated on a sample of 50 tubers as well as physical defects such as netting, cracking, damage etc.

Chemicals were selected for testing on the following criteria:
- Previous evidence of efficacy against powdery scab
- Evidence of efficacy from the pot trials at SCRI
- Evidence for control from the scientific literature
- Ad hoc reports of control from agricultural sources

The aggregated results for all six trials shown below are for a single assessment of emergence at or after 50% emergence (Table 7.5), total yield and total tuber number (Table 7.6) and incidence and severity of powdery scab (Table 7.7).

TABLE 7.5 CHEMICAL CONTROL OF POWDERY SCAB – SUMMARY OF RESULTS – EMERGENCE (%)

	Site and year	**Tillycorthie 2000**		**Lochgreens 2000**		**Tillycorthie 2001**		**South Fornet 2001**		**Tillycorthie 2002**		**W Fingask 2002**	
	Main source of inoculum	**Soil**		**Seed**		**Soil**		**Seed**		**Soil**		**Seed**	
A.I.(s)	**Product, dose and method of application**	**E**	**12/6**	**E**	**22/6**	**E**	**28/6**	**E**	**20/6**	**E**	**21/6**	**E**	**19/6**
Control	-	82.0 ab		73		73.2 a		81.0 a		75.2 abc		97 a	
Soil treatments													
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting	91.2 a		65		71.2 a		80.0 a		77.2 ab		95 a	
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied to split ridge during planting	90.4 a		78									
Flusulphamide	Experimental product (Hortichem) 1.2 kg a.i./ha applied to soil and incorporated prior to planting	86.4 ab											
Hydrogen peroxide	Herlisil (50% a.i.) 14 l/ha applied to soil and incorporated prior to planting									90.8 a			
Mancozeb	15 kg/ha applied onto beds and incorporated prior to planting											94 a	
Mancozeb	33 l/ha applied onto beds and incorporated prior to planting					73.2 a				70.4 abc			
Maneb	15.0 kg a.i./ha (18.75 kg/ha product) applied onto beds and incorporated prior to planting	46.0 d		76.5									
Sulphur	Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S, 125 kg product/ha applied onto beds and incorporated prior to planting	80.4 ab		76.5		74.0 a				73.6 abc		96 a	
Sulphur	Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S, 250 kg product/ha applied onto beds and incorporated prior to planting									83.8 a			
Zinc oxide	(Sigma) 81.38% Zinc, 15.0 kg a.i./ha applied onto beds and incorporated prior to planting	87.6 ab		80.5		72.8 a		78.5 a		74.0 abc		97 a	
Prawn shells	10 t/ha applied to beds and incorporated prior to planting					58.8 a				62.4 bc			
Tuber treatments													
Antec FFS	(Antec Farm Fluid S) 1% solution: seed dipped for 5 minutes prior to planting	1.2 e											
Fentin hydroxide	Farmatin 560 (Aventis Crop Science) 43.7% a.i. 20g in 10 l water: seed dipped for 5 minutes prior to planting	62.0 c		82.5									
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.15 l product/t seed in 1 litre of water					22.4 b		44.5 b					
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.3 l product/t seed in 1 litre of water					21.2 b		36.0 b					
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.6 l product/t seed in 1 litre of water					18.0 b		35.0 b					

Fluazinam	Shirlan (Zeneca) 500g/l a.i. 1.0 l product/t seed in 1 litre of water	69.6 bc		74									
Hydrogen peroxide	Herlisil (50% a.i.) 3% solution: seed dipped for 5 minutes prior to planting											97 a	
Mancozeb	3.3 l/tonne tuber treatment					15.6 b				30.4 e		93 a	
Mancozeb	6.6 l/tonne tuber treatment							36.0 b					
Mancozeb	1.88 kg/tonne tuber treatment											74 c	
Peracetic acid	Jet 5 (Hortichem) 5% w/w. 3% solution: seed dipped for 5 minutes prior to planting	35.2 d		70		7.2 b		48.5 b				85 b	
Peracetic acid	Jet 5 (Hortichem) 5% w/w. 3% solution: sprayed onto seed prior to planting							37.5 b					
Zinc Oxide	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting							37.5 b		23.6 e		74 c	
Combination treatments													
Zinc oxide + fluazinam	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									29.6 de			
Mancozeb + fluazinam	1.88 kg/tonne tuber treatment + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									18.8 e			
Hydrogen peroxide + fluazinam	Herlisil (50% a.i.) 3% solution: seed dipped for 5 minutes prior to planting + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									50.0 cd			
Mancozeb + prawn shells	1.88 kg/tonne tuber treatment + 10 t/ha applied to beds and incorporated prior to planting									14.0 e			
Zinc oxide + sulphur	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting + Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S 125 kg product/ha applied onto beds and incorporated prior to planting									18.4 e			
Other treatments													
Unknown	Biomex incorporated at 1.0 l/ha before planting					69.6 a							
	LSD and significance	11.9 ***		12.0 Ns		11.7 ***		12.4 ***		17.2 ***		5.7 ***	

*E=Emergence (%) at date stated. Where significant differences exist, means with the same letter are not significantly different

TABLE 7.6 CHEMICAL CONTROL OF POWDERY SCAB – SUMMARY OF RESULTS – TOTAL YIELD (Y - T/HA) AND TOTAL TUBER NUMBER (TN - 000'S/HA)

	Site and year	**Tillycorthie 2000**		**Lochgreens 2000**		**Tillycorthie 2001**		**South Fornet 2001**		**Tillycorthie 2002**		**W Fingask 2002**	
	Main source of inoculum	**Soil**		**Seed**		**Soil**		**Seed**		**Soil**		**Seed**	
A.I.(s)	**Product, dose and method of application**	**Y**	**TN**	**Y**	**TN**	**Y**	**TN**	**Y**	**TN**	**Y**	**TN**	**Y**	**TN**
Control	-	42.98 a	519.6 a	68.75	526.3 a	73.7 a	518.1 a	43.7 abc	331.0	53.4 ab	444.7 abc	44.6	446.3
Soil treatments													
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting	44.50 a	519.8 a	68.88	535.0 ab	67.3 abc	493.2 abc	44.4 ab	349.8	58.2 a	470.9 a	44.2	443.0
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied to split ridge during planting	40.78 a	453.6 bcde	69.0	495.8 b								
Flusulphamide	Experimental product (Hortichem) 1.2 kg a.i./ha applied to soil and incorporated prior to planting	44.18 a	510.6 ab										
Hydrogen peroxide	Herlisil (50% a.i.) 14 l/ha applied to soil and incorporated prior to planting									55.3 ab	442.5 abc		
Mancozeb	15 kg/ha applied onto beds and incorporated prior to planting											47.6	423.4
Mancozeb	33 l/ha applied onto beds and incorporated prior to planting					67.0 abc	477.6 abc			56.1 ab	471.8 a		
Maneb	15.0 kg a.i./ha (18.75 kg/ha product) applied onto beds and incorporated prior to planting	37.88 ab	466.6 abcd	70.52	539.5 ab								
Sulphur	Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S, 125 kg product/ha applied onto beds and incorporated prior to planting	41.5 a	496.4 abc	74.95	556.0 ab	65.8 abc	494.8 abc			51.8 abc	436.2 abc	43.0	402.3
Sulphur	Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S, 250 kg product/ha applied onto beds and incorporated prior to planting									56.3 ab	471.1 a		
Zinc oxide	(Sigma) 81.38% Zinc, 15.0 kg a.i./ha applied onto beds and incorporated prior to planting	42.16 a	507.2 ab	68.65	518.8 ab	69.5 ab	497.4 abc	48.0 a	368.5	50.3 abc	424.0 abc	44.3	423.0
Prawn shells	10 t/ha applied to beds and incorporated prior to planting					65.0 abc	436.6 c			54.4 ab	453.9 ab		
Tuber treatments													
Antec FFS	(Antec Farm Fluid S) 1% solution: seed dipped for 5 minutes prior to planting	10.50 c	142.8 f										
Fentin hydroxide	Farmatin 560 (Aventis Crop Science) 43.7% a.i. 20g in 10 l water: seed dipped for 5 minutes prior to planting	41.08 a	486.6 abcd	70.18	586.5 a								
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.15 l product/t seed in 1 litre of water					63.1 bc	488.5 abc	38.3 abc	329.0				
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.3 l product/t seed in 1 litre of water					61.6 bc	482.5 abc	33.5 c	296.0				
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.6 l product/t seed in 1 litre of water					58.8 c	449.0 bc	39.1 abc	340.0				

Fluazinam	Shirlan (Zeneca) 500g/l a.i. 1.0 l product/t seed in 1 litre of water	40.62 a	488.0 abcd	70.08	602.0 a								
Hydrogen peroxide	Herlisil (50% a.i.) 3% solution: seed dipped for 5 minutes prior to planting											45.7	420.4
Mancozeb	3.3 l/tonne tuber treatment					64.1 abc	504.6 ab			50.8 abc	445.7 abc	45.3	458.4
Mancozeb	6.6 l/tonne tuber treatment							34.1 bc	313.5				
Mancozeb	1.88 kg/tonne tuber treatment											39.5	400.7
Peracetic acid	Jet 5 (Hortichem) 5% w/w. 3% solution: seed dipped for 5 minutes prior to planting	34.72 ab	438.2 de	67.1	562.0 ab	59.3 bc	451.6 bc	34.6 bc	303.3			41.8	402.1
Peracetic acid	Jet 5 (Hortichem) 5% w/w. 3% solution: sprayed onto seed prior to planting							36.2 bc	317.0				
Zinc Oxide	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting							38.9 abc	328.5	49.9 bc	444.3 abc	44.6	397.8
Combination treatments													
Zinc oxide + fluazinam	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									53.9 abc	487.8 a		
Mancozeb + fluazinam	1.88 kg/tonne tuber treatment + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									48.4 bc	438.0 abc		
Hydrogen peroxide + fluazinam	Herlisil (50% a.i.) 3% solution: seed dipped for 5 minutes prior to planting + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									50.5 abc	476.6 a		
Mancozeb + prawn shells	1.88 kg/tonne tuber treatment + 10 t/ha applied to beds and incorporated prior to planting									44.9 c	391.9 c		
Zinc oxide + sulphur	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting + Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S 125 kg product/ha applied onto beds and incorporated prior to planting									45.3 c	404.1 bc		
Other treatments													
Unknown	Biomex incorporated at 1.0 l/ha before planting					68.3 abc	490.2 abc						
	LSD and significance	4.08 ***	37.5 ***	5.31 Ns	56.2 *	6.22 ***	39.3 **	6.43 ***	51.1 Ns	4.71 ***	37.49 ***	5.27 Ns	78.24 Ns

*Y=Total yield (t/ha), TN=Total tuber number (000's/ha). Where significant differences exist, means with the same letter are not significantly different

TABLE 7.7 CHEMICAL CONTROL OF POWDERY SCAB – SUMMARY OF RESULTS – CONTROL OF POWDERY SCAB INCIDENCE (I) AND SEVERITY (S)

	Site and year	**Tillycorthie 2000**		**Lochgreens 2000**		**Tillycorthie 2001**		**South Fornet 2001**		**Tillycorthie 2002**		**W Fingask 2002**	
	Main source of inoculum	**Soil**		**Seed**		**Soil**		**Seed**		**Soil**		**Seed**	
A.I.(s)	**Product, dose and method of application**	**I***	**S***	**I**	**S**	**I**	**S**	**I**	**S**	**I**	**S**	**I**	**S**
Control	-	44.8	9.51 ab	26.5	1.5	37.8	1.4	70.0	9.6	91 a	17.9 a	82	2.4
Soil treatments													
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting	42.8	3.86 cd	25.5	1.0	25.6	0.5	65.0	5.6	65 d	6.0 de	84	3.2
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied to split ridge during planting	45.6	6.02 bcd	31.5	1.3								
Flusulphamide	Experimental product (Hortichem) 1.2 kg a.i./ha applied to soil and incorporated prior to planting	43.4	6.99 abcd										
Hydrogen peroxide	Herlisil (50% a.i.) 14 l/ha applied to soil and incorporated prior to planting									84 abc	11.1 bcde		
Mancozeb	15 kg/ha applied onto beds and incorporated prior to planting											68	5.1
Mancozeb	33 l/ha applied onto beds and incorporated prior to planting					36.0	1.0			76 abcd	5.3 e		
Maneb	15.0 kg a.i./ha (18.75 kg/ha product) applied onto beds and incorporated prior to planting	41.6	3.69 cd	43.0	2.8								
Sulphur	Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S, 25 kg product/ha applied onto beds and incorporated prior to planting	45.0	8.23 abc	55.5	3.6	39.6	1.0			87 ab	14.2 ab	85	3.2
Sulphur	Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S, 250 kg product/ha applied onto beds and incorporated prior to planting									88 ab	12.6 abc		
Zinc oxide	(Sigma) 81.38% Zinc, 15.0 kg a.i./ha applied onto beds and incorporated prior to planting	41.6	5.71 bcd	30.0	1.8	41.6	1.6	67.3	11.5	90 ab	15.4 abc	84	3.9
Prawn shells	10 t/ha applied to beds and incorporated prior to planting					32.2	1.2			82 abc	10.7 cde		
Tuber treatments													
Antec FFS	(Antec Farm Fluid S) 1% solution: seed dipped for 5 minutes prior to planting	40.8	2.62 d										
Fentin hydroxide	Farmatin 560 (Aventis Crop Science) 43.7% a.i. 20g in 10 l water: seed dipped for 5 minutes prior to planting	44.8	5.92 bcd	39.5	2.7								
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.15 l product/t seed in 1 litre of water					32.8	1.0	64.5	5.4				
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.3 l product/t seed in 1 litre of water					24.0	0.4	69.5	5.4				
Fluazinam	Shirlan (Zeneca) 500g/l a.i. 0.6 l product/t seed in 1 litre of water					28.4	0.9	65.0	5.1				

Fluazinam	Shirlan (Zeneca) 500g/l a.i. 1.0 l product/t seed in 1 litre of water	46.0	4.74 bcd	43.0	1.9								
Hydrogen peroxide	Herlisil (50% a.i.) 3% solution: seed dipped for 5 minutes prior to planting											76	3.2
Mancozeb	3.3 l/tonne tuber treatment					32.0	0.8			88 ab	9.9 cde	80	4.2
Mancozeb	6.6 l/tonne tuber treatment							73.0	8.4				
Mancozeb	1.88 kg/tonne tuber treatment											84	2.1
Peracetic acid	Jet 5 (Hortichem) 5% w/w. 3% solution: seed dipped for 5 minutes prior to planting	46.0	3.63 cd	44.5	2.6	23.2	0.7	64.5	5.2			78	2.7
Peracetic acid	Jet 5 (Hortichem) 5% w/w. 3% solution: sprayed onto seed prior to planting							62.3	8.0				
Zinc Oxide	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting							67.0	5.6	92 a	14.1 abc	81	4.4
Combination treatments													
Zinc oxide + fluazinam	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									68 cd	5.2 e		
Mancozeb + fluazinam	1.88 kg/tonne tuber treatment + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									82 abc	6.2 de		
Hydrogen peroxide + fluazinam	Herlisil (50% a.i.) 3% solution: seed dipped for 5 minutes prior to planting + Shirlan (Zeneca) 500g/l a.i. 3.0 l product/ha applied onto beds and incorporated prior to planting									73 bcd	5.1 e		
Mancozeb + prawn shells	1.88 kg/tonne tuber treatment + 10 t/ha applied to beds and incorporated prior to planting									85 ab	12.1 abcd		
Zinc oxide + sulphur	(Sigma) 81.38% Zinc, 1 kg/tonne seed tuber treatment applied at planting + Sulfer 95 (New-Trition) 23.5% SO3 giving 95%S 125 kg product/ha applied onto beds and incorporated prior to planting									92 a	17.4 ab		
Other treatments													
Unknown	Biomex incorporated at 1.0 l/ha before planting					38.0	1.4						
	LSD and significance	2.97 Ns	2.47 *	2.06 Ns	23.2 Ns	16.0 Ns	1.05 Ns	16.8 Ns	5.98 Ns	10.6 ***	4.26 ***	17.4 Ns	3.70 Ns

*I=Incidence (% tubers) of infection, S=Severity (% surface area) of infection. Where significant differences exist, means with the same letter are not significantly different

Results

Compared with the untreated control, tuber and soil treatments for the control of powdery scab influenced emergence to different extents. In general, tuber treatments had the greatest effect on emergence. For example, fluazinam seed treatment frequently delayed emergence. The phenolic disinfectant, Antec Farm Fluid S, had the most dramatic phytotoxic effect on emergence and this carried through into crop development and yield. Another disinfectant, peracetic acid (Jet 5) when used as a 3% dip, also significantly delayed emergence on four out of five occasions. There were few significant effects on emergence with soil-applied chemicals, although significant reductions were recorded with maneb and prawn shells. All combination treatments tested in 2002 significantly affected emergence.

No treatments significantly increased total yield or tuber number. Significant reductions were recorded with those chemicals that affected emergence, for example fluazinam seed treatments, peracetic acid, prawn shells and several of the combinations treatments. Occasionally, other chemicals affected yield or tuber number. These included a high dose mancozeb tuber treatment (2001, South Fornet) and zinc oxide tuber treatment (2002, Tillycorthie).

There were few effects of the chemicals tested on other tuber diseases or disorders. Powdery scab was the principal disease present in most trials. Moderate to high incidences of powdery scab occurred in all trials but high severity levels (>5% surface area on the untreated control) were recorded only in three trials. Significant reductions in incidence only occurred at Tillycorthie in 2002 and significant reductions in severity at Tillycorthie in 2000 and 2002.

Significant reductions in incidence of powdery scab occurred with three chemicals in the Tillycorthie 2002 trial only. These were fluazinam soil treatment (3 l product/ha) and fluazinam in combination with zinc oxide seed treatment and hydrogen peroxide seed treatment. By contrast, a number of chemicals reduced severity of powdery scab: fluazinam soil treatment (3 l product/ha) either incorporated into the soil (2 trials) or applied in the furrow (1 trial), flusulphamide (1 trial), mancozeb soil treatment (1 trial), maneb soil treatment (1 trial), zinc oxide soil treatment (1 trial), prawn shells (1 trial), Antec FFS (1 trial), fluazinam seed treatment (1.0 l product/t, 1 trial), fentin hydroxide (1 trial), peracetic acid (1 trial) and the combination treatments of fluazinam with zinc oxide, mancozeb and hydrogen peroxide (1 trial each). Note that some products that reduced disease severity also affected emergence and total yield or tuber number.

Discussion

The observation in earlier chemical control studies that chemical control can reduce severity more often than incidence has been confirmed in these trials.

Of the chemicals tested, none gave consistent reductions in powdery scab. Many gave small non-significant reductions or significant reductions infrequently. The only chemical treatment that consistently reduced powdery scab was fluazinam soil treatment. This confirms earlier evidence that this product can be effective (see below). However, its effectiveness was greatest where soil-borne inoculum was the principal source of inoculum and limited where tubers were the main source of inoculum. This suggests that effective control probably lies with combination treatments of an effective tuber treatment plus the fluazinam soil treatment. However, delays in emergence and reductions in yield may occur with combination treatments as demonstrated in these results.

In New Zealand, fluazinam soil treatment has approval for the control of powdery scab. Growers there use this treatment in conjunction with a tuber dip treatment with formaldehyde to reduce the impact of the disease. In the UK, fluazinam does not have approval for the control of powdery scab. Additionally, it is considered that a formaldehyde tuber dip would be an unlikely option for use because of health and safety issues. However, from these trials there would appear to be scope to investigate the use of a disinfectant tuber treatment in conjunction with a fluazinam soil treatment.

As the only chemical showing any evidence of control of powdery scab, it is recommended that data be submitted to PSD for Specific Off-label Approval for this product for use on seed crops. The main issue for this use is the potential impact on soil flora and fauna and assistance with the SOLA would be required from the manufacturer of fluazinam, Syngenta. The ecotoxicological package required by the Pesticide Safety Directorate might require a reasonable financial investment. This might best be funded by collaboration between BPC and Syngenta. Preliminary contact with Syngenta on this matter has been made.

Summary of data on the efficacy of fluazinam for the control of powdery scab

Only those trials where the incidence of powdery scab on the untreated control were greater than 25% are reported.

All the trials summarised below that evaluated fluazinam as a soil treatment (Table 7.8) were carried out by SAC, although two were in conjunction with Dr Richard Falloon and were funded by NZ VegFed (1994a, 1994c). Mr Willie Kyle (Trial 2000c) sponsored another trial.

A clear dose response was apparent, particularly in the 1995 trial (Table 7.8). The control of *S. subterranea* increased as the dose applied increased to 4.0 kg a.i./ha. This trial also indicated that incorporation was more effective than application in-furrow. The most effective incorporation was achieved when the chemical was applied during de-stoning (2000, seed+soil inoculum).

Although the results were variable, at higher doses, reductions were more frequently recorded, irrespective of the source of inoculum. The percentage reduction in severity was usually greater than that in incidence. Complete control was never achieved.

General comments on chemical control of powdery scab

There is evidence that certain chemicals can have a substantial effect on *S. subterranea* but their consistency of effect is poor. This variability in efficacy of both seed tuber and soil treatments is unhelpful when attempting to provide guidance to a potato grower about chemical control of *S. subterranea.* The results suggest that consistent control cannot be guaranteed and the use of a chemical as a sole control measure will depend on a financial analysis of the proportion of occasions a cost effective response is achieved.

TABLE 7.8 EVALUATION OF FLUAZINAM SOIL TREATMENT FOR THE CONTROL OF *S. SUBTERRANEA*

Year	Cv[1]	Source of inoculum	Fluazinam dose (kg a.i./ha) and application method	Incidence			Severity		
				UT	%[2]	Sig.	UT	%[2]	Sig.
1994a	E	Seed	0.5 – incorporated[4]	29	0	Ns	1	24	Ns
			1.0 – incorporated[4]	29	-3	Ns	1	-7	Ns
			2.0 – incorporated[4]	29	-45	Ns	1	-15	Ns
			4.0 – incorporated[4]	29	-17	Ns	1	-18	Ns
1994b	E	Seed	4.0 – incorporated[4]	35	62	**	1	81	**
2000a	E	Seed	1.5 - incorporated	27	4	Ns	2	29	Ns
			1.5 - in furrow	27	-19	Ns	2	8	Ns
2001	E	Seed	1.5 - incorporated	70	7	Ns	10	42	Ns
2002	E	Seed	1.5 - incorporated	82	-2	Ns	2	-33	Ns
1994c	E	Soil	0.5 – incorporated[4]	47	10	Ns	12	-15	Ns
			1.0 – incorporated[4]	47	10	Ns	12	-4	Ns
			2.0 – incorporated[4]	47	8	Ns	12	-8	Ns
			4.0 – incorporated[4]	47	9	Ns	12	21	Ns
1994d	E	Soil	4.0 – incorporated[4]	53	27	Ns	4	59	Ns
1995	E	Soil	0.25 – in furrow	81	25	**	41[3]	47	**
			0.5 – in furrow	81	20	**	41[3]	40	**
			1.2 – in furrow	81	29	**	41[3]	57	**
			2.0 – in furrow	81	27	**	41[3]	51	**
			4.0 – in furrow	81	47	**	41[3]	61	**
			4.0 - incorporated	81	57	**	41[3]	86	**
2001	E	Soil	1.5 - incorporated	38	32	Ns	1	64	Ns
2002	E	Soil	1.5 - incorporated	91	29	***	18	66	***
2000b	E	Seed+Soil	1.5 - incorporated	45	5	Ns	10	59	*
			1.5 - in furrow	45	-2	Ns	10	37	Ns
2000c	E	Seed+Soil	1.5 – incorporated[5]	51	49	***	2	70	*

[1]Variety: E = Estima
[2] % = % control relative to untreated (UT)
[3] Severity expressed as % 'unmarketable' tubers i.e. >5% surface area affected
[4] Incorporated to at least 20cm after spraying onto destoned bed
[5] Incorporated by spraying soil passing through destoner
Ns = Not significant; *,**,*** = significant at p< 0.05, 0.01, 0.001

In assessing the effectiveness of control treatments, it is important to have an understanding of inoculum. Because *S. subterranea* cannot be cultured in vitro, the initial inoculum in the trials described in this paper could not be quantified. Thus, one reason for the variability in results could be differences in inoculum between trials. Whilst every effort was made to identify trial sites for control of tuber-borne inoculum free from soil-borne inoculum, the absence of soil contamination could not be guaranteed. Conversely, where control of soil-borne inoculum was being tested, the absence of contamination of seed tubers with cystosori was not possible to confirm. This was demonstrated in section 6D above.

Another complicating factor in interpreting chemical control trials is that inoculum can increase in the roots of potato crops and, as a result, even low levels of initial inoculum can lead to severe tuber infection (Burnett, 1991). Conducive conditions for inoculum multiplication early in crop growth may increase the disease pressure for subsequent tuber infection and reduce effectiveness of chemical control. Ideal conditions for infection are free moisture in the soil matrix to permit the zoospores to swim to the host tissue and a temperature between c. 9^{o} and 17^{o}C. When soil moisture is high, the conditions may be suitable for the pathogen but they may

also improve release of the chemical control agent in the soil, although chemical release in soil may be under the control of many factors (e.g. pH). As the zoospores are the phase of the life cycle susceptible to chemical control, the chemical control agent needs to be in the free moisture in soil in sufficient quantity to interact with zoospores. An *in vitro* experiment reported by Burnett & Wale (1993), demonstrated that zinc was only effective when inoculum was low and zinc concentrations high.

In vitro studies by Fornier (1997) on the release of primary zoospores after 5 days incubation in the presence of 1 ppm of a range of fungicides, demonstrated that the four fungicides tested inhibited release. Zinc oxide significantly reduced the release to 512 zoospores/ml and fluazinam to 860 zoospores/ml from 1327/ml in the water control. At 10ppm, the release of zoospores was totally inhibited by these two fungicides. However, measurement of zinc in soil solution *in vivo* (Burnett, 1991) after zinc applications of 10 or 15 kg/ha found it rose to just 0.5-1.1 ppm.

These results suggest that chemical treatment is unlikely ever to be fully effective except where inoculum pressure is low. Since inoculum may be found at any point within the soil profile, an even distribution of a chemical within the soil profile is likely to result in more effective control of soil-borne *S. subterranea*. The results with fluazinam confirm this.

Powdery scab is such a difficult disease to control and can have such a major impact on profitability, even small reductions in disease can be valuable. Thus, for example, seed growers who can increase the proportion in a seed fraction by 1.0 tonne/ha will more than cover the cost of most treatments. For the seed grower, a reduction in severity to bring a higher percentage of tubers below the surface area tolerance for certification can be important. In these trials chemical control appears to be more consistent in reducing severity. For the ware grower, however, a reduction in incidence is more beneficial and this seems to be difficult to achieve.

Chemical control (field trials) – main conclusions:

- **There is evidence that certain chemicals can have a substantial effect on *S. subterranea* but their consistency of effect is poor.**
- **Chemical control can reduce severity more often than incidence of powdery scab.**
- **The only chemical treatment that consistently reduced powdery scab was fluazinam soil treatment.**
- **Fluazinam's effectiveness was greatest where soil-borne inoculum was the principal source of inoculum and limited where tubers were the main source of inoculum.**
- **Effective control probably lies with combination treatments of an effective tuber treatment plus the fluazinam soil treatment.**
- **It is recommended that data be submitted to PSD for Specific Off-label Approval for fluazinam as a soil treatment for use on seed potato crops.**
- **One reason for the variability in results could be differences in inoculum between trials.**

Timing of fungicide treatments

Objective: To evaluate disinfectants, fungicides, novel chemicals and biofumigants as pre-plant soil and tuber treatments by evaluating the timing of treatments on the development of secondary inoculum on roots and tubers in the developing crop (field & glasshouse). These experiments examined the potential for introducing fungicides at critical times in the pathogen life cycle. Delivery in the field was investigated using irrigation methods (SCRI/SAC).

Milestone 7: Assess efficacy of timing of fungicide application and produce interpreted report from results - *achieved*

Experiments under controlled conditions

The growth room experiments on timing and combination of treatments are described above under section 7A.

Field experiments

Control of powdery scab by application of zinc in irrigation water to the growing crop

It has been demonstrated that zinc treatments can give small reductions in severity when applied as tuber treatments (1 kg ZnO/ tonne) or incorporated into soil (15 kg Zn/ha) (Burgess *et al*., 1992). It has been shown however, that zinc treatments are most effective when used in a low pressure situation, for example when inoculum or environmental conditions are limiting or when a moderately resistant variety is used (Burnett, 1991, Fornier, 1997). Zinc is a relatively inexpensive product to purchase. It is available in slow release forms (e.g. zinc oxide) or in highly soluble forms such as zinc sulphate. Previous disease control studies have suggested that highly soluble forms are less effective because they dissociate rapidly in soil and the zinc ions are adsorbed onto soil or organic particles or washed out of the soil. Since the critical time for infection by *S. subterranea* is around tuber initiation which may occur approximately 50 days after planting, it is likely that zinc sulphate applied at or before planting may not be available at the critical time. However, if zinc sulphate is applied in irrigation water in small doses, its activity may be extended through the critical period. An experiment was carried out over three years to evaluate the effect of applying up to 5 doses of 3.0 kg zinc/ha (to give a maximum of 15 kg Zn/ha) as zinc sulphate on crop development, yield, tuber numbers and disease development. In order to ensure equality of water supply all treatments received an application of water at all timings. Applications were made using T-tape trickle irrigation.

The five application timings were targeted at 0, 10, 20, 30 and 40 days after 50% emergence in order to cover the tuber initiation period (Table 7.9). Each application supplied the equivalent of 10 mm rainfall.

Trials took the form of a randomised block with two replicates. They were planted at Tillycorthie Farm, Udny Station, Aberdeenshire in a field where soil inoculum was high. The variety used was Estima. Measurements were made of emergence, vigour, yield and tuber number and incidence of diseases and disorders on the daughter tubers.

TABLE 7.9 TIMING OF ZINC TREATMENTS APPLIED IN IRRIGATION WATER FOR THE CONTROL OF POWDERY SCAB

Treatment	**Days after 50% emergence**				
	0	10	20	30	40
1	Water	Water	Water	Water	Water
2	Zn	Water	Water	Water	Water
3	Zn	Zn	Water	Water	Water
4	Zn	Zn	Zn	Water	Water
5	Zn	Zn	Zn	Zn	Water
6	Zn	Zn	Zn	Zn	Zn
7	Water	Water	Zn	Water	Water
8	Water	Water	Zn	Zn	Water
9	Water	Water	Zn	Zn	Zn

Results

As the zinc and water treatments were applied after emergence, it is unsurprising that there were no significant differences between treatments in emergence (Table 7.10). Vigour assessments were made mostly in July after zinc treatments had been applied. However, once again, no significant differences were detected (Table 7.11). There was a suggestion in two trials that five applications of zinc reduced total yield but in each trial there were no significant differences between treatments (Table 7.12). Significant reductions in total tuber number were found in the 2000 trial when 2, 4 and 5 applications of zinc were applied from tuber initiation (Table 7.13). However, in the 2001 and 2002 trials no such effect was recorded. The incidence and severity of powdery scab in the three trials was moderate to high (Tables 7.14 & 7.15). No significant differences were recorded, although there was a suggestion in the 2000 trial that 3-5 applications of zinc might lead to a reduction in incidence and severity.

TABLE 7.10 EVALUATION OF CONTROL OF POWDERY SCAB BY USING ZINC APPLIED IN IRRIGATION WATER - % EMERGENCE

No.	**Treatment**	**2000**	**2001**	**2002**	**Mean**
	Date	7 June	18 June	17 June	
1	5 x Water	30	70	73	57.7
2	1 X Zn + 4 X Water	36	72	84	64.0
3	2 X Zn + 3 X Water	48	78	67	70.0
4	3 X Zn + 2 X Water	32	52	65	49.7
5	4 X Zn + 1 X Water	40	62	77	59.7
6	5 X Zn	48	76	62	62.0
7	2 X Water + 1 X Zn + 2 X Water	30	58	85	57.6
8	2 X Water + 2 X Zn + 1 X Water	38	62	70	56.7
9	2 X Water + 3 X Zn	44	67	54	55.0
	LSD	41.0	27.6	22.7	
	Significance	Ns	Ns	Ns	

TABLE 7.11 EVALUATION OF CONTROL OF POWDERY SCAB BY USING ZINC APPLIED IN IRRIGATION WATER - VIGOUR (0-9)

No.	Treatment	2000	2001	2002	Mean
	Date	7 July	3 July	17 June	
1	5 x Water	7.5	7.5	8	7.7
2	1 X Zn + 4 X Water	7.5	7.5	8	7.7
3	2 X Zn + 3 X Water	7.0	8.0	8	7.7
4	3 X Zn + 2 X Water	4.5	7.5	8	6.7
5	4 X Zn + 1 X Water	7.5	8.0	8	7.8
6	5 X Zn	5.0	7.0	9	7.0
7	2 X Water + 1 X Zn + 2 X Water	6.0	7.0	9	7.3
8	2 X Water + 2 X Zn + 1 X Water	8.0	6.5	7	7.2
9	2 X Water + 3 X Zn	7.5	7.5	7	7.3
	LSD	2.1	2.37	3.2	
	Significance	Ns	Ns	Ns	

TABLE 7.12 EVALUATION OF CONTROL OF POWDERY SCAB USING ZINC APPLIED IN IRRIGATION WATER - TOTAL YIELD (T/HA)

No.	Treatment	2000	2001	2002	Mean
1	5 x Water	47.2	83.4	57.5	62.7
2	1 X Zn + 4 X Water	49.1	85.4	58.6	64.4
3	2 X Zn + 3 X Water	47.9	85.0	54.7	62.5
4	3 X Zn + 2 X Water	47.4	82.6	57.6	62.5
5	4 X Zn + 1 X Water	50.0	83.2	55.5	62.9
6	5 X Zn	47.6	76.4	53.4	59.1
7	2 X Water + 1 X Zn + 2 X Water	48.6	81.1	59.4	63.0
8	2 X Water + 2 X Zn + 1 X Water	53.9	78.8	57.0	63.2
9	2 X Water + 3 X Zn	51.7	79.5	56.4	62.5
	LSD	4.77	8.09	8.5640	
	Significance	Ns	Ns	Ns	

TABLE 7.13 EVALUATION OF CONTROL OF POWDERY SCAB USING ZINC APPLIED IN IRRIGATION WATER - TOTAL TUBER NUMBER (000'S/HA)

No.	Treatment	2000	2001	2002	Mean
1	5 x Water	554.5 abc	536.3	482.9	524.6
2	1 X Zn + 4 X Water	566.5 ab	527.1	476.6	523.4
3	2 X Zn + 3 X Water	544.5 bcd	537.2	473.6	518.4
4	3 X Zn + 2 X Water	568.5 ab	537.2	491.3	532.3
5	4 X Zn + 1 X Water	507.5 d	528.0	472.2	502.6
6	5 X Zn	520.0 cd	485.6	486.9	497.5
7	2 X Water + 1 X Zn + 2 X Water	565.5 ab	513.6	488.8	522.6
8	2 X Water + 2 X Zn + 1 X Water	594.0 a	490.0	484.4	522.8
9	2 X Water + 3 X Zn	583.0 ab	509.6	490.3	527.6
	LSD	29.9	92.41	108.16	
	Significance	**	Ns	Ns	

TABLE 7.14 EVALUATION OF CONTROL OF POWDERY SCAB USING ZINC APPLIED IN IRRIGATION WATER - POWDERY SCAB INCIDENCE (% TUBERS)

No.	Treatment	2000	2001	2002	Mean
1	5 x Water	42.0	56.0	90	62.7
2	1 X Zn + 4 X Water	34.0	44.0	88	55.3
3	2 X Zn + 3 X Water	44.0	54.5	93	63.8
4	3 X Zn + 2 X Water	18.0	43.5	87	49.5
5	4 X Zn + 1 X Water	38.0	51.0	90	59.7
6	5 X Zn	24.0	51.0	80	51.7
7	2 X Water + 1 X Zn + 2 X Water	48.0	46.0	92	62.0
8	2 X Water + 2 X Zn + 1 X Water	48.0	50.0	88	62.0
9	2 X Water + 3 X Zn	34.0	41.5	78	51.2
	LSD	28.4	23.37	9.7	
	Significance	Ns	Ns	Ns	

TABLE 7.15 EVALUATION OF CONTROL OF POWDERY SCAB USING ZINC APPLIED IN IRRIGATION WATER - POWDERY SCAB SEVERITY (% TUBERS)

No.	Treatment	2000	2001	2002	Mean
1	5 x Water	7.8	2.4	7.4	5.9
2	1 X Zn + 4 X Water	5.1	0.9	7.4	4.5
3	2 X Zn + 3 X Water	6.2	2.0	11.4	6.5
4	3 X Zn + 2 X Water	1.8	0.9	8.1	3.6
5	4 X Zn + 1 X Water	5.0	1.7	10.4	5.7
6	5 X Zn	2.6	1.5	5.8	3.3
7	2 X Water + 1 X Zn + 2 X Water	6.1	1.1	8.2	5.1
8	2 X Water + 2 X Zn + 1 X Water	9.2	1.0	8.4	6.2
9	2 X Water + 3 X Zn	3.4	0.9	6.6	3.6
	LSD	4.96	1.87	3.34	
	Significance	Ns	Ns	Ns	

Discussion

Although the concept of including soluble zinc in irrigation water for the control of powdery scab (chemigation) is an attractive one, the results of three years trials suggest that it will not achieve sufficient control to warrant the practice.

Timing of fungicide treatments (field trials) – main conclusion:

- **Although the concept of including soluble zinc in irrigation water for the control of powdery scab (chemigation) is an attractive one, the results suggest that it will not achieve sufficient control to warrant the practice.**

Trial to evaluate the efficacy of calcium cyanamide as a soil applied fertiliser for control of soil-borne S. subterranea *contamination.*

Perlka is the trade name for calcium cyanamide. This nitrogen generating material is manufactured in Germany and sold for the control of clubroot of brassicas (*Plasmodiophora brassicae*). This pathogen is a member of the *Plasmodiophorales*, to which *Spongospora subterranea* also belongs. In the soil, calcium cyanamide breaks down to release cyanide, which

can kill resting spores of the clubroot pathogen. Breakdown of the chemical also releases nitrogen.

A trial was set up at Tillycorthie, Udny Station, Aberdeenshire in a soil with a high level of soil contamination to evaluate whether this product would have potential for the control of powdery scab.

Methodology

The trial was a randomised block design with four replicates. Plot sizes were 6.25 m x 4 drills. The trial was planted on 10 May 2000. Treatments applied are shown in Table 7.16. The Nitram treatment provided nitrogen equivalent to that of the calcium cyanamide. The variety used was Estima.

Emergence and vigour were scored to evaluate the effect of calcium cyanamide on crop safety. In addition, yield and tuber numbers were used to evaluate the effect on crop growth. Daughter tubers were assessed for incidence and severity of disease and disorders.

TABLE 7.16 TREATMENTS FOR THE EVALUATION OF CALCIUM CYANAMIDE FOR THE CONTROL OF POWDERY SCAB

No.	Product	Rate and method of application
1	Nitram (34.5% nitrogen)	25kg/ha applied by hand at planting
2	Calcium cyanamide	450 kg/ha applied by hand on 10 May dibbled in along drill with a hand fork
3	Calcium cyanamide	450 kg/ha applied by hand on 14 Jun dibbled in along drill with a hand fork

Results

Whilst no significant differences were seen in emergence of the three treatments in June, the calcium cyanamide treatments significantly reduced crop vigour when assessed in July (Table 7.17). These differences fed through to significant reductions in yield and tuber numbers, particularly in the 45-65 mm fraction (Table 7.18).

There were significant differences between treatments in the incidence of powdery scab (Table 7.19). However, far from reducing powdery scab, they increased incidence, significantly in the case of the early application of calcium cyanamide. There were no significant differences in the severity of powdery scab.

TABLE 7.17 EVALUATION OF CALCIUM CYANAMIDE FOR THE CONTROL OF POWDERY SCAB - EMERGENCE AND VIGOUR

	Emergence (%)			Vigour (%)
Treatment	**01/06/00**	**07/06/00**	**12/06/00**	**07/07/00**
Nitram	3.0	70.0	92.5	7.5 a
Calcium cyanamide 10 June	1.0	61.5	93.0	6.0 b
Calcium cyanamide 14 June	1.0	61.5	91.5	5.3 b
LSD	4.3	18.2	10.3	1.0
Significance	Ns	Ns	Ns	**

TABLE 7.18 EVALUATION OF CALCIUM CYANAMIDE FOR THE CONTROL OF POWDERY SCAB - GRADING SIZE FRACTION NUMBERS AND YIELDS

	Yield (t/ha)				Tuber numbers (000's/ha)			
Treatment	**<45**	**45-65**	**65-85**	**Total**	**<45**	**45-65**	**65-85**	**Total**
Nitram	10.78	28.43 a	2.43	41.65	219.8	264.5	9.5 a	493.8a
Calcium cyanamide 10 June	11.03	22.05 b	1.13	34.21 b	233.5	209.0	4.0 b	446.5a b
Calcium cyanamide 14 June	11.08	20.05 b	0.90	32.03 b	228.5	193.8	3.5 b	425.8 b
LSD	2.10	4.11	1.35	4.43	51.4	33.9	5.0	48.0
Significance	Ns	**	Ns	**	Ns	**	*	*

TABLE 7.19 EVALUATION OF CALCIUM CYANAMIDE FOR THE CONTROL OF POWDERY SCAB - POWDERY SCAB INCIDENCE AND SEVERITY

	Incidence	Severity
Treatment	**(% tubers)**	**(% surface area)**
Nitram	53.0 b	3.3
Calcium cyanamide 10 June	71.0 a	5.1
Calcium cyanamide 14 June	63.5 ab	2.7
LSD	13.3	1.97
Significance	*	Ns

Discussion

For control of clubroot, calcium cyanamide is worked into the soil a few weeks before brassicas are transplanted. This allows the chemical to break down and act upon the pathogen before the crop is present. There is also no risk of damage to the brassica crop. In this trial on potatoes, the first calcium cyanamide treatment was applied at planting. Although it had no effect on emergence, it is clear that it affected crop growth. The second treatment, which was applied a few days later, had the same effect.

Despite the effect on potato growth, the calcium cyanamide treatments might still have been useful if they had effected a reduction in powdery scab. However, this did not happen. It is possible that the potential for *S. subterranea* to multiply in the roots counteracted any reduction in inoculum achieved.

Calcium cyanamide trial – main conclusion:

- **Calcium cyanamide did not prove an effective option for the control of powdery scab.**

Integrated control

A. Integrated control trials

Objective: To evaluate agronomic methods that may reduce the incidence of powdery scab by quantifying the benefits of integrating control measures. This included monitoring of populations in the field. Experiments consisted of factorial field plot trials in situations of soil contamination and/or tuber infection. Possible mechanisms that were investigated included controlling irrigation and Zn levels. The work exploited the basic studies on inoculum and 'productivity' of the fungus under 1a and 1b (SAC).

Milestone 8: Establish field trials and determine effects of different control measures on powdery scab. Relate the results to population monitoring. Produce interpreted reports on integrated control measures as grower friendly summary sheets - *achieved*

Integrating control measures is believed to be the most effective way to optimise the control of powdery scab. However, whilst this view is stated frequently, there is little guidance on the most effective combinations of control measures. A series of trials, one in each of the three years of the project, evaluated the relative contribution of a range of established and novel control measures to the control of powdery scab and whether the control achieved was greater when used in combination.

Methodology

All trials were carried out at Tillycorthie Farm, Udny Station, Aberdeenshire in a field that had grown potatoes in 1999 on which powdery scab infection was severe. The trial design was a factorial with 2 replicates. Basic details of the trial are shown in Table 8.1.

TABLE 8.1 INTEGRATED CONTROL OF POWDERY SCAB – KEY TRIAL DATES

Year	Date planted	Date haulm destruction	Date harvested
2000	11 May	7 & 11 August	2 October
2001	26 May	13 & 21 September	7 October
2002	23 May	4 & 12 September	8 November

The control measures investigated in each year are described in Table 8.2. In each, emergence was measured at regular intervals, as well as crop vigour. Tuber yield and tuber number was determined in total and in grading fractions. After harvest, tuber disease incidence and severity were estimated on a sample of 50 tubers as well as physical defects such as netting, cracking, damage etc.

TABLE 8.2 CONTROL MEASURES (FACTORS) EVALUATED IN INTEGRATED CONTROL TRIALS AND THEIR LEVELS

Year	Main factors and levels			
2000	**Variety*** Maris Piper (3) Saxon (6)	**Fleece** +/- Fleece to end of tuber initiation	**Chemical control** +/- Fluazinam (3.0 l/ha Shirlan) incorporated	**Depth of planting** Shallow (12cm) or Deep (18cm)
2001	**Variety*** Estima (3) Saxon (6) Wilja (5)	**Fleece** +/- Fleece to end of tuber initiation		**Depth of planting** Shallow (12cm) or Deep (18cm)
2002	**Variety*** Estima (3) Saxon (6) Wilja (5)	**Fleece** +/- Fleece to end of tuber initiation	**Chemical control** +/- Zinc oxide (15 kg/ha Zinc) incorporated	

* Figures in brackets are NIAB resistance ratings to powdery scab in the 2003 Varieties of Potatoes Pocket Guide

Results

Results for each year of trials are shown in Tables 8.3 (2000), 8.4 (2001) and 8.5 (2002).

Throughout the three trials, variety had a significant impact on emergence, total yield and total tuber number and powdery scab incidence and severity. Fleece had a significant impact on emergence and vigour with those plots covered by fleece emerging more rapidly and developing faster. The effect of fleece on yield and tuber number was a tendency for an increase, sometimes significant. Depth of planting affected emergence, as expected, but had little impact on yield, tuber number or incidence and severity of powdery scab, except for a single trial where the incidence of powdery scab was significantly greater at depth. However, in another trial, the severity was greater at the shallower depth. Fungicide had limited effect on emergence, vigour, yield, tuber number or powdery scab incidence and severity. In the 2002 trial zinc treatment reduced the incidence of black dot.

The most dramatic effect on powdery scab was variety. The incidence and severity of powdery scab was significantly reduced where a more resistant variety was grown.

Interactions between control measures were infrequent for emergence, vigour, yield or tuber numbers and where recorded were always a variety x fleece interaction. With powdery scab incidence and severity, interactions were detected each year. In each trial, significant variety x fleece interactions were detected, and in the first year (2000), fleece x fungicide interactions were found.

In the fleece x variety interactions, the fleece reduced incidence (1 trial) and severity (3 trials) to a greater extent with the most susceptible variety. In the fleece x fungicide interaction (1 trial), the incidence and severity of powdery scab was reduced more by fungicide treatment where no fleece was present. Presumably, this was due to greater moisture allowing the zinc to be more available in the soil solution.

Results - Integrated control of powdery scab – 2000 trial

TABLE 8.3.1 EMERGENCE (%) – 7 JUNE 2000

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		53.0	39.0	16.0	35.0	
	Saxon		4.0	10.0	9.0	16.0	-
No Fleece	Estima		-	-	-	-	
	Saxon		-	-	-	-	

Significant interactions – N/a

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	-	-	35.8	9.8	26.5	19.0	20.5	25.0
SED	-		-		-		-	
Signif.	-		-		-		-	

TABLE 8.3.2 VIGOUR (0-9) - 7 JUNE 2000

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		8.0	8.0	8.0	8.0	
	Saxon		7.5	8.0	8.0	8.0	0.94
No Fleece	Estima		8.0	7.5	6.5	7.5	
	Saxon		5.5	5.0	5.0	5.5	

Significant interactions – None

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	7.9	6.3	7.7	6.6	7.2	7.1	7.1	7.2
SED	0.13		0.16		0.16		0.16	
Signif.	*		**		Ns		Ns	

TABLE 8.3.3 TUBER NUMBER (X1000) <45MM

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		121.0	111.0	119.0	147.5	
	Saxon		87.0	116.0	100.0	101.5	48.52
No Fleece	Estima		149.5	150.0	148.7	208.5	
	Saxon		121.5	131.5	155.0	150.0	

Significant interactions - None

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	112.9	151.8	144.4	120.3	123.4	141.3	125.2	139.5
SED	5.71		8.21		8.21		8.21	
Signif.	Ns		*		Ns		Ns	

TABLE 8.3.4 YIELD (T/HA) <45MM

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		4.80	4.35	4.80	6.20	
	Saxon		3.45	4.60	3.85	4.25	2.03
No Fleece	Estima		6.35	6.60	6.58	8.60	
	Saxon		4.60	5.25	5.70	5.85	

Significant interactions – None

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	4.54	6.19	6.03	4.69	5.0	5.73	5.02	5.71
LSD	0.18		0.35		0.35		0.35	
Signif.	Ns		*		Ns		Ns	

TABLE 8.3.5 TUBER NUMBER (X1000) 45-65MM

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		317.5	244.5	282.8	246.0	
	Saxon		279.0	249.0	226.0	208.5	80.07
No Fleece	Estima		224.0	263.5	211.4	221.5	
	Saxon		213.5	203.0	190.5	184.5	

Significant interactions – None

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	256.7	214.0	251.4	219.3	249.3	221.4	243.1	227.6
SED	6.42		13.75		13.75		13.75	
Signif.	Ns		*		Ns		Ns	

TABLE 8.3.6 YIELD (T/HA) 45-65MM

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		37.0	29.0	32.5	27.4	
	Saxon		28.8	26.3	24.7	22.0	7.09
No Fleece	Estima		24.7	27.2	20.6	23.0	
	Saxon		21.2	21.3	19.5	19.1	

Significant interactions - Fleece x Depth (*)

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	28.4	22.1	27.7	22.8	26.9	23.6	26.1	24.4
SED	0.53		1.22		1.22		1.22	
Signif.	Ns		*		Ns		Ns	

TABLE 8.3.7 TUBER NUMBER (X1000) 65-85MM

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		45.5	43.5	42.4	30.0	
	Saxon		45.5	42.0	38.5	38.0	16.64
No Fleece	Estima		18.5	23.5	7.6	12.5	
	Saxon		11.5	16.5	15.0	18.5	

Significant interactions - Fleece x Depth (*)

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	40.9	15.5	28.2	28.2	30.8	25.6	28.1	28.3
SED	4.14		1.96		1.96		1.96	
Signif.	Ns		Ns		*		Ns	

TABLE 8.3.8 YIELD (T/HA) 65-85MM

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		10.2	10.9	9.0	7.5	
	Saxon		10.0	9.3	8.6	9.2	4.41
No Fleece	Estima		4.2	5.6	1.7	2.8	
	Saxon		2.4	3.9	3.3	4.4	

Significant interactions - Variety x Fungicide (*)

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	9.3	3.5	6.5	6.4	7.0	5.8	6.2	6.7
SED	1.08		0.40		0.40		0.40	
Signif.	Ns		Ns		*		Ns	

TABLE 8.3.9 TUBER NUMBER (X1000) >85MM

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		1.5	3.5	0.4	1.0	
	Saxon		0.0	1.0	3.5	4.0	2.98
No Fleece	Estima		0.0	2.0	0.0	0.5	
	Saxon		0.0	0.0	0.5	0.0	

Significant interactions - Variety x Fungicide (*)

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	1.9	0.4	1.1	1.1	1.0	1.2	0.7	1.5
SED	0.50		0.48		0.48		0.48	
Signif.	Ns		Ns		Ns		Ns	

TABLE 8.3.10 YIELD (T/HA) >85MM

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		0.6	1.4	0.1	0.4	
	Saxon		0.0	0.4	1.2	1.8	1.06
No Fleece	Estima		0.0	0.7	0.0	0.2	
	Saxon		0.0	0.0	0.2	0.0	

Significant interactions - Variety x Fungicide (*)

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	0.7	0.1	0.4	0.4	0.4	0.5	0.3	0.6
SED	0.20		0.17		0.17		0.17	
Signif.	Ns		Ns		Ns		Ns	

TABLE 8.3.11 OVERALL YIELD (T/HA) - 12 OCTOBER 2000

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		52.6	46.8	45.5	41.4	
	Saxon		42.2	38.3	40.5	37.1	9.54
No Fleece	Estima		35.2	28.3	40.0	34.5	
	Saxon		28.2	28.7	30.4	29.3	

Significant interactions – None

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	43.0	31.8	40.5	34.3	39.3	35.5	37.5	37.3
SED	1.56		1.56		1.56		1.56	
Signif.	***		**		*		Ns	

TABLE 8.3.12 POWDERY SCAB SEVERITY (% SURFACE AREA)

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	Estima		2.5	3.3	3.6	3.9	
	Saxon		0.4	0.2	0.6	1.1	2.89
No Fleece	Estima		9.0	13.0	7.1	8.1	
	Saxon		0.5	1.9	1.0	0.3	

Significant interactions - Fleece x Variety (**) and Fleece x Fungicide (*)

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	1.9	5.1	6.3	0.7	3.8	3.2	3.1	4.0
SED	0.65		0.42		0.42		0.42	
Signif.	Ns		**		Ns		*	

TABLE 8.3.13 POWDERY SCAB INCIDENCE (% TUBERS)

		Fungicide	No fungicide		Fungicide		Overall
Treatment	**Variety**	**Depth**	**Deep**	**Shallow**	**Deep**	**Shallow**	**LSD**
Fleece	*Estima*		32.0	29.0	38.0	46.0	
	Saxon		12.0	6.0	18.0	27.0	19.5
No Fleece	Estima		59.0	66.0	55.0	59.0	
	Saxon		12.0	21.0	20.0	11.0	

Significant interactions - Fleece x Variety (*) and Fleece x Fungicide (*)

Overall means

	Fleece	No Fleece	Estima	Saxon	No Fung.	Fung.	Deep	Shallow
Mean	26.0	37.9	48.0	15.9	29.6	34.2	30.8	33.1
SED	1.87		3.4		3.4		3.4	
Signif.	Ns		**		Ns		Ns	

Results - Integrated control of powdery scab – 2001 trial

TABLE 8.4.1 % EMERGENCE

		Depth of planting	
	Variety	**Shallow**	**Deep**
Fleece	Estima	75.0	41.0
	Saxon	76.0	71.0
	Wilja	88.0	87.0
No Fleece	Estima	17.0	2.0
	Saxon	44.0	13.0
	Wilja	49.0	16.0

Significant interactions – None

Overall means

	Fleece	No Fleece	Estima	Saxon	Wilja	Shallow	Deep
Mean	73.0	23.5	33.7	51.0	60.0	58.2	38.3
SED	5.13		6.04			4.93	
Signif.	*		**			**	

TABLE 8.4.2 % EMERGENCE

		Depth of planting	
	Variety	**Shallow**	**Deep**
Fleece	Estima	86.0	65.0
	Saxon	91.0	94.0
	Wilja	99.0	99.0
No Fleece	Estima	41.0	33.0
	Saxon	87.0	51.0
	Wilja	86.0	75.0

Significant interactions – None

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**Shallow**	**Deep**
Mean	89.0	62.2	56.2	80.7	89.7	81.7	69.5
SED	3.22		5.38			4.39	
Signif.	*		***			*	

TABLE 8.4.3 % EMERGENCE

		Depth of planting	
	Variety	**Shallow**	**Deep**
Fleece	Estima	99.0	99.0
	Saxon	97.0	100.0
	Wilja	100.0	100.0
No Fleece	Estima	99.0	99.0
	Saxon	97.0	100.0
	Wilja	100.0	100.0

Significant interactions – None

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**Shallow**	**Deep**
Mean	99.2	99.2	99.0	98.5	100.0	99.7	98.7
SED	-		-			-	
Signif.	-		-			-	

TABLE 8.4.4 YIELD (T/HA)

		Depth of planting									
		Shallow					Deep				
Variety*		<45	45-65	65-85	>85	Tot	<45	45-65	65-85	>85	Tot
Fleece	**E**	3.0	35.2	39.1	5.1	82.3	2.0	39.3	36.8	3.7	81.8
	S	1.4	26.9	40.2	6.3	74.8	0.9	29.1	40.0	2.8	72.9
	W	4.0	46.5	27.3	1.2	79.0	3.0	42.1	26.3	0.8	72.2
No Fleece	**E**	3.3	44.4	24.6	1.4	73.6	3.6	47.6	23.3	2.0	76.4
	S	1.6	31.3	35.9	4.5	73.3	1.5	31.8	33.7	2.3	69.3
	W	5.8	51.0	15.7	1.1	73.6	3.8	46.0	19.3	0.4	69.5

* E = Estima, S = Saxon, W = Wilja

Overall means

	<45		45-65		65-85		>85		Total	
	Mean	**SED**	**Mean**	**SED**	**Mean**	**SED**	**Mean**	**SED**	**Mean**	**SED**
Fleece	2.4	0.31	36.5	1.04	35.0	1.20	3.3	0.07	77.2	0.96
No Fleece	3.3	Ns	42.0	*	25.4	*	2.0	**	72.6	*
Estima	3.0	0.35	41.6	1.48	30.9	1.63	3.0	0.55	78.5	1.66
Saxon	1.4	***	29.8	***	37.4	***	4.0	***	72.6	*
Wilja	4.1		46.4		22.2		0.9		73.6	
No Zinc	3.2	0.29	39.2	1.21	29.9	1.33	3.3	0.45	73.7	1.36
Zinc	2.5	*	39.2	Ns	30.5	Ns	2.0	*	76.1	Ns
Sig. Interact's	None		Variety x depth (*)		None		None		None	

TABLE 8.4.5 TUBER NUMBERS (000'S/HA)

		Depth of planting									
		Shallow					Deep				
Variety*		<45	45-65	65-85	>85	Tot	<45	45-65	65-85	>85	Tot
Fleece	**E**	65.6	252.8	126.9	9.2	454.5	43.7	277.3	124.2	7.0	452.3
	S	33.7	203.8	150.9	13.1	401.6	21.0	212.2	151.8	6.1	391.1
	W	83.6	327.6	91.9	2.2	505.3	63.0	300.5	93.6	1.3	458.4
No Fleece	**E**	73.1	327.2	85.3	2.6	488.2	77.0	351.7	81.8	3.5	514.0
	S	42.0	239.7	133.9	9.6	425.2	35.4	224.4	127.3	4.4	391.5
	W	113.3	368.8	54.7	1.8	538.5	78.7	330.7	91.9	0.9	477.0

* E = Estima, S = Saxon, W = Wilja

Overall means

	<45		45-65		65-85		>85		Total	
	Mean	**SED**	**Mean**	**SED**	**Mean**	**SED**	**Mean**	**SED**	**Mean**	**SED**
Fleece	51.8	5.15	262.4	3.82	123.2	6.62	6.5	0.39	443.9	3.12
No Fleece	69.9	Ns	307.1	**	91.6	*	3.8	*	472.4	*
Estima	64.9	6.83	302.3	9.57	104.5	5.64	5.6	1.06	477.2	10.67
Saxon	33.0	***	220.0	***	141.0	***	8.3	***	402.3	***
Wilja	84.6		331.9		76.8		1.5		494.9	
No Zinc	68.5	5.58	282.8	7.82	107.2	4.60	6.4	0.87	447.4	8.72
Zinc	53.1	*	286.7	Ns	107.6	Ns	3.9	*	468.9	*
Sig. Interact's	None		Variety x depth (*)		None		Variety x depth (*)		None	

TABLE 8.4.6 POWDERY SCAB – INCIDENCE (% TUBERS)

		Depth of planting	
	Variety	**Shallow**	**Deep**
Fleece	Estima	9.0	37.0
	Saxon	3.0	1.0
	Wilja	6.0	7.0
No Fleece	Estima	34.0	38.0
	Saxon	8.0	0.0
	Wilja	6.0	14.0

Significant interactions – None

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**Shallow**	**Deep**
Mean	10.5	16.7	29.5	3.0	8.3	11.0	16.2
SED	10.3		6.2			5.1	
Signif.	Ns		***			*	

TABLE 8.4.7 POWDERY SCAB – SEVERITY (% SURFACE AREA)

		Depth of planting	
	Variety	**Shallow**	**Deep**
Fleece	Estima	0.1	0.6
	Saxon	0.0	0.0
	Wilja	0.1	0.8
No Fleece	Estima	1.5	1.2
	Saxon	0.1	0.0
	Wilja	0.1	0.3

Significant interactions – Variety x Fleece (***)

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**Shallow**	**Deep**
Mean	1.5	0.5	0.9	0.0	0.1	0.4	0.3
SED	0.26		0.22			0.18	
Signif.	Ns		**			Ns	

Results - Integrated control of powdery scab – 2002 trial

TABLE 8.5.1 % EMERGENCE – 10 JUNE 2002

		Zinc treatment	
	Variety	**No Zinc**	**Zinc**
Fleece	Estima	0	0
	Saxon	0	0
	Wilja	59	45
No Fleece	Estima	0	0
	Saxon	0	0
	Wilja	36	20

Significant interactions – Fleece x Variety (*)

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**No Zinc**	**Zinc**
Mean	17.3	9.3	0	0	40	10.8	15.8
SED	1.05		4.47			3.65	
Signif.	*		***			Ns	

TABLE 8.5.2 % EMERGENCE – 13 JUNE 2002

		Zinc treatment	
	Variety	**No Zinc**	**Zinc**
Fleece	Estima	37.0	33.0
	Saxon	10.0	15.0
	Wilja	99.0	97.0
No Fleece	Estima	2.0	0
	Saxon	1.0	0
	Wilja	54.0	48.0

Significant interactions - Fleece x Variety (***)

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**No Zinc**	**Zinc**
Mean	48.5	17.5	18.0	6.5	74.5	33.8	32.2
SED	3.6		2.9			2.4	
Signif.	*		***			Ns	

TABLE 8.5.3 % EMERGENCE – 17 JUNE 2002

		Zinc treatment	
	Variety	**No Zinc**	**Zinc**
Fleece	Estima	82.0	81.0
	Saxon	61.0	47.0
	Wilja	99.0	98.0
No Fleece	Estima	17.0	52.0
	Saxon	26.0	4.0
	Wilja	70.0	82.0

Significant interactions – None

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**No Zinc**	**Zinc**
Mean	78.0	41.4	58.0	34.5	87.2	59.2	60.7
SED	4.13		7.55			6.16	
Signif.	*		***			Ns	

TABLE 8.5.4 % EMERGENCE – 21 JUNE 2002

		Zinc treatment	
	Variety	**No Zinc**	**Zinc**
Fleece	Estima	100.0	100.0
	Saxon	100.0	100.0
	Wilja	100.0	100.0
No Fleece	Estima	73.0	84.0
	Saxon	50.0	39.0
	Wilja	97.0	98.0

Significant interactions – Fleece x Variety (***)

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**No Zinc**	**Zinc**
Mean	100.0	73.5	89.2	72.2	98.7	86.7	86.8
SED	2.50		2.66			2.17	
Signif.	**		***			Ns	

TABLE 8.5.5 % EMERGENCE – 1 JULY 2002

		Zinc treatment	
	Variety	**No Zinc**	**Zinc**
Fleece	Estima	100	100
	Saxon	100	100
	Wilja	100	100
No Fleece	Estima	99	100
	Saxon	97	96
	Wilja	100	100

Significant interactions –

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**No Zinc**	**Zinc**
Mean	100	98.7	99.8	98.3	100	99.3	99.3
SED	0.0		0.84			0.68	
Signif.	Ns		Ns			Ns	

TABLE 8.5.6 YIELD (T/HA)

		Zinc treatment									
		No Zinc					Zinc				
Variety*		**<45**	**45-65**	**65-85**	**>85**	**Tot**	**<45**	**45-65**	**65-85**	**>85**	**Tot**
Fleece	**E**	3.64	35.8	19.5	0		4.09	42.6	16.2	0	
	S	2.41	33.9	12.1	0.5		2.07	32.7	9.5	0.2	
	W	5.71	26.7	7.2	0		5.12	27.1	10.7	0	
No Fleece	**E**	7.34	38.1	8.1	0		6.30	40.1	8.0	0.3	
	S	2.76	30.2	13.1	0		2.66	31.0	14.1	0.2	
	W	8.52	32.8	5.9	0		8.57	32.1	5.6	0	

* E = Estima, S = Saxon, W = Wilja

Overall means

	<45		**45-65**		**65-85**		**>85**		**Total**	
	Mean	**SED**	**Mean**	**SED**	**Mean**	**SED**	**Mean**	**SED**	**Mean**	**SED**
Fleece	3.84	0.46	33.1	0.22	12.5	1.55	0.12	0.12		
No Fleece	6.02	*	34.0	Ns	9.1	Ns	0.1	Ns		
Estima	5.34	0.40	39.1	1.16	12.9	1.14	0.1	0.14		
Saxon	2.47	***	31.9	***	12.2	***	0.2	Ns		
Wilja	6.98		29.7		7.34		0			
No Zinc	5.06	0.33	32.9	0.95	11.0	0.93	0.1	0.11		
Zinc	4.80	Ns	34.3	Ns	10.7	Ns	0.1	Ns		
Sig. Interact's	Fleece x variety (*)		Fleece x variety (*)		Fleece x variety (***)		-			

TABLE 8.5.7 TUBER NUMBERS (000'S/HA)

		Zinc treatment									
		No Zinc					Zinc				
Variety*		<45	45-65	65-85	>85	Tot	<45	45-65	65-85	>85	Tot
Fleece	**E**	82.2	274.2	69.9	0	426.3	89.6	317.0	58.6	0	465.2
	S	54.6	272.2	47.3	0.99	375.1	48.2	266.3	38.4	0.49	353.5
	W	114.7	185.1	25.1	0	324.9	93.0	191.5	35.9	0	320.5
No Fleece	**E**	159.0	322.0	30.0	0	511.0	143.3	335.8	30.5	0.49	510.0
	S	63.0	239.3	50.2	0	352.5	67.9	253.0	51.7	0.49	373.2
	W	158.0	240.7	20.2	0	419.0	158.0	237.8	21.2	0	417.0

* E = Estima, S = Saxon, W = Wilja

Overall means

	<45		45-65		65-85		>85		Total	
	Mean	**SED**	**Mean**	**SED**	**Mean**	**SED**	**Mean**	**SED**	**Mean**	**SED**
Fleece	80.4	8.38	251.1	4.69	45.9	4.41	0.25	0.25	377.6	8.39
No Fleece	124.9	*	271.4	*	34.0	Ns	0.16	Ns	430.4	*
Estima	118.5	7.48	312.2	8.64	47.3	4.33	0.12	0.26	478.2	11.13
Saxon	58.5	***	257.7	***	46.9	***	0.49	Ns	363.6	***
Wilja	131.0		213.8		25.6		0.0		370.3	
No Zinc	105.3	6.10	255.6	7.06	40.5	3.54	0.16	0.21	401.5	9.09
Zinc	100.0	Ns	266.9	Ns	39.4	Ns	0.25	Ns	406.6	Ns
Sig. Interact's	Fleece x variety (*)		Fleece x variety (**)		Fleece x variety (**)		-		Fleece x variety (**)	

TABLE 8.5.8 POWDERY SCAB – INCIDENCE (% TUBERS)

		Zinc treatment	
	Variety	**No Zinc**	**Zinc**
Fleece	Estima	74.0	94.0
	Saxon	62.0	79.0
	Wilja	80.0	83.0
No Fleece	Estima	85.0	94.0
	Saxon	79.0	53.0
	Wilja	73.0	80.0

Significant interactions – None

Overall means

	Fleece	No Fleece	Estima	Saxon	Wilja	No Zinc	Zinc
Mean	78.9	77.3	86.7	68.2	79.0	75.5	80.5
SED	9.89		9.12			7.44	
Signif.	Ns		Ns			Ns	

TABLE 8.5.9 POWDERY SCAB – SEVERITY (% SURFACE AREA)

		Zinc treatment	
	Variety	**No Zinc**	**Zinc**
Fleece	Estima	3.2	3.7
	Saxon	0.7	1.2
	Wilja	2.7	1.7
No Fleece	Estima	6.6	4.4
	Saxon	1.0	0.8
	Wilja	1.6	2.4

Significant interactions – Variety x Fleece (***)

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**No Zinc**	**Zinc**
Mean	2.2	2.8	4.5	0.9	2.1	2.63	2.35
SED	0.43		0.32			0.26	
Signif.	Ns		***			Ns	

TABLE 8.5.10 % INCIDENCE OF BLACK DOT

		Zinc treatment	
	Variety	**No Zinc**	**Zinc**
Fleece	Estima	20	28
	Saxon	2	0
	Wilja	18	4
No Fleece	Estima	27	1
	Saxon	1	1
	Wilja	28	2

Significant interactions – Fleece x Zinc treatment

Overall means

	Fleece	**No Fleece**	**Estima**	**Saxon**	**Wilja**	**No Zinc**	**Zinc**
Mean	12	10	19	1	13	16	6
SED	5.4		3.5			2.8	
Signif.	Ns		***			**	

Discussion

In the trials in this series, variety had the greatest impact on powdery scab. Simply by changing from a susceptible variety to a moderately resistant one, the incidence and severity of disease fell significantly. To a grower, variety choice is not always an option but where several fields are available to grow potatoes, it is clear that placing the most resistant variety in the field with the highest risk of disease is a sensible option. Thus, matching variety to disease risk is a clear option for growers to reduce powdery scab risk. However, in the absence of a cost effective soil test, assessing risk is difficult. Previous studies (in Scotland) have shown that where potatoes have been grown before, the risk of powdery scab can be high. Better still would be to have knowledge of the disease burden on the tubers lifted from the field on the previous occasion potatoes were grown. With rented fields, this information is not always possible. The trend of seed growers (particularly those growing high grade seed) to source fields with no history of potato production is a wise one, but each year finding such fields is more difficult.

Fleece had a marked affect on emergence and growth, yield and tuber number but surprisingly little effect on powdery scab. The exception was for a greater reduction of powdery scab under fleece with susceptible varieties. This effect was either due to a less favourable environment for infection and disease development under the fleece or, more likely, a reduction in the wetting of the ridges by shedding water into furrows. In measurements of soil temperatures under fleece in Aberdeenshire, at 9 am in the morning, temperatures were c. 2^{o}C greater under fleece. This differential probably increased during the day.

The effect of depth of planting was unclear. Originally it was hypothesised that deeper planting would lead to more powdery scab but the results from trials were ambiguous. Fungicide treatments only had a small effect and, surprisingly, did not add much to the control provided by variety resistance.

Although, the results of these trials do not unambiguously confirm it, it seems logical that to effect the greatest control of powdery scab, control measures should be integrated.

From 1991 to 1993, the BPC (as the PMB) funded work on integrated trials. Table 8.6 summarises the control measures (factors) tested in these and the current integrated control trials. It is clear that reduction in incidence of powdery scab was consistently achieved by growing a more resistant variety. Apart from depth of planting (2001 trial), incidence was only reduced by chitting (1992 Bilbo, presumably through earlier tuber initiation and disease avoidance) and by planting seed with less powdery scab infection (1993 Sunnybrae). This last finding conflicts with the findings of the Experiment to determine the relationship between inoculum and disease development reported in section 6D. The severity of powdery scab was similarly reduced consistently by a more resistant variety. In the 1991-1993 trials, the only other control measure that reduced severity was planting seed with less powdery scab.

Interactions between control measures were minimal in the 1991-1993 trials except for 1993 where variety x disease level and variety x irrigation were significant.

These earlier trials confirm the importance of variety resistance as a control measure and the infrequent significant effect of other control measures.

TABLE 8.6 INTEGRATED TRIALS – SUMMARY OF RESPONSES TO CONTROL MEASURES (FACTORS) TESTED IN 9 TRIALS - 1991 TO 2002

		Significant response		
Year, site and source of inoculum	**Main factors tested**	**Incidence of powdery scab**	**Severity of powdery scab**	**Significant interactions**
1991 Waterside Soil-borne	Variety	***	***	None
	Oxygen provider	Ns	Ns	
	Chemical control	Ns	Ns	
		Ns	Ns	
1991 Sunnybrae Seed-borne	Variety	-	-	Very low disease levels
	Irrigation	-	-	
	Chemical control	-	-	
	Disinfectant	-	-	
1992 Bilbo Soil-borne	Variety	***	***	Severity - Variety x chitting (**)
	Chitting	*	Ns	
	Chemical control	Ns	Ns	
	Chemical soil treat.	Ns	Ns	
1992 Sunnybrae Seed-borne	Variety	***	***	None
	Irrigation	Ns	Ns	
	Chemical control	Ns	Ns	
	Disease level on seed	Ns	Ns	
1993 Bilbo Soil-borne	Variety	*	**	None
	Chitting	Ns	Ns	
	Chemical control	Ns	Ns	
	Chemical soil treat.	Ns	Ns	
1993 Sunnybrae Seed-borne	Variety	***	***	Incidence – Variety x disease level. Severity – Variety x irrigation, Variety x disease level
	Irrigation	Ns	Ns	
	Chemical control	Ns	Ns	
	Disease level on seed	***	***	
2000 Tillycorthie	Variety	**	**	Incidence + severity – Variety x Fleece (*, **) & Fleece x Chem. control (*, *)
	Fleece	Ns	Ns	
	Chemical control	Ns	Ns	
	Depth of planting	Ns	*	
2001 Tillycorthie	Variety	***	Ns	Low severity of infection. Variety x Fleece (***)
	Fleece	Ns	**	
	Depth of planting	*	Ns	
2002 Tillycorthie	Variety	Ns	***	Severity – Variety x Fleece (***)
	Fleece	Ns	Ns	
	Chemical control	Ns	Ns	

Evaluation of the impact of disease resistance on the reduction of powdery scab incidence and severity.

Taking the data from the 9 trials carried out from 1991 to 2002, it is possible to determine the effect of disease resistance on powdery scab. The varieties used in the trials and their resistance ratings are shown in Table 8.7.

TABLE 8.7 VARIETIES TESTED AND THEIR POWDERY SCAB RESISTANCE RATING

Variety*	Rating
Estima	3
Maris Piper	3
Wilja	5
Record	5+
Saxon	6
Nadine	7++
Pentland Dell	7

+ Rating at time of trials (now rated 7)
++Rating at time of trials (now rated 3)
* NIAB Pocket guide to varieties of potatoes

All trials included a variety with a resistance rating of 3 (usually Estima). Using the incidence and severity of this variety as 100%, disease levels of all other varieties were ranked proportionately. The results are shown in Tables 8.1 and 8.2.

FIG. 8.1 RELATIVE REDUCTION IN INCIDENCE OF POWDERY SCAB BY GROWING A MORE RESISTANT VARIETY (RESISTANCE RATING 3 = 100%)

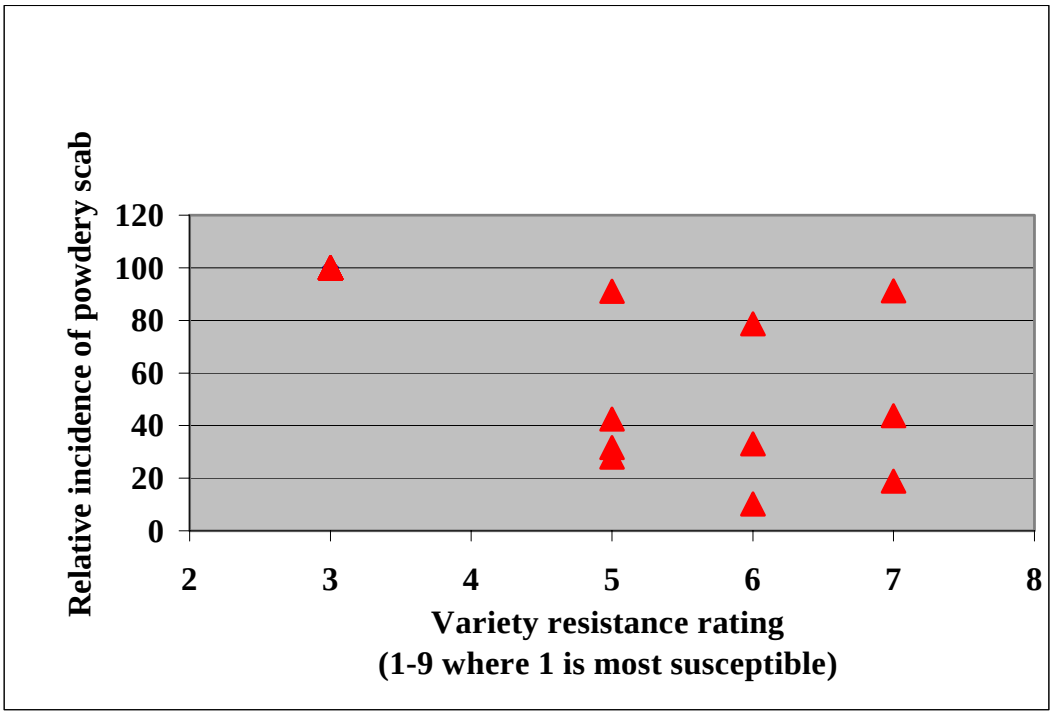
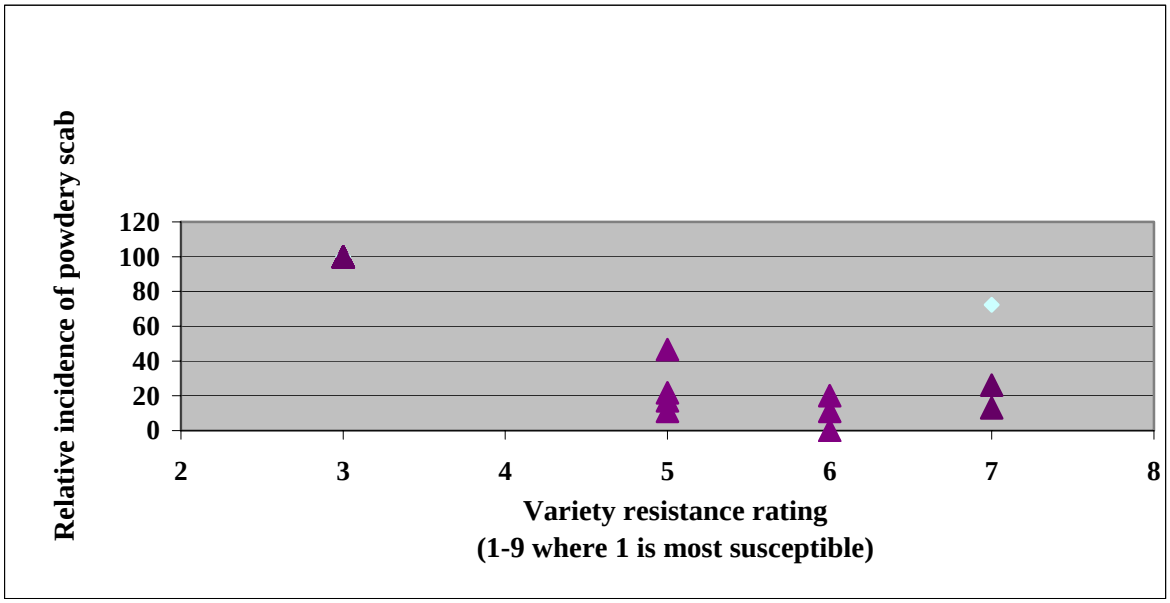


FIG. 8.2 RELATIVE REDUCTION IN SEVERITY OF POWDERY SCAB BY GROWING A MORE RESISTANT VARIETY (RESISTANCE RATING 3 = 100%)



These graphs demonstrate that, in common with other control measures, variety resistance is more consistent at reducing severity than incidence.

- **Integrated control trials – main conclusions:**

- **Variety had the greatest impact on powdery scab. Changing from a susceptible variety to a more resistant one gave the most consistent reduction in incidence and severity of powdery scab of all control measures evaluated.**

- **However, as with other control measures, changing to a resistant variety has a more consistent effect on severity than incidence.**

- **Matching variety to disease risk is a clear option for growers to reduce powdery scab risk, although identifying high risk fields remains subjective.**

- **There were few interactions between control measures in the control of powdery scab.**

- **The use of fleece resulted in a reduction of powdery scab with susceptible varieties only.**

- **Although, the results of the trials do not unambiguously confirm it, it seems logical that to effect the greatest control of powdery scab, combinations of control measures should be integrated.**

B. Bio-fumigation

Objective: To evaluate agronomic methods that may reduce the incidence of powdery scab by investigating 'bio-fumigation' with break crops such as brassicas as a method of controlling soil inoculum (SAC).

Milestone 9: Identify a range of brassica species for evaluation as bio-fumigants. Carry out trials on contaminated land and assess relative efficacy. Prepare grower friendly report on potential of bio-fumigation - *achieved*

Evaluation of brassica species has been carried out in controlled environment experiments (see section 7a above). Evaluation in the field has been carried out in the SEERAD funded projects 'Biotic and abiotic control of potato pathogens and disorders – soil-borne *Rhizoctonia solani*' (SAC project 647026) and its predecessor 'Evaluation of Brassica derived biofumigants' SAC project 609012).

Population structure of *Spongospora subterranea*.

Objective: The aim is to provide information on the population structure of the fungus that will be valuable to the breeders in the longer term. Tubers from selected farms were sampled in a structured fashion so as to provide information on the populations within single lesions, individual tubers, whole field and nationally. The fingerprinting method of choice was amplified fragment length polymorphism (AFLP). Stocks sampled were selected by choosing two popular varieties, one susceptible and one moderately resistant and identifying the stocks at random from those growers who produce the variety. Help with selection of stocks, the appropriate grade and sampling was sought from BioSS (SCRI/SAC).

Milestone 10: Determine smallest number of sporeballs that can reliably be tested by AFLP – *achieved*

Milestone 11: Complete sampling of five lesions per tuber per stock, ten stocks for across Scotland. Extract DNA for AFLP analysis – *achieved*

Milestone 12: Analyse AFLP patterns of above samples – *achieved where applicable*

An AFLP (Amplified Fragment Length Polymorphism) protocol for the study of genetic variation in fungal pathogens developed at SCRI was used to study the genetic variation in Scottish isolates of S. subterranea. An initial trial was carried out with sporeballs from tubers with powdery scab from four different origins. Tubers with powdery scab were washed and air dried. Sporeballs were scraped from the lesions. Sporeballs from each sample were treated in three different ways:

a. not sieved, washed three times in SDW
b. sieved, then washed three times in SDW
c. sieved, then washed three times in SDW, then washed three times in hypochlorite solution (Domestos, 1:10)

DNA from the sporeballs was then extracted using the protocol for soil extraction of Bell *et al.* (1999) but without filtering of the DNA through Sephadex columns. This DNA was amplified in a standard AFLP protocol modified for fungal pathogens by SCRI.

The first AFLP gel yielded only bands for one sample and then only for the DNA from sieved sporeballs. A second gel with the four initial samples showed bands for two samples but not for DNA from sporeballs washed in hypochlorite solution. Thus it was decided not to disinfect sporeballs before DNA extraction.

Potato tubers with powdery scab were collected by SAC from farms around Northern Britain so that a wide range of samples could be used to study the genetic variation in *S. subterranea* using AFLP. Table 9.1 gives the details on the origin of the samples. Some samples of sporeballs of *S. subterranea* from Australia and New Zealand were included as outliers.

TABLE 9.1 ORIGIN OF SAMPLES OF *SPONGOSPORA SUBTERRANEA* USED FOR AFLP.

Sample no.	Geographic origin	Potato variety	Disease symptoms
1	Skene, Scotland	Penta	severe canker, severe powdery scab
2	Coupar Angus, Scotland	Cara	severe powdery scab
3	Arbroath, Scotland	Maris Piper	severe canker, severe powdery scab
4	Kirrimuir, Scotland	Nadine	severe powdery scab
5	Montrose, Scotland	Estima	severe canker, light powdery scab
6	Montrose, Scotland	Nadine	severe powdery scab
7	Arbroath, Scotland	Red Cara	severe canker, severe powdery scab
8	Northumbria, England	Estima	light powdery scab
9	Newmachar, Scotland	Nadine	light powdery scab
10	Inverbervie, Scotland	Estima	light to moderate powdery scab
11	Forfar, Scotland	Estima	moderate powdery scab
12	Coupar Angus, Scotland	Estima	moderate to severe powdery scab
13	Aberdeen, Scotland	Maris Bard	light canker, moderate to severe powdery scab
14	Arbroath, Scotland	Estima	severe powdery scab
15	Portmahomack, Scotland	Maris Piper	severe canker, severe powdery scab
16	New Zealand	Agria	unknown
17	Ballarat, Australia	Toolangi Delight	unknown
18	Colac, Australia	unknown	unknown
19	Ballarat, Australia	unknown	unknown

DNA was extracted as described above from different amounts of sporeballs (0, 1, 5, 15, 50, 500 and 1000) from sample no. 3 in Table 9.1. The sporeballs were scraped from washed and dried tubers. Using a stereomicroscope, single sporeballs were then counted and transferred with a glass rod with a narrow tip to tubes containing SDW. Sporeballs at the two highest concentrations were measured by establishing the sporeball concentration of a solution of sporeballs in SDW in a haemocytometer and pipetting the correct amount into plastic tubes. There were five replicates of each amount of sporeballs. The DNA extracted from the different amounts of sporeballs was used for the AFLP protocol. Vague band patterns were observed for most of the DNA samples, but those for DNA from 50 sporeballs appeared much brighter than from the other amounts. On the basis of this result, 50 sporeballs were counted out for each sample listed in Table 9.1 (3 replicates) and the DNA extracted from these sporeballs was used for another AFLP. Although most samples showed band patterns on the AFLP, these were too faint to analyse. This was probably because the DNA concentrations in the samples were too low. To test this, DNA was extracted from scrapings with large numbers of sporeballs (1000's) and used for AFLP. This yielded a gel with bright bands for most samples. However, interpretation of the results was difficult since it was unknown which bands were the result of the presence of *S. subterranea* DNA and which of potato DNA co-extracted from skin tissue attached to sporeballs. Due to the nature of powdery scab, separation of *S. subterranea* sporeballs from the host tissues is extremely difficult unless sporeballs are selected singly under the microscope or zoospores are used. However, not enough DNA can be extracted with the latter methods to obtain bands bright enough for analysis.

Population structure of *S. subterranea* - main conclusion:

- **The AFLP protocol works for DNA from *S. subterranea*. However, due to the nature of the pathogen, not enough pure DNA could be obtained to successfully use AFLP for the study of genetic variation.**

Seed tuber survey

Objective: To survey (confidentially) seed stocks to determine the amount and distribution of inoculum carried symptomlessly on seed tubers. A structured sample of tubers will be collected from a limited number of certified stocks and tested for the presence of inoculum. Sampling would be made of a susceptible and a moderately resistant variety (20 stocks) of each. The stocks would be chosen at random from the seed grower register (SASA assistance) but confined to a single grade and regulated to ensure a geographical distribution in relation to acreages grown. Statistical advice would be sought from BioSS on the sampling. The results in combination with the results of the glasshouse work on inoculum will provide some estimate of how widespread undetected low levels of inoculum are on stocks and the possible hazard that they represent to ware growers. It is essential for several reasons that such work is undertaken in confidence and without prejudice to the tested crops (SCRI).

Milestone 13: Collect washings for three 20 tuber samples from 40 seed stocks to be representative of stocks throughout Scotland (SASA assistance). Freeze sample. *– achieved (for 10 individual tuber samples from 11 seed stocks, sap was also tested)*

Milestone 14: Test washings by PCR and produce confidential report of survey – *achieved*

In order to investigate how common contamination is of certified Scottish seed potato stocks with *S. subterranea*, tubers were sampled by SASA from a selection of seed stocks around Scotland in 2002. Two different varieties were sampled, 'Estima', which is susceptible to powdery scab, and 'Hermes', which is more resistant to the disease. Twenty-five tubers were sampled from each of the stocks listed in Table 10.1 of which ten tubers were randomly selected and tested in the laboratory for the presence of *S. subterranea*.

TABLE 10.1 STOCKS OF CERTIFIED SCOTTISH SEED POTATOES SAMPLED FOR THE TESTING OF SEED TUBERS FOR THE PRESENCE OF *SPONGOSPORA SUBTERRANEA*.

Variety	County
Estima	Aberdeen 1
	Angus
	Aberdeen 2
	Berwick
	Ross
	Perth
Hermes	Moray
	Banff
	Kincardine
	Aberdeen 3
	Roxburgh

Tubers were randomly selected from each sample and treated individually. Each tuber was washed in a sealed plastic bag in 20 ml SDW and then again in another 20 ml SDW. The washings were collected in centrifuge tubes. Washed tubers were dried, weighed and then assessed for symptoms of powdery scab. Powdery scab severity was assessed using the scale of Merz (2000) as described before. Every tuber was peeled using a sterile scalpel and the skin

peelings were transferred to a 20 ml sterile disposable syringe which was squeezed after adding 10 ml SDW. The sap from the syringe was collected in centrifuge tubes.

The tubes with the washing and sap samples were centrifuged at 3200 *g* for 10 min after which the superfluent was discarded. The pellets were transferred individually to 2 ml sterile tubes, 0.4 g beats and 1 ml of extraction buffer were added per tube, and DNA was then extracted following the protocol of Bell *et al*. (1999).

DNA extracted from the tuber washings and peelings was used in a real-time PCR assay and the amount of *S. subterranea* DNA present in each sample was quantified.

S. subterranea DNA was detected in all stocks tested, but there were considerable differences between stocks and within the two varieties (Table 10.2). All 'Estima' stocks contained tubers with powdery scab symptoms. One stock stood out significantly from the rest in the incidence and severity of powdery scab. All tubers sampled from the 'Estima' stock from Ross had severe powdery scab, most with more than 25% of surface covered with mature symptoms. Extremely high levels of *S. subterranea* DNA were detected in the skins of these tubers (on average 76,282 sporeball equivalents (units) per tuber). The other 'Estima' stocks did not differ significantly from each other in powdery scab incidence or severity. About 50% of tubers sampled from these stocks showed mature powdery scab symptoms, but in most cases less than 10% of surface area was covered with scabs. There was some variation between these stocks in amount of *S. subterranea* DNA detected in the skin and washings (Table 10.2). The tuber skin results for the 'Estima' stocks from Angus and Aberdeen-2 were similar (4 to 5 units per g tuber weight), as were those for Aberdeen-1 and Berwick (230 to 340 units per g tuber weight). Results for the Perth stock were very variable. The *S. subterranea* DNA levels in the 'Estima' tuber washings were on average lower than from tuber skin peelings. Stocks that tested low for DNA in the skin peelings also tested lowest for DNA in the tuber washings. These were the stocks with, on average, the least severe powdery scab symptoms (not significant).

Two of the 'Hermes' stock samples did not contain tubers with powdery scab (Moray and Banff) and not many tubers with powdery scab were found in the other 'Hermes' samples. However, most tuber washings and skin peelings from the 'Hermes' stocks still tested positive for *S. subterranea*. Tubers from the Roxburgh stock in particular contained relatively high levels of *S. subterranea* DNA in their skin (9 units per g tuber weight) compared with the other 'Hermes' stocks, which did not differ much from each other (1.5 or less units per g tuber weight). As with 'Estima', 'Hermes' stocks that tested lowest for DNA in the tuber skin peelings also tested lowest for DNA in the tuber washings. However, the highest amount of DNA (7 units per g tuber weight) was found in the Banff stock which did not contain tubers with powdery scab. Unlike 'Estima', the average amount of *S. subterranea* DNA in 'Hermes' tuber washing was generally not significantly different from the amount of DNA detected in tuber skin peelings.

On average, *S. subterranea* DNA levels were significantly lower in 'Hermes' stocks than in Estima stocks. Tuber washings from 'Hermes' contained on average only 4% of the amount of DNA detected in 'Estima' washings. *S. subterranea* DNA levels in tuber peelings from 'Hermes' were also much lower (less than 1% of the average amount in 'Estima' peelings). However, the cleanest 'Estima' stocks (Angus and Aberdeen-2) did not differ significantly from the 'Hermes' stocks in the average level of *S. subterranea* DNA in washing or skin peelings.

TABLE 10.2 DETECTION AND QUANTIFICATION OF SPONGOSPORA SUBTERRANEA DNA IN WASHINGS AND SKIN PEELINGS OF CERTIFIED SCOTTISH POTATO SEED TUBERS FROM DIFFERENT ORIGIN, USING A REAL-TIME PCR (TAQMAN®) ASSAY.

Variety	Origin	No. of tubers with powdery scab symptoms	Average powdery scab severity[1] ± S.E.	Tuber washings			Tuber skin peelings		
				Number of tubers testing *S. subterranea* DNA positive	Average amount of DNA detected (tot. units)[2] ± S.E.	Average amount of DNA detected (units per g tuber weight)[2] ± S.E.	Number of tubers testing *S. subterranea* DNA positive	Average amount of DNA detected (tot. units)[2] ± S.E.	Average amount of DNA detected (units per g tuber weight)[2] ± S.E.
Estima	Aberdeen-1	5/10	1.8 ± 0.3	9/10	788 ± 329	13.9 ± 5.4	10/10	10889 ± 9455	227.0 ± 206.2
	Angus	5/10	1.5 ± 0.2	9/10	176 ± 57	1.8 ± 0.5	9/10	371 ± 119	3.9 ± 1.3
	Aberdeen-2	5/10	1.5 ± 0.2	9/10	471 ± 293	7.6 ± 4.9	9/10	306 ± 180	5.1 ± 3.0
	Berwick	5/10	1.9 ± 0.3	10/10	5727 ± 3026	287.3 ± 169.3	10/10	6157 ± 5877	339.1 ± 326.8
	Ross	10/10	4.5 ± 0.3	8/10	6218 ± 2464	110.3 ± 46.1	9/10	76282 ± 21232	1227.7 ± 328.0
	Perth	4/10	1.5 ± 0.2	5/10	466 ± 387	10.4 ± 9.1	10/10	728 ± 639	16.0 ± 15.0
	average	*34/60*	*2.1 ± 0.2*	*50/60*	*2445 ± 790*	*80.3 ± 36.7*	*57/60*	*15269 ± 5139*	*297.4 ± 99.2*
Hermes	Moray	0/10	1.0 ± 0	9/10	54 ± 16	1.0 ± 0.4	10/10	11 ± 5	0.2 ± 0.1
	Banff	0/10	1.0 ± 0	10/10	153 ± 61	7.3 ± 2.9	7/10	45 ± 33	1.5 ± 1.0
	Kincardine	5/10	1.7 ± 0.3	10/10	78 ± 19	1.2 ± 0.4	9/10	35 ± 8	0.5 ± 0.1
	Aberdeen-3	4/10	1.4 ± 0.2	8/10	154 ± 65	2.8 ± 1.2	10/10	33 ± 17	0.7 ± 0.3
	Roxburgh	2/10	1.4 ± 0.3	10/10	240 ± 159	2.6 ± 1.7	10/10	872 ± 434	9.3 ± 4.4
	average	*11/50*	*1.3 ± 0.1*	*47/50*	*137 ± 38*	*3.1 ± 0.8*	*46/50*	*213 ± 105*	*2.5 ± 1.1*

[1]based on the percentage tuber surface area covered with powdery scab on a scale from 1 (no powdery scab) to 7 (> 75% of surface covered with powdery scab); [2]positive samples only; one unit corresponds with the amount of DNA detected after extraction from one average sized sporeball.

TABLE 10.3 DETECTION AND QUANTIFICATION OF *SPONGOSPORA SUBTERRANEA* DNA IN WASHINGS AND SKIN PEELINGS OF CERTIFIED SCOTTISH POTATO SEED TUBERS CV. 'ESTIMA' WITH AND WITHOUT POWDERY SCAB SYMPTOMS, USING A REAL-TIME PCR (TAQMAN®) ASSAY.

Powdery scab symptoms	Origin	Average powdery scab severity[1] ± S.E.	Tuber washings			Tuber skin peelings		
			Number of tubers testing *S. subterranea* DNA positive	Average amount of DNA detected (tot. units)[2] ± S.E.	Average amount of DNA detected (units per g tuber weight)[2] ± S.E.	Number of tubers testing *S. subterranea* DNA positive	Average amount of DNA detected (tot. units)[2] ± S.E.	Average amount of DNA detected (units per g tuber weight)[2] ± S.E.
+	Aberdeen-1	2.6 ± 0.2	4/5	578 ± 416	12.7 ± 9.9	5/5	21663 ± 18555	452.0 ± 407.4
	Angus	2.0 ± 0	5/5	202 ± 94	2.0 ± 0.8	5/5	491 ± 151	5.2 ± 1.8
	Aberdeen-2	2.0 ± 0	5/5	685 ± 519	12.0 ± 8.6	5/5	451 ± 322	8.0 ± 5.3
	Berwick	2.8 ± 0.4	5/5	8180 ± 6019	436.7 ± 337.9	5/5	12117 ± 11733	670.8 ± 652.4
	Ross	4.5 ± 0.3	8/10	6218 ± 2464	110.3 ± 46.1	9/10	76282 ± 21232	1227.7 ± 328.5
	Perth	2.3 ± 0.3	4/4	575 ± 481	12.9 ± 11.3	4/4	1626 ± 1615	37.7 ± 37.6
	average	*3.0 ± 0.2*	*31/34*	*3216 ± 1229*	*104.5 ± 57.9*	*33/34*	*26262 ± 8418*	*511.5 ± 162.3*
-	Aberdeen-1	1.0 ± 0	5/5	957 ± 520	14.9 ± 6.8	5/5	115 ± 34	2.1 ± 0.5
	Angus	1.0 ± 0	4/5	143 ± 63	1.6 ± 0.6	4/5	220 ± 184	2.3 ± 2.0
	Aberdeen-2	1.0 ± 0	4/5	203 ± 148	2.1 ± 1.3	4/5	124 ± 27	1.6 ± 0.4
	Berwick	1.0 ± 0	5/5	3274 ± 1406	137.8 ± 60.2	5/5	196 ± 135	7.4 ± 5.0
	Perth	1.0 ± 0	1/6	28 ± 0	0.3 ± 0	6/6	129 ± 62	1.4 ± 0.6
	average	*1.0 ± 0*	*19/26*	*1188 ± 474*	*41.0 ± 20.1*	*24/26*	*154 ± 41.6*	*3.0 ± 1.1*

[1]based on the percentage tuber surface area covered with powdery scab on a scale from 1 (no powdery scab) to 7 (> 75% of surface covered with powdery scab);
[2]positive samples only; one unit corresponds with the amount of DNA detected after extraction from one average sized sporeball.

TABLE 10.4 DETECTION AND QUANTIFICATION OF *SPONGOSPORA SUBTERRANEA* DNA IN WASHINGS AND SKIN PEELINGS OF CERTIFIED SCOTTISH POTATO SEED TUBERS CV. 'HERMES' WITH AND WITHOUT POWDERY SCAB SYMPTOMS, USING A REAL-TIME PCR (TAQMAN®) ASSAY.

Powdery scab symptoms	Origin	Average powdery scab severity[1] ± S.E.	Tuber washings			Tuber skin peelings		
			Number of tubers testing *S. subterranea* DNA positive	Average amount of DNA detected (tot. units)[2] ± S.E.	Average amount of DNA detected (units per g tuber weight)[2] ± S.E.	Number of tubers testing *S. subterranea* DNA positive	Average amount of DNA detected (tot. units)[2] ± S.E.	Average amount of DNA detected (units per g tuber weight)[2] ± S.E.
+	Kincardine	2.4 ± 0.2	5/5	99 ± 30	1.8 ± 0.7	4/5	31 ± 15	0.5 ± 0.3
	Aberdeen-3	2.0 ± 0	3/4	118 ± 101	1.6 ± 1.3	4/4	59 ± 43	1.0 ± 0.8
	Roxburgh	3.0 ± 0	2/2	198 ± 184	2.2 ± 2.0	2/2	1165 ± 1141	15.5 ± 15.2
	average	*2.4 ± 0.2*	*10/11*	*124 ± 42*	*1.8 ± 0.6*	*10/11*	*269 ± 227*	*3.7 ± 3.0*
-	Moray	1.0 ± 0	9/10	54 ± 16	1.0 ± 0.38	10/10	11 ± 5	0.2 ± 0.1
	Banff	1.0 ± 0	10/10	153 ± 61	7.3 ± 2.9	7/10	45 ± 33	1.5 ± 1.0
	Kincardine	1.0 ± 0	5/5	57 ± 24	0.6 ± 0.3	5/5	38 ± 11	0.4 ± 0.1
	Aberdeen-3	1.0 ± 0	5/6	175 ± 93	3.5 ± 1.8	6/6	16 ± 5.7	0.4 ± 0.2
	Roxburgh	1.0 ± 0	8/8	251 ± 198	2.7 ± 2.1	8/8	798 ± 503	7.8 ± 4.7
	average	*1.0 ± 0*	*37/39*	*140 ± 47*	*3.4 ± 1.0*	*36/39*	*197 ± 119*	*2.2 ± 1.1*

[1]based on the percentage tuber surface area covered with powdery scab on a scale from 1 (no powdery scab) to 7 (> 75% of surface covered with powdery scab);
[2]positive samples only; one unit corresponds with the amount of DNA detected in one average sized sporeball.

Comparison of the results for 'Estima' tubers with and without powdery scab symptoms, showed that on average there was no significant difference in amount of *S. subterranea* DNA in tuber washings between the two groups, although the average for tubers with disease was higher (Table 10.3). In contrast, the amount of *S. subterranea* DNA in skin peelings from symptomless tubers was on average significantly lower than in skin peelings of tubers with powdery scab. The average amount of *S. subterranea* DNA found in skin peelings from tubers without powdery scab was less than 1% of that detected in skin from tubers with symptoms.

When the same comparisons were made for 'Hermes' tubers, no significant differences in *S. subterranea* levels in tuber washings or peelings were found between the groups with and without powdery scab symptoms (Table 10.4). Even though the average disease severity was similar for 'Estima' and 'Hermes' tubers with powdery scab, the average amount of *S. subterranea* DNA detected in tuber skin peelings of diseased 'Hermes' tubers was at the same level as that of 'Estima' and 'Hermes' tubers without powdery scab (i.e. less than 1% of the amount found in 'Estima' tubers with powdery scab).

Although the average amount of *S. subterranea* DNA found in skin of tubers without powdery scab was on average similar for 'Estima' and 'Hermes', the washings from healthy 'Estima' tubers contained much more *S. subterranea* DNA than those from 'Hermes' tubers, possibly due to contamination of the 'Estima' tubers with sporeballs from tubers with powdery scab in the same stocks. Washings from 'Hermes' tubers (regardless of the presence of powdery scab symptoms) contained on average only 4-8% of the amount of *S. subterranea* DNA found in washings from 'Estima' tubers without powdery scab.

Powdery scab severity and the amount of *S. subterranea* DNA detected in tuber skin appeared to be correlated, but there were some outliers at higher disease levels. Although there was some variation in DNA levels in tubers with medium to high levels of powdery scab, tubers with low powdery scab scores (score 1 or 2, no disease at all or less than 5% of surface covered with powdery scab) always yielded < 40 units (sporeball equivalents) per g tuber weight. No relationship existed between powdery scab severity and the amount of *S. subterranea* DNA detected in tuber washing. Tubers without powdery scab symptoms sometimes yielded relatively high amounts of *S. subterranea* DNA when washed.

Not all 25 tubers sampled from each stock were tested because the testing of single tubers was time consuming and the results for ten tubers per stock did not differ much from the results for five tubers. The number of stocks sampled in this survey was too small to determine whether there was a relationship between supplier, geographical origin, final grade or acreage and the powdery scab and *S. subterranea* DNA levels in the samples. In a future survey, it would be better to sample a smaller number of tubers from a much wider range of stocks.

The results of this survey showed that most certified seed tubers were contaminated and/or infected with *S. subterranea*. Even the powdery scab "resistant" variety 'Hermes' was found to be widely contaminated with the pathogen, although DNA levels were much lower than in the more susceptible variety 'Estima'. The role of seed-borne inoculum of *S. subterranea* in disease epidemiology is still not fully understood but is probably not significant in situations where fields have already been contaminated with the pathogen. However, the presence of *S. subterranea* on Scottish potato seed could have an important impact on disease incidence if seed is planted in fields previously free from inoculum.

Seed tuber survey - main conclusions:

- **Certified Scottish seed tubers (cvs. 'Estima' and 'Hermes') were widely contaminated and infected with the powdery scab pathogen *S. subterranea*.**

- **In the case of the susceptible variety, powdery scab symptoms were common and contamination and infection levels were generally very high. In symptomless 'Estima' tubers, pathogen infection levels were low but contamination with large numbers of sporeballs was widespread.**

- **In case of the resistant variety ('Hermes'), the incidence of contamination and infection with *S. subterranea* was high, but pathogen DNA levels were generally low compared with the susceptible variety irrespective of the presence of powdery scab symptoms.**

Main conclusions

Detection methods:

- **The primer/probe designed to detect *S. subterranea* DNA is effective. Sensitive, selective quantification of *S. subterranea* DNA by real-time PCR assay is now possible.**
- **The real-time PCR assay can be used to detect *S. subterranea* DNA from sporeballs in water, soil and host tissues, and from zoosporangia/plasmodia in roots and zoospores in water.**
- **Combination of the tomato bait plant test with a PCR assay is a reliable method for the detection of *S. subterranea*.**
- ***Quantification of* S. subterranea *using the bait plant method is not reliable at high inoculum levels.***
- ***Quantification of* S. subterranea *DNA in soil samples after direct extraction can be inaccurate if soil chemicals inhibit the PCR reaction.***
- **Soil type has an effect on the detection of *S. subterranea* in soil after direct extraction. Substances in loamy and sandy soils in particular strongly inhibit the PCR reaction.**
- **The detection of *S. subterranea* in soil samples using a PCR assay can be improved by flooding dried soil samples for three days and by then extracting DNA from soil and floodwater together.**
- **The detection of *S. subterranea* in soil samples using a conventional PCR assay can be improved further by using nested PCR.**

Viability of sporeballs in soil:

- **The amount of *S. subterranea* DNA detected in soil samples is often lower than expected. This is probably due to inhibition of PCR by co-extracted soil chemicals.**
- **The viability of sporeballs of *S. subterranea* in clay soil decreases in time, but the speed of the decline depends on temperature.**

Disease threshold and environmental factors:

- **Both the incidence and severity of potato powdery scab, caused by soil-borne inoculum, are influenced by a range of environmental factors, including temperature, soil type and soil moisture regime.**

- **Soil inoculum level does not have a significant influence on infection and disease development and even low levels of inoculum can result in severe disease symptoms.**

- **Latent tuber infections appear to be common, but more research needs to be conducted into their importance in the spread and occurrence of powdery scab.**

- **There is no relationship between the occurrence of powdery scab on tubers and galls on roots. Both symptoms may occur independently and even when they do occur together their severity is usually not correlated.**

- **The influence of environmental factors, especially temperature, is different for powdery scab and root gall development.**

- **There is little evidence that a consistent or simple relationship exists between the level of inoculum on seed and subsequent disease developing on the progeny crop.**

- **Environmental conditions are the major driver of disease development in the field.**

- **There is no evidence from the field trial that tightening the SEERAD standard for potato seed will reduce the risk of powdery scab in a subsequent crop.**

- **Where soil is already contaminated, it seems unlikely that a low level of further contamination would influence the risk of disease, if the view that environmental conditions are the major driver of disease development is accepted.**

- **It is a reasonable aim of seed production to minimise disease development and a reasonable aim of purchasers of seed to buy seed with limited levels of powdery scab.**

Chemical and integrated control:

- **Several treatments have been identified in pot tests, which are more effective against soil borne inoculum of *S. subterranea* than zinc.**

- **In pot tests, fluazinam and mancozeb are the most effective chemicals for the control of disease caused by soil-borne *S. subterranea*. However, phytotoxic side effects may occur.**

- **Application of prawn shell waste to the soil before planting can eliminate powdery scab but is not effective against root galling. Applying an additional chemical treatment after tuber initiation reduces root gall incidence.**

- ***Brassica juncea* is the most effective biofumigant crop tested in pots and application of freeze dried powder of this species to the soil before planting may significantly reduce powdery scab incidence and severity.**

- **There is evidence from the field that certain chemicals can have a substantial effect on *S. subterranea* but their consistency of effect is poor.**

Chemical control can reduce severity more often than incidence of powdery scab.

The only chemical treatment that consistently reduced powdery scab in the field was fluazinam soil treatment.

Fluazinam's effectiveness in the field was greatest where soil-borne inoculum was the principal source of inoculum and limited where tubers were the main source of inoculum.

Effective control probably lies with combination treatments of an effective tuber treatment plus the fluazinam soil treatment.

It is recommended that data be submitted to PSD for Specific Off-label Approval for fluazinam as a soil treatment for use on seed potato crops.

One reason for the variability in results could be differences in inoculum between trials.

Host variety had the greatest impact on powdery scab. Changing from a susceptible variety to a more resistant one gave the most consistent reduction in incidence and severity of powdery scab of all control measures evaluated.

However, as with other control measures, changing to a resistant variety has a more consistent effect on severity than incidence.

- **Matching variety to disease risk is a clear option for growers to reduce powdery scab risk, although identifying high risk fields remains subjective.**
- **There were few interactions between control measures in the control of powdery scab.**
- **The use of fleece resulted in a reduction of powdery scab with susceptible varieties only.**
- **Although, the results of the trials do not unambiguously confirm it, it seems logical that to effect the greatest control of powdery scab, control measures should be integrated.**

Population structure of S. subterranea:

- **The AFLP protocol works for DNA from *S. subterranea*. However, due to the nature of the pathogen, not enough pure DNA could be obtained to successfully use AFLP for the study of genetic variation.**

Seed tuber survey:

- **Certified Scottish seed tubers (cvs. 'Estima' and 'Hermes') were widely contaminated and infected with the powdery scab pathogen *S. subterranea*.**
- **In the case of the susceptible variety, powdery scab symptoms were common and contamination and infection levels were generally very high. In symptomless 'Estima' tubers, pathogen infection levels were low but contamination with large numbers of sporeballs was widespread.**

- **In case of the resistant variety ('Hermes'), the incidence of contamination and infection with *S. subterranea* was high, but pathogen DNA levels were generally low compared with the susceptible variety irrespective of the presence of powdery scab symptoms.**

Discussion

Spongospora subterranea is a very difficult pathogen to study, since it cannot be cultured and 'pure' single isolate cultures are not available. Its sporeballs, which are used as inoculum by most researchers, are highly variable and may release different numbers of motile zoospores, which are the actual infectious units. The zoospores can infect root cells, forming zoosporangia, which release secondary zoospores. Thus, a low level of inoculum can quickly increase to a much higher level making it impossible to know the exact amount of inoculum present during the different stages of disease development. In order to be able to accurately measure the presence of S. subterranea during experiments, diagnostic methods are needed that permit precise determination of pathogen DNA levels, regardless of whether the DNA originates from sporeballs, plasmodia, zoosporangia or zoospores. Serological methods have been tried but are generally too insensitive. New real-time PCR methodologies for the detection and quantification of S. subterranea were developed at SCRI and optimised for use in this project. The method has enabled greater insight into the pathogen than was previously possible and it is now possible to accurately measure the level of S. subterranea DNA in host plant tissues. The use of real-time PCR has highlighted the presence of 'latent infections' in plant tissues.

Measurement of inoculum in soils using real-time PCR has been less successful and in experiments using standard levels of soil inoculum, the proportion of sporeballs detected was relatively low. All soil types were found to inhibit the PCR reaction to some extent, but the inhibition was greatest with sandy or loamy soils and least with a clay loam. It is believed that heavy metals or substances from organic matter such as humic acids may be the cause of the inhibition. In order to overcome the poor detection of inoculum in soil, DNA extraction methods that successfully remove the soil inhibitory element need to be developed.

Another issue that needs to be addressed in the detection of soil-borne inoculum is the threshold of detection. In the experiments described in this report, the limit of detection was 2 sporeballs per gram of soil. In earlier bioassays, detection of under 0.05 sporeballs per gram was achieved (Brereton, 1991) and significant disease developed from levels as low as this. Success in establishing a robust diagnostic method for the detection of soil-borne inoculum is a crucial objective, not just for research purposes but also to enable growers to make objective and practical decisions on disease evasion and control. Provided a robust PCR test can be established that is commercially viable, has a sufficiently rapid throughput and a low threshold of detection, growers would be willing to pay for a soil test to help decide the risk of powdery scab. The answer might lie in combining the tomato bait plant and real-time PCR techniques into one sensitive, reliable test.

There are two other steps allied to diagnostics that need to be clarified before a test can be marketed. These are clear guidelines for soil sampling and straightforward guidelines for interpretation of the test results. The sampling guidelines cannot be established until a robust test is available and, to a certain extent, this is true also for the interpretation. However, by using data from this and other projects it should be possible to develop a risk evaluation as the basis for the interpretation of soil test results.

New discoveries about the biology of the pathogen have been made during this project and most of these have practical significance. For example, the increased longevity of sporeballs under cool conditions can explain, in part, why the pathogen appears to be a more persistent problem in northern Britain. The converse finding, that in warm temperatures survival is shorter, is also useful information. It would be interesting to know the cause of the differences in survival between warm

and cool conditions. It may be that the natural death of individual spores or the microbial breakdown of the whole sporeball is greater at higher temperatures or that the sporeballs mature and germinate more rapidly under warmer conditions.

The finding that there is a poor relationship between the level of visible infection of seed with S. subterranea and the development of powdery scab on the progeny is supported by data from a MAFF (now DEFRA) potato disease survey (Wale et al., 2002) and other studies carried out in the UK and overseas. This has implications for seed growers, seed certification and buyers of seed. The data presented in this report indicate that the acceptance of a slightly higher level of seed tuber contamination poses little risk of additional disease. A higher level of seed inoculum will contribute more to the contamination of the field where the seed is planted but as environmental conditions are a bigger driver for disease than inoculum level, this is unlikely to be of major significance except in cases where the soil is completely free from S. subterranea.

A survey to identify the spread and severity of soil contamination with S. subterranea in different regions of the UK would be useful, but would require a robust diagnostic test. Experience with powdery scab in Scotland over many years, particularly knowledge of levels of the disease in seed stocks (supported by the survey of stocks in this project), highlights the fact that a high proportion of varieties grown are susceptible or moderately susceptible. In addition, difficulties in finding fields free from S. subterranea suggest that soil contamination in potato growing regions might be widespread. If this was confirmed, concern amongst growers about planting seed with low levels of powdery scab would probably diminish.

During the seed tuber trial in this project, powdery scab failed to develop in many sites in south and east England despite the fact that it was one of the wettest summers for years. It is known that there are some parts of England (e.g. Cheshire, Norfolk) where powdery scab can be a problem but there are other parts (e.g. heavy soils in Essex) where significant levels of powdery scab never seem to occur. This may be related to soil type, to the suppressive character of some soils or to the inter-relationship between inoculum and environmental conditions. Powdery scab is a major disease in the northern latitudes of Europe primarily, which suggests that climate has a large impact on the disease. Climate may explain, in part, the regional differences found in the experiment. The view that S. subterranea is mainly a cool climate pathogen has been reinforced by further important findings in this project. The discovery that disease occurs as low as 9^{o}C extends the known temperature range for powdery scab development to 9^{o}C – 20^{o}C. The optimum temperature for powdery scab appears to lie around 12^{o}C. The temperature range of 9^{o}C-12^{o}C is typically that found during wet spells (at least for part of the day) in Scotland after planting and before tuber initiation is complete, thus forming the ideal conditions for infection of the potato crop by S. subterranea.

Controlled environment studies have suggested that more disease develops in sandy and loam soils than in clay soils, which agrees with field observations in some countries (de Boer, 2000; Wale, 2000). This difference between soil types may be related to oxygen availability and/or pore size or presence of a suppressive character. Further study on the interaction of disease and soil type is required to help growers to identify disease risks and select the most suitable fields.

Two areas of the studies undertaken in this project that potentially have the most impact are the significance of root galls and latent infections. Root galls have been observed in the field by growers but are often dismissed as a curiosity. However, the galls produce sporeballs that persist in the soil and act as a source of infection in the same way as sporeballs originating from tubers with powdery scab. What is clear from the experiments presented here is that root galls are far more frequent in warmer soil conditions (17^{o}C) than cool soil conditions (9-12°C). It is likely, therefore, that galls may occur more frequently in the south of the UK than the north. The identification of this

relationship should enable breeders to screen varieties during selection more effectively. Such screening is not part of variety evaluation currently, but the fact that some varieties can exhibit high levels of root galling whilst having a high degree of tuber resistance (Schwärzel, 2002) may have important practical implications. When varieties with a high degree of tuber resistance are grown, powdery scab is usually rare but root galling can be common and if it remains undetected, the level of sporeball inoculum in the soil can rise, also unnoticed. If a susceptible variety is subsequently grown in the same field, severe powdery scab may occur. This situation has been recognised in the USA and it would therefore be advisable for breeders to screen varieties for root gall susceptibility in order to alert growers to the risk of root galling and inoculum build-up.

The use of the real-time PCR test has indicated that latent and immature tuber infections by *S. subterranea* could be common, especially under conditions sub-optimal for symptom development. These infections may continue to develop during storage and immature *S. subterranea* infections may even form sporeballs without acquiring the typical powdery appearance (Kole, 1954). This could form an important source of inoculum, and there are therefore direct implications for current seed inspection procedures and certification standards. Given the finding in the seed survey that DNA can be detected in the skin of washed, symptomless tubers of a resistant variety, it is important to establish the significance of latent infections. The potential of latent infection appears to add to the risk of contamination of seed tubers as another potential source of the pathogen.

Powdery scab is such an important disease to seed growers that many are looking for a chemical to control it. However, as this and previous projects have demonstrated, effective chemical control is difficult to achieve and where there is an effect, it results in reduced severity more than incidence. Confirmation that fluazinam is the most effective chemical that has been tested provides the spur to develop a SOLA for soil treatment. If this can be obtained, it will add an important weapon to the armoury for control of the disease. However, there is a need to evaluate the combination of tuber disinfection and soil treatment with fluazinam to determine if a major reduction in disease is possible. This is the current approach in New Zealand and should form part of any further investigation here. At the moment, there is a reluctance to dip tubers in the UK because of the increased risk of blackleg incidence.

The overwhelming significance of varietal resistance as the most important control measure was the main finding in the integrated control trials. No other measure resulted in a reduction of disease of the same degree. This re-emphasises the need to place powdery scab resistance higher in the screening programme for new varieties. Given the increase in significance of powdery scab across northern Europe and the higher profile it has in seed importing countries, GB cannot afford to release varieties with susceptibility to powdery scab.

The discovery that fleece covers can reduce disease in susceptible varieties may provide an opportunity for growers of high-grade seed. The cost of using fleece is high but for relatively small acreages the expense would be limited. Other future components of integrated control may include biofumigation using *B. juncea* or the application of prawn shell waste, which both proved to be very effective in the controlled environment trials carried out during this project. However, further research is needed before these methods can be applied successfully in the field.

Although the results of this project have contributed considerably to a better understanding of *S. subterranea* and to disease prevention and control, powdery scab remains a major problem for which no easy solutions exist. More research into epidemiology, detection and control has to be carried in order to progress further towards a fully effective integrated management system for potato powdery scab.

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Project milestones

Milestone 1: Establish glasshouse system for testing viability and productivity and measuring inoculum levels in soil. (SCRI)

Milestone 2: Preliminary estimate on inoculum viability in soil (provided it declines enough to be measurable within 30 months).(SCRI)

Milestone 3: Establish field experiments to tests of standard stocks. (SAC)

Milestone 4: Preliminary assessments for field work. (SAC/SCRI)

Milestone 5: Assess glasshouse trials for relative efficacy of different chemicals, identify promising new candidate chemicals for field trials and produce interpreted grower report on results. (SCRI/SAC)

Milestone 6: Assess field trials for relative efficacy of different chemicals, and produce interpreted grower report on results. (SAC)

Milestone 7: Assess efficacy of timing of fungicide application and produce interpreted reports from results of (i) the glasshouse (SCRI) (2) the field (SAC).

Milestone 8: Establish field trials and determine effects of different control measures on powdery scab. Relate the results to population monitoring. Produce interpreted reports on integrated control measures as grower friendly summary sheets. (SAC)

Milestone 9: Identify a range of brassica species for evaluation as bio-fumigants. Carry out trials on contaminated land and assess relative efficacy. Prepare grower friendly report on potential of bio-fumigation. (SAC)

Milestone 10: Determine smallest number of spore balls that can reliably be tested by AFLP. (SCRI)

Milestone 11: Complete sampling of five lesions per tuber, ten tubers per stock, ten stocks selected for across Scotland. Extract DNA for AFLP analysis. (SCRI/SAC)

Milestone 12: Analyse AFLP patterns of above samples. (SCRI)

Milestone 13: Collect washings for three 20 tuber samples from 40 seed stocks selected to be representative of stocks throughout Scotland (SASA assistance). Freeze sample. (SAC/SCRI)

Milestone 14: Test washing by PCR and produce confidential report of survey. (SCRI/SAC)

Communicated outputs

Publications

Lees, A.K., van de Graaf, P., Cullen, D.W. & Duncan, J.M. (2002) The development of a quantitative real-time PCR assay for *Spongospora subterranea* f.sp. *subterranea* and its use for epidemiological studies. *Abstracts 5th Symposium International Working Group of Plant Viruses with Fungal Vectors, Zürich, Switzerland, July 22-25, 2002*.

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Lees, A.K., van de Graaf, P., Cullen, D.W. & Duncan, J.M. (2003) The development of a quantitative real-time PCR assay for *Spongospora subterranea* f.sp. *subterranea* and its use for epidemiological studies. *Proceedings of the Plasmodiophorid workshop,* ICPP2003, Christchurch, NZ.

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Press articles

"Experts home in on supply chain worries", *Potato Review*, March 2001, p. 28-29.

"Bio-fumigation solution to soil-borne fungal disease", *Arable Farming*, September 22, 2001, p. 38.

"Research pinpoints system to cut losses from disease", *The Scottish Farmer*, February 2, 2002, p. 23.

"Battling against scab", *Crops*, February 16, 2002, p. 38.

"Putting the lid on powdery scab", *Grower*, December 12, 2002, p. 14-15.

Oral presentations

Oral presentations related to this project were given by P. van de Graaf, A.K. Lees and/or S.J. Wale at the following meetings:

- First European Powdery Scab Workshop, SAC, Aberdeen, Scotland, July 2000

- Syngenta Potato Colloquium, Peterborough, February 2001

- BPC MITT 'Ask the expert' meeting, November 2001

- Crop Protection in Northern Britain, Dundee, February 2002

- 5th Symposium International Working Group of Plant Viruses with Fungal Vectors, Zürich, Switzerland, July 2002

- Powdery Scab Scoring Workshop, La Fretaz, Switzerland, August 2002

- Plasmodiophorid workshop, International Congress of Plant Pathology, Christchurch, New Zealand, February 2003.

Poster presentations and demonstrations

Posters or demonstrations related to this project were presented at the following meetings:

- Potatoes in Practice, Dundee, August 2000

- Potatoes in Practice, Dundee, August 2001

- Potatoes in Practice, Dundee, August 2002

- British Potato 2001, September 2001

- 15th Triennial Conference of the European Association for Potato Research, Hamburg, Germany, July 2002

- 5th Symposium International Working Group of Plant Viruses with Fungal Vectors, Zürich, Switzerland, July 2002

- Powdery Scab Scoring Workshop, La Fretaz, Switzerland, August 2002

- Plasmodiophorid workshop, International Congress of Plant Pathology, Christchurch, New Zealand, February 2003

Other outputs

Potatoes in Practice 2000, 2001 & 2002 at SCRI, Dundee:
Field trials by SAC and SCRI. These events were widely reported in the press. Reports on the events were produced and made available by the British Potato Council to levy payers.

An EU-proposal for the funding of a European workshop on powdery scab was prepared, submitted and approved in 2000. As a result the first European powdery scab workshop took place at SAC Aberdeen on 20-22 July 2000 and the proceedings were published:

Merz, U. & Lees, A.K. (2000) *Proceedings First European Powdery Scab Workshop, SAC, Aberdeen, Scotland, July 20-22, 2000.* ISBN 09-0587-516-8.

The proceedings are also available on-line at:

http://www.pa.ipw.agrl.ethz.ch/spongospora/

Future R&D needs

Areas for future research are discussed in section 12 above. In summary they are:

- Improvement of the PCR diagnostic tool to
  - Reliably detect sporeballs in soil
  - Reduce the threshold of detection
  - Develop a useful commercial test for soil-borne contamination
- Development of an effective soil sampling procedure that accounts for the possible heterogeneity of distribution
- Production of an interpretation guide to accompany a soil test
- Survey GB soils for contamination with *S. subterranea*
- Determine the epidemiological significance of 'latent' infections
- Investigate the importance of root infection and root galls in the epidemiology of the pathogen, particularly in relation to varietal resistance
- Further investigate the relationship between soil type, inoculum level and environment
- Investigate the practical use of tuber disinfectant dips in conjunction with fluazinam soil treatment

- Other areas of research that have not been discussed above include
- The role of alternative hosts
  The literature suggests that a wide range of crop and weed species may act as alternative hosts to *S. subterranea*. Large numbers of zoosporangia are reportedly formed in the roots of common weed species such as fat hen, common chickweed, stinging nettle and shepherd's-purse. Some hosts may even form root galls and possibly sporeballs as in potato. Oilseed rape, spinach and sugar beet are also susceptible, but it is unknown whether their cultivation in rotation with potato affects the incidence of spraing and powdery scab.
- The relative importance of sources of inoculum
- The detection, biology and significance of Potato Mop Top Virus (PTMV)
  Detection of *S. subterranea* and Potato Mop Top Virus in host plants has thus far relied on visual observations by microscope and the use of indicator plants respectively. Real-time PCR (Taqman) would be a much more reliable method and could be used for the simultaneous detection and quantification of *S. subterranea* and PMTV in soil and host plant tissue samples. Many fundamental questions remain concerning the concurrence of *S. subterranea* and PMTV and must be answered before further progress in disease control can be made. Inconsistencies between the incidence of powdery scab and spraing in the same cultivars are frequently observed, but are not understood. Tuber, soil and host plant samples collected to test for the presence of *S. subterranea* could also be tested for the incidence of PMTV, and thus yield important information on the relationship between the two pathogens. It should be noted that despite the importance of powdery scab, research presents difficulties in terms of expertise and for that reason very little research has been previously, or is currently, undertaken. At SCRI and SAC we have the expertise required in both *S. subterranea* and PMTV research and have been at the forefront of such research internationally.

Based on some of the areas for future research described above a concept note was written and sent for consideration to the BPC. The description of the proposed research is reproduced below.

Description of the proposed research:

Part 1. Symptomless tuber infections and tuber contamination: Aim: To investigate the incidence and development of symptomless infections of potato tubers by *S. subterranea* and the incidence and importance of the contamination of seed tubers with *S. subterranea* sporeballs. Seed tubers of selected potato cultivars from a wide range of sources will be sampled and tested for symptomless infection and surface contamination using a real-time PCR assay. The conditions under which symptomless and immature infections are formed during growth, continue to develop during storage, and cause disease in the next crop will be studied in controlled environment trials. The effect of different levels of tuber inoculum on disease incidence and severity will be studied under controlled conditions and in the field. The different levels can be quantified using a real-time PCR assay.

Part 2. Root infection and alternative hosts: Scottish potato crops and common alternative hosts will be sampled and tested for root infection by *S. subterranea* (root galling). The susceptibility of different potato varieties and other Scottish host plants to root infection by *S. subterranea* will be determined and the role of root infection as a source of inoculum will be investigated under controlled conditions and in the field.

Part 3. Pathogen distribution in soil: An effective field sampling strategy for sporeballs of *S. subterranea* will be developed and used to determine the incidence of the pathogen in soil in Scotland. The lowest soil inoculum level needed for infection in different soil types will be determined under controlled conditions.

Plant and soil samples collected (parts 2 & 3) will also be tested for PMTV forming the basis of a Scottish survey for the disease. A real-time PCR assay can be used for the detection and quantification of both pathogens in soil and plant tissues.

Objectives:

- To determine the incidence of symptomless infection of seed tubers with *S. subterranea*, and PMTV, and to understand under which conditions symptomless infections by *S. subterranea* are formed, develop during storage and may cause powdery scab in the next crop.
- To determine the incidence of seed tuber contamination with *S. subterranea*, and to understand the effect of seed inoculum level on powdery scab incidence and severity.
- To determine the distribution of *S. subterranea* and PMTV in roots of potato and alternative hosts in Scotland, and to understand the role of root infection by *S. subterranea* in powdery scab epidemiology.
- To develop an effective field sample strategy for sporeballs of *S. subterranea* in soil.
- To determine the distribution of *S. subterranea* and PMTV in field soil in Scotland, and to understand the effect of inoculum level on powdery scab development in different soil types.
- To translate scientific findings into practical advice on prevention and control of powdery scab/ spraing.

Main milestones (description and timing):
We estimate that the project will last 3 years.

Year 1:

Part 1: Optimising real-time PCR assay for detection of *S. subterranea* and PMTV (months 1-6).
Sampling and testing of seed tubers for symptomless infections and contaminations (7-12).

Part 2: Trials on susceptibility of potato varieties and other host plants to root infection (1-24).

Part 3: Development of a sample strategy for sporeballs in field soil (1-24).

Year 2:

Part 1: Determine factors affecting the formation of latent tuber infections during growth (13-24), and to determine the effect of seed inoculum level on disease incidence in the next crop (13-36).

Part 2: Sampling of potato and other host plants in the field and testing for presence of *S. subterranea* and PMTV (13-18).

Part 3: Sampling of soil in Scottish fields and testing for presence of *S. subterranea* and PMTV (13-18).

Year 3:

Part 1: Trials to determine factors promoting the development of latent infections during storage (25-36), and to determine the role of latent infections in disease incidence in the next crop (25-36).

Part 2: Trials on the effect of root infection on disease incidence (25-36).

Part 3: Trials on the effect of soil type on minimum inoculum level needed for disease development (25-36).

Technology transfer.