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The potential for the rapid screening of potato cultivars (*Solanum tuberosum*) for resistance to powdery scab (*Spongospora subterranea*) using a laboratory bioassay

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Abstract

Powdery scab of potato, once established in a field, is difficult to control because of the longevity of the resting spores (cystosori) of the causal organism, *Spongospora subterranea* f.sp. *subterranea*. Host resistance is likely to be the most efficient in a long-term control strategy for preventing build-up of field inoculum and spread of the disease. Resistance screening of potato cultivars is mostly done in laborious field trials where disease development is likely to be unpredictable. A bioassay with potato tissue cultured plantlets and cystosori as inoculum is described and was tested for its potential to screen potato cultivars at an early stage for their relative susceptibility to powdery scab by comparing the lab results with field data. With cystosori inoculum of Swiss origin, the laboratory test showed clear differences between the potato cultivars in the severity of zoosporangial root infection which correlated better with ranked tuber infection data, compared to root galling. There are apparent differences in the relative trends in susceptibility between roots and tubers of five selected cultivars when using naturally infested soil instead of prepared cystosori as inoculum in the lab bioassay. Furthermore, differences in the severity of zoosporangial root infection of two selected cultivars were found when cystosori from different countries where used as inoculum. A possible host genotype × pathogen interaction is discussed. The bioassay has the potential to screen and select for resistant material at an early breeding stage thus making field trials not unnecessary but more economical. It will allow the use of a standard set of pathogen collections and facilitate testing for inoculum virulence in infested soils.

Introduction

Powdery scab of potato is caused by the biotrophic protozoan pathogen, *Spongospora subterranea* f.sp. *subterranea*, the only soil-borne plant pathogen which is also important as a vector of a virus, the potato mop-top furovirus. Powdery scab is difficult to control mainly because of the longevity of the resting spores (cystosori) in soil (de Boer, 2000). Once introduced into a field, the inoculum level build up through potato cropping without any apparent disease consequences, especially when cultivars with a high root susceptibility to galling combined with low tuber susceptibility to lesions (both structures contain cystosori) are cropped (de Boer, 2000). At a certain inoculum level and with the coincidence of favourable environmental conditions farmers can be confronted with sudden disease outbreaks when crops of susceptible cultivars are grown (Merz, 1993). This epidemiological situation, characteristic for the disease, is one reason for the cyclic pattern which powdery scab showed through the last century. The disease has increased in importance in recent decades in Europe and in other parts of the world (Walsh et al., 1996; Wale, 2000). For the current situation, factors such as intensification of potato production, increasing use of susceptible cultivars, more frequent irrigation and banning of mercury, which was previously used as an efficient

seed treatment, are all contributing to higher disease incidence.

The availability of resistant potato cultivars with commercially acceptable quality is essential for long-term control of powdery scab. The disease has not been considered in breeding programmes until 1996 in New Zealand (Falloon et al., 1999). Powdery scab resistance screening is commonly conducted in field trials with naturally infested soils, where tuber infection is scored. Field trials are costly, laborious and time consuming. Even more important, their success (establishment of infection) depends strongly on the environmental conditions, i.e. cool and wet weather during the first half of the cultivation period. Some attempts were made to encompass these difficulties by using artificially inoculated small-scale plots (Kirkham, 1986; Wastie et al., 1988) or cultivating the plants in pots either outside (Gans and Vaughan, 2000) or in a greenhouse (Jellis et al., 1987; de Boer, 1991). These methods still remain costly and are unsuitable for mass screening. A laboratory-based system does not have these disadvantages and, if the level of root infection is a useful resistance indicator, would allow a quick test of breeding lines at early stages of selection.

Root infection of potato by *S. subterranea* results in production of either zoosporangial or galls which contain cystosori. The process until gall maturity takes up to 4 months. However, mature zoosporangial can be found 3–4 days after infection. Merz (1989) introduced a versatile bioassay where scoring of zoosporangial root infection of bait plants was used as a measure for infectivity of cystosori inoculum or infested soil. There is little information available on the relationship between the susceptibility of roots (to zoosporangial) and tubers for a particular cultivar. Previous findings are rather contradictory. Fornier (1997), using a liquid culture with artificial infection, found a high level of zoosporangial root infection in a cultivar considered to be rather resistant to tuber infection. However, Mäkäräinen et al. (1994) observed higher zoosporangial infection in roots of a cultivar which is highly susceptible to potato mop-top virus, transmitted by *S. subterranea,* compared to the root infection level of a more resistant cultivar.

The aim of this study was to compare laboratory-based data on zoosporangial root infection with field data and to investigate the possibility of using a bioassay system for pre-screening potato for powdery scab resistance.

Materials and methods

Cultivation of tissue cultured plantlets

Stem cuttings of 11 potato cultivars (Agria, Bintje, Charlotte, Desirée, Ditta, Erntestolz, Gladiator, Granola, Nicola, Sirtema, Urgenta) were cultivated in tissue culture containers ($80 \times 10 \times 10\,mm^3$; reversed use; Phytatray II, Sigma, Switzerland) with 60 ml of MS medium (with vitamins; Duchefa, Haarlem, the Netherlands) containing $40\,mg\,l^{-1}$ ascorbic acid, $500\,mg\,l^{-1}$ casein hydrolysate (Fluka), $30\,g\,l^{-1}$ sucrose and $6\,g\,l^{-1}$ agar (Oxoid No 1). The tissue cultures were grown at 20 °C and 16 h photoperiod (cool white fluorescent lamps, $190\,\mu mol\,m^{-2}\,s^{-1}$) and were subcultered every 4 weeks (16 cuttings/container).

Bioassay

The bioassay was a modification of Merz (1989). The main differences were the use of potato plantlets rather than tomato seedlings as bait plants, Petri dishes instead of plastic containers and different lengths of inoculum incubation and infection period.

Preparation of plantlets

The roots of 4-week-old plantlets were washed free of agar medium, trimmed to a length of 60 mm and transferred to plastic containers ($26 \times 20 \times 6\,cm^3$). In each container, 16 plantlets were held in slit Neopren plugs inserted into holes in a polyvinyl (PVC) cover and supplied with 250 ml of nutrient solution (Merz, 1989; stock solution 1 : 24 diluted, hereafter designated NS) to induce extra root growth. Plantlets were kept in a growth chamber for 1 week with 16 h of light ($190\,\mu mol\,m^{-2}\,s^{-1}$, cool white fluorescent tubes) at 18 °C and a 8 h dark period at 15 °C and 75% RH. The roots were washed before use in the bioassay.

Preparation of inoculum

Cystosori inoculum was prepared from infected tubers of cv Bintje (obtained from a potato field which is at a distance of about 100 km from the trial site reported here) (Merz, 1989), or obtained from different countries (Japan, USA or New Zealand). The cystosori material ($20\,mg\,l^{-1}$ NS) was soaked

in 5 ml NS for 30 min, mixed for 2 min with an Ultra-Turrax (TP 18/10, Janke and Kunkel GmbH, Staufen, DE) at 6000 rev min^{-1}, and then additional NS was added to the suspension to make up a total volume of 360 ml.

Soil inoculum (infested soil from the trial site, dried at room temperature) was prepared by mixing 36 g of soil with 360 ml NS (MSE Homogenizer at 4000 rev min^{-1}) for 5 min. Then, 60 ml of suspension of either cystosori or infested soil were added to Petri dishes (diameter 75 mm, depth 30 mm, painted black outside) which were covered and incubated in a growth chamber in the dark (16 h at 18 °C, 8 h at 15 °C, 75% RH) for 9 days. There were six replicate Petri dishes for each treatment. The experiment with Bintje cystosori inoculum was repeated three times.

Plant infection

Two potato bait plantlets were used per Petri dish (held by two opposite slits made into the Petri dish lid) and grown for 1 day. The roots were washed in tap water and the bait plants cultivated in Petri dishes with 60 ml fresh NS for another 7 days (16 h of light, 190 $\mu mol\ m^{-2}\ s^{-1}$, cool white fluorescent tubes) at 18 °C and an 8 h dark period at 15 °C, 75% RH) to allow multiplication of zoospores.

Disease assessment

Whole roots were washed, stained and root infection evaluated (Merz, 1989). A modified scale was used for rating of root infection: 0 = no sporangia, 1 = only a few sporangia (1–50), 2 = several roots with little infection, 3 = several roots with moderate infection, 4 = sporangia regularly present, moderate infection, 5 = sporangia regularly present, heavy infection.

Field trial data

The field data originated from a resistance screening trial conducted regularly at a federal research station in a field with high *S. subterranea* infestation, located at an altitude of 1200 m. Each year, a set of Swiss and foreign cultivars is planted there to evaluate their relative susceptibility to powdery scab (Schwärzel, 2002). In 1999, the set included the same 11 varieties used in the bioassay. These varieties were selected based on their different relative susceptibilities ranging from low to high. Tubers were planted at the beginning of May in two-row plots of 12.5 m^2 size, with two repetitions per cultivar and 50 tubers per repetition. Thirty days before harvest, a total of 20 plants per cultivar (2 × 10) were examined for the presence or absence of root galls. Root galling scores were based on the following scale: 0 = no galls, 2 = 1 gall, 4 = 3 galls, 6 = 10 galls, 8 ≥ 10 galls. Tubers were visually assessed for disease severity one month after harvest. Scoring of the percentage of the surface of each tuber covered with powdery scab lesions was based on calibrated photographs of diseased potatoes (0 = no disease, 2 = 2%, 4 = 12%, 6 = 50%, 8 = 90%). A total of 400 tubers were scored per cultivar (2 × 200).

Statistical analysis

For the analysis of the data of zoosporangial root infection, tuber infection and root galling, multiple t-test statistics were used (Tukey's HSD test; $P \leq 0.05$; with log-transformed data). The ranked tuber and zoosporangial root infection data were compared using Spearman's ranking correlation coefficient (r_s).

Results

Bioassay with Swiss cystosori inoculum and 11 cultivars

Zoosporangial of *S. subterranea* were found in roots of all cultivars. None was immune to the pathogen. A significant difference in average severity of root infection was obtained between the most susceptible cultivars (Erntestolz, Sirtema, Urgenta, Charlotte, Desiree and Agria) and the least susceptible cultivars (Nicola and Ditta (Figure 1)). The cultivars Bintje, with intermediate susceptibility, and Desirée had the highest variation of root infection level.

Comparison of bioassay data with field data

Ranking of tuber infection and severity of zoosporangial root infection resulted in similar ranking positions for Erntestolz, Charlotte, Granola and Nicola, followed by Urgenta, Agria and Ditta in the two assays (Table 1; $r_s = 0.52$). The remaining cultivars showed different rankings in each category with Desirée and Bintje showing the greatest differences. All cultivars had

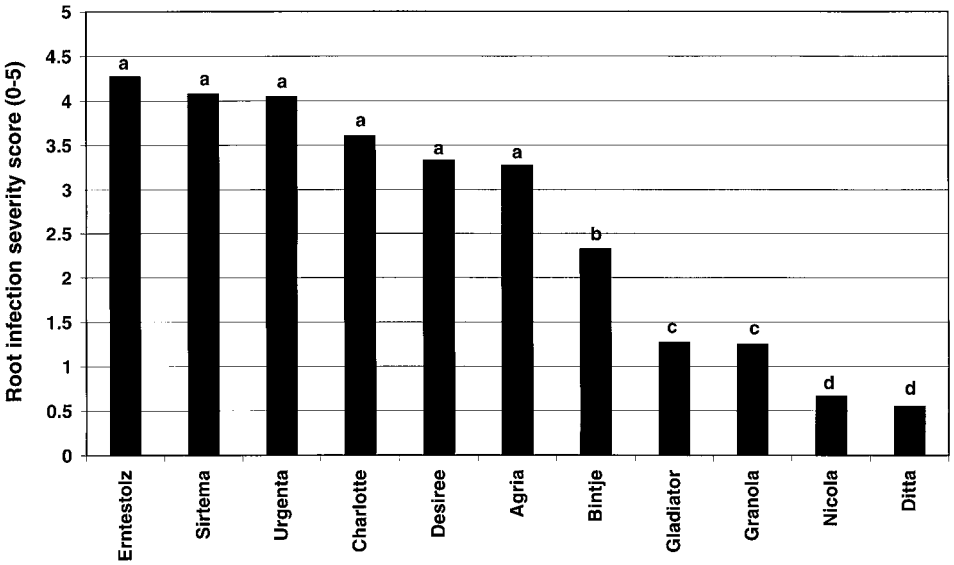

Figure 1. Mean *S. subterranea* zoosporangial root infection scores for tissue cultured plantlets of 11 potato cultivars in a bioassay using Swiss cystosori inoculum. Values are means of three experiments with six replicates each and two plants per replicate; means accompanied by the same letters are not significantly different at $P \leq 0.05$ (Turkey's HSD test).

Table 1. Ranking of 10 potato cultivars according to their tuber infection score, root galling score (both data sets from a field trial in 1999) and zoosporangial root infection score of tissue cultured plantlets, artificially inoculated with cystosori of *S. subterranea* in a bioassay

Cultivar	Tuber infection score[1]		Root galling score[2]		Zoosporangial root infection score[3]	
Erntestolz	1	5.2^{a}	7	1.5^{cde}	1	4.3^{a}
Bintje	2	4.3^{b}	3	3.1^{bc}	7	2.3^{b}
Agria	3	4.1^{b}	1	7.6^{a}	6	3.3^{a}
Charlotte	4	1.7^{c}	9	0.4^{ef}	4	3.6^{a}
Urgenta	5	1.6^{c}	2	4.0^{ab}	3	4.1^{a}
Sirtema	6	1.5^{c}	6	1.6^{cde}	2	4.1^{a}
Ditta	7	1.1^{d}	10	0^{f}	10	0.5^{d}
Granola	8	0.6^{e}	4	1.8^{cd}	8	1.3^{c}
Nicola	9	0.6^{e}	5	1.8^{cde}	9	0.7^{d}
Desirée	10	0.5^{e}	8	0.6^{def}	5	3.3^{a}

[1]Tuber infection was rated at harvest on a visual scale from 0 (no infection) to 8 (infected surface >90%). Values are means of samples of 400 tubers (200 tubers per replicate).
[2]Root galling score based on the following scale: 0 = no galls, 2 = 1 gall, 4 = 3 galls, 6 = 10 galls, 8 ≥ 10 galls; values are means of 20 plants.
[3]Whole stained roots were scored under a stereo microscope on a scale from 0 (no zoosporangial) to 5 (heavy zoosporangial infection). Values are means of three repetitions with 6 replicates each and 2 plants per replicate.
Means accompanied by the same letters are not significantly different at $P \leq 0.05$ (Turkey's HSD test).

infected tubers. When comparing ranked root galling score and zoosporangial root infection, no obvious relationship was obtained with the exception of Ditta which was the least susceptible cultivar in both categories (Table 1; $r_s = 0.09$).

Comparison of bioassay data with field data when field soil was used as inoculum

A better correlation was obtained when severity of tuber infection was compared with severity of

zoosporangial root infection of five selected cultivars, which were inoculated with trial site field soil, with special emphasis on Desirée (Table 2; $r_s = 0.90$).

Table 2. Ranking of five potato cultivars according to their tuber infection severity score (data set from a field trial in 1999) and zoosporangial root infection severity score of tissue culture plantlets, inoculated with soil infested with *S. subterranea* (from the field trial site) in a bioassay

Cultivar	Tuber infection score[1]		Zoosporangial root infection score[2]	
Erntestolz	1	5.2[a]	1	2.66[a]
Bintje	2	4.3[a]	3	1.83[a]
Charlotte	3	1.7[a]	2	2.58[a]
Ditta	4	1.1[d]	4	0.58[b]
Desirée	5	0.5[e]	5	0.08[b]

[1]Tuber infection was rated at harvest on a visual scale from 0 (no infection) to 8 (heavy infected). Values are means of samples of 400 tubers.
[2]Whole stained roots were scored under a stereo microscope on a scale from 0 (no zoosporangial) to 5 (heavy zoosporangial infection). Values are means of six replicates with two plants per replicate.
Means accompanied by the same letters are not significantly different at $P \leq 0.05$ (Turkey's HSD test).

Bioassay data of two selected cultivars with cystosori inoculum from different countries

When the two cultivars Erntestolz and Ditta where inoculated with cystosori originating from different countries, the biggest difference in severity of zoosporangial root infection between the two cultivars was obtained with New Zealand inoculum followed by Swiss inoculum. In contrast, no difference in susceptibility was found with inoculum from Japan or the USA (Figure 2).

Discussion

The bioassay described here allows results to be obtained after only 17 days. Significant differences in zoosporangial root infection between some of the cultivars were obtained; e.g. Erntestolz was most susceptible and Ditta and Nicola the least susceptible cultivars. Gladiator, known to be resistant to tuber infection in New Zealand, had a similar low level of zoosporangial root infection to Ditta and Nicola in the bioassay.

Comparison with the field data showed a more mixed situation. Surprisingly there was no correlation

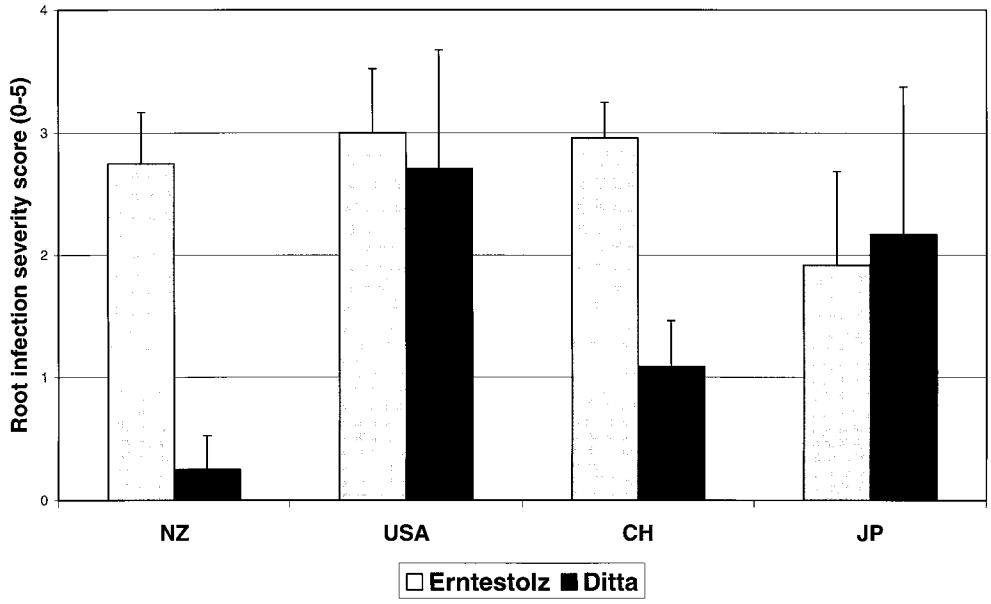

Figure 2. Mean *S. subterranea* zoosporangial root infection scores for tissue cultured plantlets of two potato cultivars baited with cystosori inoculum originating from different countries (NZ = New Zealand, CH = Switzerland, JP = Japan) in a bioassay. Values are means of six replicates with two plants per replicate; bars represent standard deviations.

between the root galling score and the level of zoosporangial root infection, with the exception of Ditta which produced no galls in the field. It is likely that the development of zoosporangial and root galls may be subject to different resistance mechanisms. Hughes (1980) tried to find a relationship between root galling and tuber infection level but the number of aberrant results was too high to consider using root ratings for assessment of tuber resistance. A better picture was obtained when comparing zoosporangial root infection with the ranked tuber infection data. Erntestolz ranked as most susceptible and Granola, Ditta and Nicola ranked as most resistant in both systems. The remaining cultivars differed in their positions. The greatest differences were shown by Desirée, followed by Bintje and Sirtema. On the other hand, Desirée ranked similar in both the zoosporangial root infection severity and the tuber infection severity when the original field soil was used as inoculum in the bioassay.

Desirée is known to be moderately susceptible in Swiss soils, whereas it has ranked as more susceptible or more resistant in screening trials in other countries (Lees, 2000; Bus, 2000). The same situation applies for Bintje. A possible explanation for these findings may be the existence of pathogen field population interactions with different host genotypes. This hypothesis is supported by the clear differences in zoosporangial root infection reaction of Erntestolz and Ditta to cystosori inoculum from several world locations, especially to material from USA and New Zealand. Little is known about the presence of race-specific host–pathogen reaction for potato and *S. subterranea*. Bulman and Marshall (1998) found two different types of ITS-sequences in DNA material of cystosori of Australasian/European, Scottish and Peruvian collections of the pathogen. Genetic analysis of pathogen populations and pathogen virulence is urgently needed for the development of a successful breeding strategy. In addition, a standard protocol for assessing resistance that includes known reference cultivars and an illustrated scoring scale (Merz, 2000) would be beneficial to the production of more reliable data thus allowing better comparison of relative resistance.

The data presented here show that there is a relationship between susceptibility to tuber infection and to zoosporangial root infection especially with more resistant cultivars. The bioassay has the potential to screen and select for resistant material at an early breeding stage making field trials not unnecessary but more economical. A set of reference cultivars together with reliable relative tuber susceptibility data would allow standardizing the test. If race specificity is important, a relevant set of pathogen isolates also has to be included in the test. Monoclonal zoosporangial antiserum or quantitative PCR, using existing primers (Bulman and Marshall, 1998; Bell et al., 1999), will allow automated assessment of root infection making the system more efficient. Furthermore the bioassay has the potential to be used for inoculum virulence analysis of infested soils with the help of appropriate potato plant differentials.

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References

Bell KS, Roberts J, Verrall S, Cullen DW, Williams NA, Harrison JG, Toth LK, Cooke DEL, Duncan JM and Claxton JR (1999) Detection and quantification of *Spongospora subterranea* f.sp. *subterranea* in soils and on tubers using specifique PCR primers. European Journal of Plant Pathology 105: 905–915

Bulman SR and Marshall JW (1998) Detection of *Spongospora subterranea* in potato tuber lesions using the polymerase chain reaction (PCR). Plant Pathology 47: 759–766

Bus CB (2000) Powdery scab control in the Netherlands. In: Merz U and Lees AK (eds) Proceedings of the First European Powdery Scab Workshop (pp 79–83) Aberdeen, Scotland, July 20–22 (http://www.pa.ipw.agrl.ethz.ch/spongospora/EUworkshop/proceedings.html)

de Boer RF (1991) Evaluation of potato cultivars in the greenhouse and field for resistance to powdery scab. Australian Journal of Experimental Agriculture 31: 699–703

de Boer RF (2000) Research into the biology and control of powdery scab of potatoes in Australia. In: Merz U and Lees AK (eds) Proceedings of the First European Powdery Scab Workshop (pp 79–83) Aberdeen, Scotland, July 20–22

Falloon R, Genet R, Wallace A and Nott H (1999) IPM use for powdery scab. Commercial Grower 54(3): 33–34

Fornier N (1997) Epidemiology of *Spongospora subterranea*, the cause of powdery scab of potatoes. University of Aberdeen, Scotland, PhD thesis

Gans PT and Vaughan JE (2000) Cultivar susceptibility to powdery scab of potatoes, caused by *Spongospora subterranea*.

In: Merz U and Lees AK (eds) Proceedings of the First European Powdery Scab Workshop (pp 39–41) 2000, Aberdeen, Scotland, July 20–22

Hughes IK (1980) Powdery scab (*Spongospora subterranea*) of potatoes in Queensland: Occurrence, cultivar susceptibility, time of infection, effect of soil pH, chemical control and temperature relations. Australian Journal of Experimental Agriculture and Animal Husbandry 20: 625–632

Jellis GJ, Phul PS and Starling NC (1987) Evaluation of potato germplasm for resistance to powdery scab (*Spongospora subterranea*). Annals of Applied Biology 110(Suppl 8): 154–155

Kirkham RP (1986) Screening for resistance to powdery scab disease of potatoes, Australian Journal of Experimental Agriculture 26: 245–247

Lees AK (2000) Resistance to powdery scab in potato. In: Merz U and Lees AK (eds) Proceedings of the First European Powdery Scab Workshop (pp 35–38) 2000, Aberdeen, Scotland, July 20–22

Mäkäräinen E, Rita H, Teperi E and Valkonen JPT (1994) Resistance to *Spongospora subterranea* in tuber-bearing and non tuber-bearing *Solanum* spp. Potato Research 37: 123–127

Merz U (1989) Infectivity, inoculum density and germination of *Spongospora subterranea* resting spores: A solution-culture test system. Bulletin OEPP 19: 585–592

Merz U (1993) Epidemiological aspects of powdery scab of potatoes caused by *Spongospora subterranea*. In: Hiruki Ch (ed) Proceedings of the 2nd Symposium of the International Working Group on Plant Viruses with Fungal Vectors (pp 104–106) 1993, Montreal, Canada, July 25–27

Merz U (2000) Powdery scab. Research in Switzerland. In: Merz U and Lees AK (eds) Proceedings of the First European Powdery Scab Workshop (pp 67–71) 2000, Aberdeen, Scotland, July 20–22

Schwärzel R (2002) Sensibilité des racines et tubercules des variétés de pommes de terre à la gale poudreuse et quelques résultats de lutte chimique. Revue Suisse d'Agriculture 34: 261–266

Wale SJ (2000) Summary of the session on national potato production and the powdery scab situation. In: Merz U and Lees AK (eds) Proceedings of the First European Powdery Scab Workshop (pp 3–9) 2000, Aberdeen, Scotland, July 20–22

Walsh JA, Merz U and Harrison JG (1996) Serological detection of spore balls of *Spongospora subterranea* and quantification in soil. Plant Pathology 45(5): 884–895

Wastie RL, Caligari PDS and Wale SJ (1988) Assessing the resistance of potatoes to powdery scab (*Spongospora subterranea* (Wallr.) Lagerh.). Potato Research 31: 167–171