J Plant Growth Regul (2009) 28:282–288
DOI 10.1007/s00344-009-9092-9

Detection and Measurement of *Plasmodiophora brassicae*

Robert Faggian · Stephen E. Strelkov

Received: 5 March 2009 / Accepted: 10 March 2009 / Published online: 1 May 2009

Abstract Clubroot, caused by *Plasmodiophora brassicae*, is one of the most important diseases of brassicas. Management of clubroot is difficult, and the best means of avoiding the disease include planting in areas where *P. brassicae* is not present and using plants and growing media free from pathogen inoculum. As *P. brassicae* is not culturable, its detection has traditionally relied on plant bioassays, which are time-consuming and require large amounts of glasshouse space. More recently, fluorescence microscopy, serology, and DNA-based methods have all been used to test soil, water, or plant samples for clubroot. The use of fluorescence microscopy to detect and count pathogen spores in the soil requires significant operator skill and is unlikely to serve as the basis for a routine diagnostic test. By contrast, serologic assays are inexpensive and amenable to high-throughput screening but need to be based on monoclonal antibodies because polyclonal antisera cannot be reproduced and are therefore of limited quantity. Several polymerase chain reaction (PCR)-based assays have also been developed; these are highly specific for *P. brassicae* and have been well-correlated with disease severity. As such, PCR-based diagnostic tests have been adopted to varying extents in Canada and Australia, but wide implementation has been restricted by sample processing costs. Efforts are underway to develop inexpensive serologic on-farm diagnostic kits and to improve quantification of pathogen inoculum levels through real-time PCR. Proper detection and quantification of *P. brassicae* will likely play an increasingly important role in the development of effective clubroot management strategies.

Keywords Clubroot · *Plasmodiophora brassicae* · Crucifers · Detection · Quantification · Plant disease

R. Faggian (✉)
Department of Primary Industries, P. O. Box 4166, Parkville, VIC 3052, Australia
e-mail: Robert.Faggian@dpi.vic.gov.au

S. E. Strelkov
Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada

Introduction

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is one of the most important diseases of brassicas, causing annual losses of 10–15% worldwide (Dixon 2006) (see Dixon, this issue). Infection by *P. brassicae* leads to the formation of galls on the roots of susceptible plants, resulting in stunted growth and increased susceptibility to wilting (Karling 1968; Voorips 1995). The pathogen forms resting spores that are released from the decaying galls and have a half-life of 3.6 years (Wallenhammar 1996). These long-lived spores can build up in the soil, especially when brassicas are repeatedly grown, leading to increased disease severity in subsequent crops. A number of control measures such as application of fungicides (Shimotori and others 1996; Donald and others 2001), liming of the soil (Murakami and others 2002), and use of resistant host genotypes are recommended for clubroot, but may not always be practical or effective.

Given the difficulty in managing clubroot, the best means of avoiding the disease is to plant in areas where the pathogen is not present and use plants and growing media that are free from contaminating pathogen inoculum. Therefore, a sensitive and reliable technique for the detection of clubroot inoculum is, or has the potential to be, an essential component of clubroot control strategies. As *P. brassicae* is nonculturable, its detection in soil has

traditionally relied on plant bioassays, either by visual assessment of root symptoms or by microscopic examination of root-hair infection (Toxopeus and Janssen 1975). More recently, fluorescence microscopy, serology, and DNA-based methods have all been used to test soil, water, or plant samples for clubroot. In this review we evaluate the current and historical approaches that have been used to detect and quantify *P. brassicae*, and analyze the situation in Australia and Canada where clubroot is increasing in significance as a disease of brassicas.

Bait Plants

The use of bait plants is the most reliable diagnostic method for assessing soils for the presence of *P. brassicae* and is still heavily relied upon today despite the availability of more modern techniques.

Following problematic early attempts to examine soil microscopically for the presence of *P. brassicae* resting spores (Fedotova 1933), bait plant methods were developed. Samuel and Garrett (1945) developed the infected root-hair count in which cabbage seedlings were grown for 1 week in the suspect soil sample. A 2-cm length of root was stained with acetocarmine and root hairs were examined microscopically for the presence of zoosporangia. MacFarlane (1952) used this method to assess resting-spore survival in soil. However, questions relating to the relationship between root-hair infection and subsequent gall development led to further refinements in the method. Therefore, Colhoun (1957) grew plants under controlled conditions for 5 weeks in suspect soil before examining the roots visually for clubroot symptoms. Melville and Hawken (1967) adapted the method further after encountering problems, including the poor growth of test plants, the prevalence of other pathogens (such as *Rhizoctonia solani* Kuhn), and difficulty maintaining the maximum water-holding capacity of the test soil. As a result, a variety of bait plant techniques (or bioassays) are currently in use to provide a reliable means of establishing the presence of viable *P. brassicae* inoculum in soils (for example, Wallenhammar 1996), or to provide the only means of validating newly developed diagnostic procedures (for example, Cao and others 2007).

These methods are useful, however, only when inoculum reaches levels of greater than 1000 spores per gram dry soil, which is the generally accepted threshold for symptom development (depending on soil type and environmental conditions). Bait plant techniques are also time-consuming (typically taking 5 weeks or longer), labor-intensive, or both. Five weeks is also an unacceptably long time to wait for a test result, whereas examining root hairs microscopically is a laborious task requiring trained personnel. Such methods also require large amounts of glasshouse space (Melville and Hawken 1967) and therefore, for practical and economic reasons, they cannot form the basis of a routine diagnostic test for large numbers of samples (Wakeham and White 1996).

Fluorescent Microscopy

Microscopic examination of soil was revisited by Takahashi and Yamaguchi (1987), who developed a fluorescence assay for *P. brassicae* detection. Soil suspensions were stained with a single fluorochrome and fluorescing resting spores were counted under a fluorescent microscope. The method was modified after fluorescing soil particles prevented unambiguous counts (Takahashi and Yamaguchi 1988). The updated assay involved the use of two fluorochromes and an altered soil-preparation protocol that improved spore detection and provided a means of determining spore viability. One fluorochrome, Calcofluor White M2R, bound to chitin (a major component of the resting spore wall) in undamaged cells, whereas the second fluorochrome, ethidium bromide, penetrated damaged and nonviable cells. This enabled the differential staining of viable (blue fluorescence) and nonviable (red fluorescence) resting spores. A correlation was later found between blue fluorescing spores and disease severity (Takahashi and Yamaguchi 1989; Takahashi 1991). However, Donald and others (2002) later determined that the correlation between spore viability and disease severity, as predicted by the fluorescent-staining method, was less clear.

Again, although potentially useful for observing and counting spores in soil, it is unlikely that microscopic examination of soil would form the basis of a routine diagnostic assay. Fluorescing soil particles and other artifacts can make it difficult to identify *P. brassicae* resting spores, so sample throughput is restricted by operator skill.

Serology

Arie and others (1988) made the first attempt at a serology-based detection method for *P. brassicae*, developing an immunofluorescence assay for use with soil samples. However, the cross-reactivity of the antiserum with other organisms was not tested and detection limits were not discussed.

Lange and others (1989) prepared a polyclonal antiserum for use in a dot immunobinding protocol to detect *P. brassicae*. The antiserum did not cross-react with *Polymyxa graminis* Ledingham, another plasmodiophorid, or the common root pathogens *R. solani*, *Pythium ultimum* Trow, and *Fusarium oxysporum* Schlectend:Fr. The technique

allowed detection of *P. brassicae* in infected plant tissue up to a dilution of 1 in 2048, prompting the authors to state that the "sensitivity obtained was within the range permissible for a routine test." It was suggested that a fluorescent antibody technique be developed to detect *P. brassicae* in soil samples.

Wakeham and White (1996) prepared several polyclonal antisera against *P. brassicae*, and used them in the development of soil diagnostic tests in the form of Western blots, dip-sticks, dot-blots, immunoblotting assays, indirect enzyme-linked immunosorbent assays (ELISA), and indirect immunofluorescence assays. A range of soil fungi, including the closely related plasmodiophorid *Spongospora subterranean* Tomlinson, generally showed low cross-reactivity with the antisera. A detection limit of 100 spores per gram of soil was achieved with one of the polyclonals, PAb 15/2, when used in the dip-stick method, indirect ELISA, and immunofluorescence.

Orihara and Yamamoto (1998) developed another polyclonal-based assay, but the detection limit in artificially inoculated soil was 10,000 spores per gram, whereas in field soil some nonspecific reactions were observed. It was suggested that improvements in the soil preparation method might help to increase the detection sensitivity of the assay.

In general, the specificity of antisera raised against fungal plant pathogens can vary greatly (Savage and Sall 1981; Hardham and others 1986), and as polyclonal antisera cannot be reproduced and are therefore limited in quantity, their usefulness as diagnostic tools is limited. However, serologic assays based on monoclonal antibodies are likely to be a significant advance, improving specificity and providing an infinite source of antibodies. Serologic assays are also inexpensive and amenable to high-throughput sample processing. A number of monoclonal antibodies have recently been developed for the detection of *P. brassicae* (A. Wakeham, personal communication), and preliminary experiments, in a range of formats that include a lateral-flow device, indicate that the monoclonal antibodies are highly specific and can detect *P. brassicae* in naturally infested soil.

Polymerase Chain Reaction

Polymerase chain reaction (PCR)-based assays are now routine components of plant pathogen diagnostic procedures, and because they require only DNA, they are ideally suited to the detection of obligate pathogens such as *P. brassicae*. Also, PCR is applicable to soil and small tissue samples, making it an attractive alternative to more limited and time-consuming methods such as bioassays. Several PCR-based diagnostic assays have been developed for the detection of *P. brassicae*.

Buhariwalla and others (1995) developed PCR primers for the specific amplification of polymorphic DNA from *P. brassicae.* This provided a rapid means of studying the genetic diversity of the pathogen. The primers, however, also facilitated the amplification of host brassica DNA, making them of little use for diagnostic purposes.

Ito and others (1997) developed a seminested PCR for specific amplification of an *iso*pentyltransferase-like gene from purified *P. brassicae* DNA. These primers were later used to develop a detection protocol for *P. brassicae* in soil (Ito and others 1999). The PCR protocol could detect as little as one resting spore per gram of artificially infested soil. However, detection was difficult in naturally infested soil, necessitating a "double" PCR that involved another round of PCR with the nested set of primers. PCR results were not correlated with disease severity.

A number of PCR assays have been developed that were directed at the ribosomal genes (rDNA) or internal transcribed spacer (ITS) regions of *P. brassicae.* Chee and others (1998) developed a PCR detection protocol directed at the rDNA of *P. brassicae* and were able to detect the organism in infected roots of Chinese cabbage and radish. Detection in soil was not attempted.

Faggian and others (1999) also developed a nested PCR detection protocol directed at the rDNA and ITS regions. The assay was used with artificially inoculated soil and potting mix, naturally infested soil, and with plant tissue, enabling detection down to 10 spores per gram potting mix or fentogram (fg) levels of *P. brassicae* DNA, although PCR results were not correlated with disease severity.

Wallenhammar and Arwidsson (2001) developed an rDNA-directed nested PCR protocol that enabled detection of *P. brassicae* in a range of naturally infested soils. Positive test results were obtained with soils containing inoculum that corresponded with a disease severity index higher than 21% (35% of bioassay plants showing symptoms). In some of the soils tested, however, plant bioassays returned positive results below the PCR detection limit (down to inoculum levels corresponding to a disease severity index of 8%), and also on one occasion what appeared to be a false negative for the PCR assay (inoculum level corresponding to a disease severity index of 88%).

Finally, Cao and others (2007) developed an rDNA-directed, one-step PCR protocol that enabled consistent detection of *P. brassicae* in naturally infested soil down to inoculum levels corresponding to a disease index of 11% (14% of plants infected), or fg quantities of DNA. The assay also allowed the detection of *P. brassicae* DNA on/in symptomless roots 3 days after inoculation. The PCR assay could still reliably detect *P. brassicae* in naturally infested

Table 1 Summary of PCR-based methods for the detection of *P. brassicae* DNA

Author	Target	Sensitivity	Validated in soil?	Correlated with disease?
Chee and others (1998)	rDNA/ITS region	–	X	X
Ito and others (1999)	*Iso*pentyltransferase-like gene	1 resting spore per gram of soil	✔	X
Faggian and others (1999)	rDNA/ITS region	0.1 fg *P. brassicae* DNA	✔	X
Wallenhammar and Arwidsson (2001)	rDNA/ITS region	260 pg DNA from infected roots	✔	✔
Cao and others (2007)	rDNA/ITS region	100 fg *P. brassicae* DNA	✔	✔

soil that had been diluted up to five times with "clean" soil of the same type. However, as with Wallenhammar and Arwidsson (2001), some variation between soils was observed. Both groups of authors suggested that this resulted from the patchy distribution of the pathogen in soil and from sampling inadequacies. Table 1 summarizes all the PCR-based methods for the detection of *P. Brassicae* DNA.

None of the PCR-based assays mentioned above reported any nonspecific amplification, and progressive improvements in sensitivity and improved correlation with disease severity indicate that PCR may be a useful foundation to a routine diagnostic assay. Problems with sampling, sample size, and consistency between soil types require further research.

Situation Analysis: Australia

Clubroot is a problem in all states of Australia and in most of the major vegetable brassica-growing regions. The spread of the disease, within and between regions, has been facilitated by the reliance of the Australian vegetable brassica industry on cell-grown transplants. The transplants are generally grown in reusable plastic trays that are prone to contamination by soil that has previously been shown to harbor *P. brassicae* (Faggian and others 1999). This problem is compounded by ineffective commercial disinfectants (Donald and others 2002) and in some cases by poor farm hygiene. Other components of the brassica production cycle, such as seed and potting mix, have also been implicated in the spread of clubroot, but their involvement has never been confirmed.

However, some farms, as well as some new and emerging brassica-growing regions (including the Australian canola industry), still remain free of clubroot. Therefore, researchers in Australia have worked toward the development of integrated programs to control the disease and to prevent its spread (Donald and others 2006). These programs are used in conjunction with DNA-based detection (Faggian and others 1999) and quantification methods (Faggian and Parsons 2002). The ability to detect *P. brassicae* in soil, water, and plant tissue has enabled farm hygiene protocols to be improved (following the detection of *P. brassicae* in dust and irrigation water) and seed sources to be screened. The ability to quantify *P. brassicae*, through real-time PCR, is now being used to assess the efficacy of clubroot control measures.

Adoption of the diagnostic assays by the Australian brassica industry has varied over time. The predominant current use is for (1) resolution of disputes between growers, or between a grower and seed or seedling supplier, where one party seeks evidence of contamination, and (2) within research programs to determine inoculum loads in soil. Routine use of the diagnostic assays by industry has been limited by the high cost of DNA-based methods. At the time of its introduction as a commercial test in 2001 (at the Victoria Department of Primary Industries), the clubroot DNA-based diagnostic assay cost approximately $110 (AUD). Since then, a shift in government policy toward full cost recovery within diagnostic facilities now means that a DNA-based test can cost up to $450 per sample (depending on the complexity of the DNA extraction procedure). As a result, Australian clubroot researchers are working with colleagues in the UK, where new inexpensive serologic on-farm diagnostic kits are being developed.

Situation Analysis: Canada

Clubroot has long been an important pathogen of vegetable brassicas in Canada, particularly in British Columbia, Ontario, Quebec, and the Maritime provinces (Rimmer and others 2003). However, it was not until recently that clubroot was identified on *Brassica napus* L. canola (oil seed rape) on the Canadian prairies (Tewari and others 2005), despite extensive production of the crop in this region for many decades. Initially, a dozen clubroot-infested fields were found clustered near the city of Edmonton, Alberta, but additional surveying has revealed its occurrence in hundreds of fields throughout the central part of the province, as well as isolated cases in southern Alberta (Strelkov and others 2008). Yield losses in severely

infested canola fields have ranged from 30 to 100%, with the most severe infestations observed in fields under very short rotations. The primary mechanism of spread between fields appears to be the movement of infested soil on farm machinery (Strelkov and others 2007).

The identification of clubroot on canola in Alberta has been a cause for concern among farmers and the agricultural industry in general, especially because there is no genetic resistance in Canadian canola cultivars (Strelkov and others 2006) and no strategies have been developed, aside from long rotations, for control of clubroot in fields in which it is already present. In an effort to address industry concerns and prevent or slow down further clubroot spread, the province of Alberta recently made *P. brassicae* a "declared pest" under its Agricultural Pests Act. Under this Act, municipalities are responsible for local enforcement of clubroot control measures, which at present consist mainly of sanitation of equipment and a minimum 5-year rotation without canola (and other crucifers) on infested fields (Alberta Clubroot Management Committee 2008). Given the significant economic implications for a farmer when a field is declared to be clubroot-infested, municipalities generally obtain independent verification of their diagnosis in the form of a positive PCR result. As such, two private diagnostic laboratories now offer a commercial version of the PCR test developed by Cao and others (2007). The test, which costs about $90 (CAD) per sample, is also subscribed to by the oil and gas industry, which is active in Alberta and contributes to the movement of soil and equipment, as well as by some growers concerned about the presence of clubroot on their fields. Although detection of *P. brassicae* in suspect soil or plant tissue using a PCR-based method provides strong confirmation of the presence of the disease, concerns have arisen regarding the inadequate sampling of fields, which could lead to false negatives depending on where the sample(s) were taken. Currently, under the Pest Management Act clubroot-infested fields are treated the same, regardless of the degree of infestation.

Challenges and the Future for Detection of *P. brassicae*

One of the main challenges associated with testing a particular field for the presence of *P. brassicae* is the patchy distribution in which it often occurs (Cao and others 2007). Ideally, fields should be sampled in a standardized pattern, such as a "W" transect, with samples collected at multiple points along the arms of each transect. However, processing of multiple samples from one field increases glasshouse space requirements in bioassays and can be prohibitively expensive when samples are submitted for PCR testing in a molecular laboratory. Cao and others (2007) suggested pooling multiple samples from the same field as one way to deal with this issue, but stressed that proper validation would be required before such an approach could be applied as part of a routine diagnostic service. Similarly, strategic soil sampling based on the likely spatial distribution of *P. brassicae* (Faggian and others 2001) might reduce the number of samples required to confirm presence or absence of the pathogen. Indeed, among the recommendations made by the private laboratories offering clubroot testing in Alberta is that surveyors collect samples at the field entrances, because this is where the highest incidence of clubroot has been found (Strelkov and others 2007); similarly, sampling at low-lying points (representing higher moisture areas) and homestead (domestic) garden sites (where vegetable brassicas may have been grown) within each canola field is also recommended. The availability of global positioning systems may facilitate strategic sampling and the production of clubroot risk maps for individual fields and paddocks.

Another challenge associated with PCR-based detection of soilborne pathogens, including *P. brassicae*, is the extraction of high-quality genomic DNA. Several published protocols (for example, Steffan and others 1988; Volossiuk and others 1995; Yeats and others 1998) and commercial kits for extraction of DNA from soil are available. However, the effectiveness of any particular technique may vary with the type of soil tested and/or the specific detection protocol used. For instance, Wallenhammar and Arwidsson (2001) obtained satisfactory results using a published DNA extraction procedure (Volossiuk and others 1995), whereas Cao and others (2007) and Faggian and others (2001) preferred a commercial kit for the extraction of good-quality DNA. The presence of PCR inhibitors such as humic acids and other organic compounds in the soil extracts (Yeats and others 1998) could also confound results, necessitating inclusion of appropriate controls prior to analysis.

The DNA-based assays for *P. brassicae* currently in use for routine diagnostic services, although correlated with levels of inoculum and disease severity, are based on conventional PCR and are therefore not truly quantitative. Work is ongoing in several laboratories on the use of real-time PCR with TaqMan® probes as a more accurate tool for quantification of inoculum levels in plant and soil samples (Faggian and others 2001; D. Rennie and S.E. Strelkov, unpublished data). As noted above, the ability to quantify *P. brassicae* through real-time PCR is already being used to evaluate the efficacy of clubroot control measures in Australia, and has the potential to serve as an important research tool for quantifying soil inoculum loads. However, the widespread adoption of real-time PCR for clubroot testing may be hampered by its high cost relative to other methods. The development of serologic diagnostic

kits could represent an inexpensive alternative to quantitative PCR and, if successful, could be readily used by growers in many clubroot-affected countries.

Given the increasing importance of *P. brassicae* as a worldwide pathogen of brassicas and other crucifers, it may also be worthwhile for researchers to work together to develop a consistent definition of the limits of detection, particularly with respect to diagnostic assays. However, regardless of the exact metholodogy used, detection and quantification of *P. brassicae* is likely to play an increasingly important role in the development of effective strategies for the management of clubroot.

References

Alberta Clubroot Management Committee (2008) Alberta clubroot management plan. AGDEX 140/638-2. Alberta Agriculture and Rural Development. 8 pp. http://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/agdex11519

Arie T, Namba S, Yamashita S, Doi Y (1988) Detection of resting spores of *Plasmodiophora brassicae* Woron. from soil and root by fluorescent antibody technique. Ann Phytopathol Soc Jpn 54:242–245

Buhariwalla H, Greaves S, Magrath R, Mithen R (1995) Development of specific primers for the amplification of polymorphic DNA from the obligate root pathogen *Plasmodiophora brassicae*. Physiol Mol Plant Pathol 47:83–94

Cao T, Tewari J, Strelkov SE (2007) Molecular detection of *Plasmodiophora brassicae*, causal agent of clubroot of crucifers, in plant and soil. Plant Dis 91:80–87

Chee HY, Kim WG, Cho WD, Jee HJ, Choi YC (1998) Detection of *Plasmodiophora brassicae* by using polymerase chain reaction. Kor J Plant Pathol 14:589–593

Colhoun J (1957) A technique for examining soil for the presence of *Plasmodiophora brassicae* Woron. Ann Appl Biol 45:559–565

Dixon GR (2006) The biology of Plasmodiophora brassicae Wor.—A review of recent advances. Acta Hort 706:271–282

Donald EC, Porter IJ, Lancaster RA (2001) Band incorporation of fluazinam (Shirlan) into soil to control clubroot of vegetable brassica crops. Aust J Exp Agric 41:1223–1226

Donald EC, Lawrence JM, Porter IJ (2002) Evaluation of a fluorescent staining technique as an indicator of pathogenicity of resting spores of *Plasmodiophora brassicae*. Australas Plant Pathol 31:373–379

Donald EC, Porter IJ, Faggian R, Lancaster RA (2006) An integrated approach to the control of clubroot in vegetable Brassica crops. Acta Hort 706:283–300

Faggian R, Parsons S (2002) A rapid diagnostic test for clubroot. Final report for project no. VG 99008. Horticulture Australia, Sydney

Faggian R, Bulman SR, Lawrie AC, Porter IJ (1999) Specific polymerase chain reaction primers for the detection of *Plasmodiophora brassicae* in soil and water. Phytopathology 89:392–397

Faggian R, Parsons S, Porter IJ, Lawrie AC (2001) Detection of clubroot in Australia: progress towards a reliable molecular diagnostic assay. In: Porter IJ (ed), Proceedings of the 2nd Australasian soilborne diseases symposium, Lorne, Australia

Fedotova T (1933) Contribution to the evolution of a method for the evaluation of soil infection with clubroot (*Plasmodiophora brassicae* Wor.). Trudjjw zashchite rastenii Ser 2(3):51–81; in Russian

Hardham AR, Suzaki E, Perkin JL (1986) Monoclonal antibodies to isolate species and genus specific components on the surface of zoospores and cysts of the fungus *Phytophthora cinnamomi*. Can J Bot 64:311–325

Ito S, Maehara T, Tanaka S, Kameya-Iwaki M, Yano S, Kishi F (1997) Cloning of a single-copy DNA sequence unique to *Plasmodiophora brassicae*. Physiol Mol Plant Pathol 50:289–300

Ito S, Maehara T, Maruno E, Tanaka S, Kameya-Iwaki M, Kishi F (1999) Development of a PCR-based assay for the detection of *Plasmodiophora brassicae* in soil. J Phytopathol 147:83–88

Karling JS (1968) The Plasmodiophorales. Hafner Publishing, New York, 239 pp

Lange L, Heide M, Hobolth L, Olson LW (1989) Serological detection of *Plasmodiophora brassicae* by dot immunobinding and visualization of the serological reaction by scanning electron microscopy. Phytopathology 79:1066–1071

MacFarlane I (1952) Factors affecting the survival of *Plasmodiophora brassicae* Wor.in the soil and its assessment by a host test. Ann Appl Biol 39:239–256

Melville IE, Hawken RW (1967) Soil testing for club root in Devon and Cornwall. Plant Pathol 16:145–147

Murakami H, Tsushima S, Kuroyanagi Y, Shishido Y (2002) Reduction of resting spore density of *Plasmodiophora brassicae* and clubroot disease severity by liming. Soil Sci Plant Nutr 48:685–691

Orihara S, Yamamoto T (1998) Detection of resting spores of *Plasmodiophora brassicae* from soil and plant tissues by enzyme immunoassay. Ann Phytopathol Soc Jpn 64:569–573

Rimmer SR, Kutcher HR, Morrall RAA (2003) Diseases of canola and mustard. In: Bailey KL, Gossen BD, Gugel RK, Morrall RAA (eds) Diseases of field crops in Canada. Canadian Phytopathological Society, Saskatoon, pp 129–146

Samuel G, Garrett SD (1945) The infected root-hair count for estimating the activity of *Plasmodiophora brassicae* Woron. in the soil. Ann Appl Biol 32:96–101

Savage SD, Sall MA (1981) Radioimmunosorbent assay for *Botrytis cinerea*. Phytopathology 71:411–415

Shimotori H, Yanagida H, Enomoto Y, Igarashi K, Yoshinari M, Umemoto M (1996) Evaluation of benzenesulfonanilide derivatives for the control of crucifers clubroot. J Pestic Sci 21:31–35

Steffan RJ, Goksoyr J, Bej AK, Atlas RM (1988) Recovery of DNA from soils and sediments. Appl Env Microbiol 54:2908–2915

Strelkov SE, Tewari JP, Smith-Degenhardt E (2006) Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. Can J Plant Pathol 28:467–474

Strelkov SE, Manolii VP, Cao T, Xue S, Hwang SF (2007) Pathotype classification of *Plasmodiophora brassicae* and its occurrence in *Brassica napus* in Alberta, Canada. J Phytopathol 155:706–712

Strelkov SE, Manolii VP, Hwang SF, Howard RJ, Manolii AV, Zhou Q, Holtz M, Yang Y (2008) Incidence of clubroot on canola in Alberta in 2007. Can Plant Dis Surv 88:101–102

Takahashi K (1991) Correlation between fluorescent staining reaction and germination in resting spores of *Plasmodiophora brassicae*. Ann Phytopathol Soc Jpn 57:160–164

Takahashi K, Yamaguchi T (1987) An improved method for estimating the number of resting spores of *Plasmodiophora brassicae* in soil. Ann Phytopathol Soc Jpn 53:507–515

Takahashi K, Yamaguchi T (1988) A method for assessing the pathogenic ability of resting spores of *Plasmodiophora brassicae* by fluorescence microscopy. Ann Phytopathol Soc Jpn 54:466–475

Takahashi K, Yamaguchi T (1989) Assessment of pathogenicity of resting spores of *Plasmodiophora brassicae* in soil by fluorescence microscopy. Ann Phytopathol Soc Jpn 55:621–628

Tewari JP, Strelkov SE, Orchard D, Hartman M, Lange RM, Turkington TK (2005) Identification of clubroot of crucifers on canola (*Brassica napus*) in Alberta. Can J Plant Pathol 27:143–144

Toxopeus H, Janssen AMP (1975) Clubroot resistance in turnip. II. The slurry screening method and clubroot races in the Netherlands. Euphytica 24:751–755

Volossiuk T, Robb EJ, Nazar RN (1995) Direct DNA extraction for PCR-mediated assays of soil organisms. Appl Env Microbiol 61:3972–3976

Voorips ER (1995) *Plasmodiophora brassicae*: aspects of pathogenesis and resistance in *Brassica oleracea*. Euphytica 83:139–146

Wakeham AJ, White JG (1996) Serological detection in soil of *Plasmodiophora brassicae* resting spores. Physiol Mol Plant Pathol 48:289–303

Wallenhammar AC (1996) Prevalence of *Plasmodiophora brassicae* in spring oilseed rape growing area in Central Sweden and factors influencing soil infestation levels. Plant Pathol 45:710–719

Wallenhammar AC, Arwidsson O (2001) Detection of *Plasmodiophora brassicae* by PCR in naturally infested soils. Eur J Plant Pathol 107:313–321

Yeats C, Gillings MR, Davison AD, Altavilla N, Veal DA (1998) Methods for microbial DNA extraction from soil for PCR amplification. Biol Proc Online 1:40–47