European Journal of Plant Pathology **107:** 305–311, 2001.
© 2001 *Kluwer Academic Publishers. Printed in the Netherlands.*

Diversity of *Phytophthora clandestina* isolated from subterranean clover in southern Australia: analysis of virulence and RAPD profiles

Agus Purwantara[1], André Drenth[2], Sze P. Flett[3], Wendy Guppy[3] and Philip J. Keane[1]
[1]*School of Botany, La Trobe University, Bundoora, Vic. 3083 (Present address: Biotechnology Research Unit for Estate Crops, Jalan Taman Kencana 1, P.O. Box 179, Bogor 16151, Indonesia (E-mail: briec@indo.net.id.);*
[2]*Cooperative Research Centre for Tropical Plant Pathology, The University of Queensland, Qld. 4072,*
[3]*Agriculture Victoria, Institute of Sustainable Irrigated Agriculture, Ferguson Road, Tatura, Vic. 3616, Australia*

Accepted 2 January 2000

Key words: oomycetes, population genetics, races, subterranean clover, virulence

Abstract

The virulence spectrum of 112 isolates of *Phytophthora clandestina* collected from 56 sites in four subterranean clover-growing states in southern Australia was determined using differential cultivars of subterranean clover. Five races were detected, with race 0 in all states except New South Wales, race 1 in all states, race 2 only in Victoria, race 3 only in New South Wales, and race 4 in Victoria and Western Australia. The level of genotypic diversity among the different *P. clandestina* populations was investigated using five RAPD primers. Among 30 bands amplified, only two were polymorphic. This enabled identification of four multilocus RAPD genotypes. Three of the four genotypes occurred in all four states. Races 2 and 3 occurred with RAPD genotypes 1 and 2 only whereas races 0 and 1 occurred in all four multilocus RAPD genotypes. These results indicate that the pathogenicity spectrum of *P. clandestina* can change rapidly.

Introduction

Subterranean clover (*Trifolium subterraneum* L.) is the most important pasture legume in southern Australia. Over the last 40 years there has been a marked decline in its productivity. Root rot caused by *Phytophthora clandestina* Taylor, Pascoe and Greenhalgh is an important disease associated with this decline (Greenhalgh and Clarke, 1985; Greenhalgh and Taylor, 1985; Taylor et al., 1985a–c). *P. clandestina* is widespread in both dryland and irrigated pastures (Greenhalgh and Taylor, 1985; Taylor and Greenhalgh, 1987). The disease reduced the annual production of dry matter of subterranean clover in clay loam soils by 47% and in light clay loam soils by 94% (Greenhalgh, 1992).

Control of root rot by cultivation, grazing management and fungicide application has been recommended (Greenhalgh and Clarke, 1985; Taylor et al., 1985b; Smiley et al., 1986; Flett et al., 1994; Greenhalgh et al., 1994), but an important strategy for controlling the disease is planting resistant cultivars (Taylor et al., 1985b; Flett et al., 1994). This has resulted in selection of specific races of *P. clandestina* able to overcome resistance (Flett, 1994; Purwantara et al., 1995, 1996a). At first, only race 0, able to attack cultivar Woogenellup (which does not carry any known resistant genes) was found in Victoria, until race 1 was isolated from Katamatite, Victoria in 1990 and race 2 from Rutherglen, Victoria in 1992 (Flett et al., 1994) (Table 1). So far, only races 1 and 3 have been reported from New South Wales (Dear et al., 1994), race 1 from South Australia (Flett et al., 1994) and race 0 from Western Australia (Barbetti, 1994). Cultivars which were previously resistant to root rot may not be appropriate for areas where virulent races have become common.

P. clandestina was first identified in Australia in 1982 (Taylor, 1984; Taylor et al., 1985a,c) and has not been reported elsewhere (Erwin and Ribeiro, 1998). The pathogen is specific to subterranean clover. The fungus is homothallic and oospores are readily produced in infected roots. The origin of this pathogen and the way in which new races evolve and spread are not known.

Table 1. Distribution of races of *P. clandestina* by state and year of isolation, and RAPD genotypes found within each race

State	Race	No. of isolates*	Year	RAPD genotype
New South Wales	1	12 (80)	91, 93, 95, 96	1, 2, 4
	3	3 (20)	93	1
South Australia	0	3 (19)	96	1, 2
	1	13 (81)	92, 96	1, 2, 3, 4
Victoria	0	20 (51)	92, 93, 96	1, 2, 3, 4
	1	14 (36)	92, 93, 96	1, 2, 4
	2	3 (8)	92	2
	4	2 (5)	96	1
Western Australia	0	11 (26)	85, 96	1, 2, 3, 4
	1	30 (71)	96	1, 2, 3, 4
	4	1 (3)	96	4

*Percentage of isolates calculated within each state in parentheses.

Studies of the diversity of the pathogen population are needed to address these questions. Recent advances in molecular techniques offer methods to assess levels of genetic diversity in populations of almost any organism. Restriction Fragment Length Polymorphism (RFLP) (Botstein et al., 1980) and Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990) have been widely used to elucidate the genetic structure of fungal populations (McDermott and McDonald, 1993). The specific aim of the current investigation was to determine the geographic distribution of races of *P. clandestina* in southern Australia and their genotypic diversity. Such information can be used to test hypotheses concerning the origin and evolution of new races of *P. clandestina* and to develop strategies to deploy resistance genes for optimal disease control.

Materials and methods

Fungal isolates

One hundred and eleven isolates of *P. clandestina* were collected from 56 locations in four states in southern Australia (Figure 1) as part of a national survey

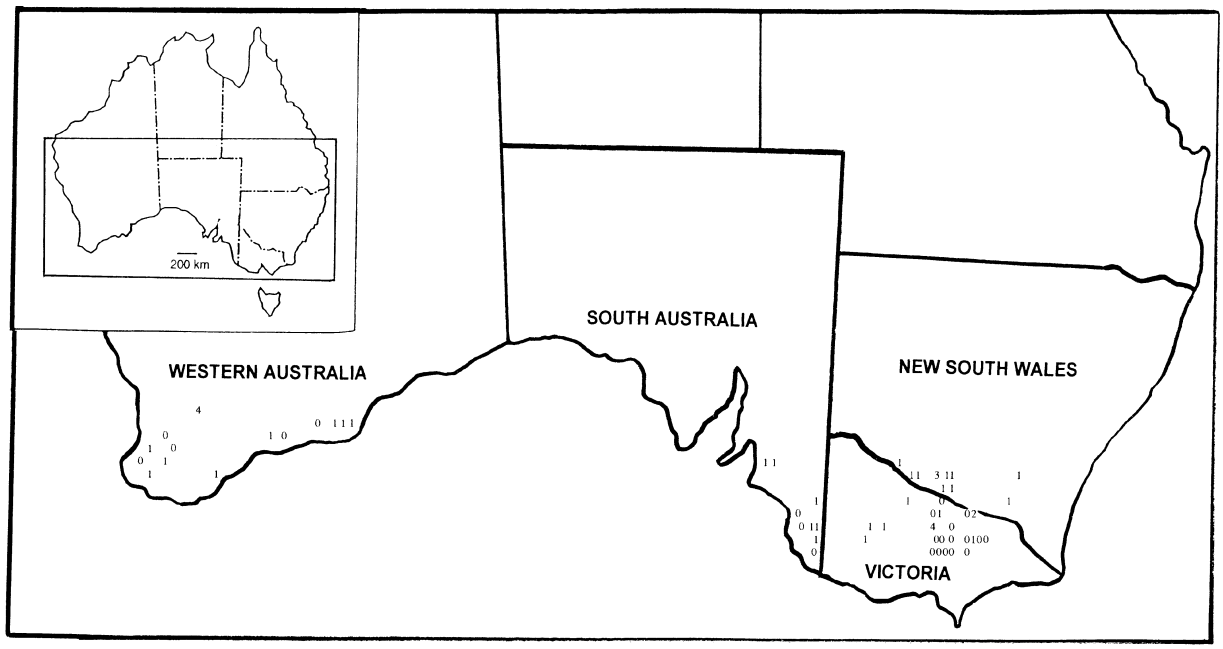

Figure 1. Distribution of races of *P. clandestina* at sites sampled in southern Australia. Each number (0, 1, 2, 3 or 4) represents the race designation. One hundred and twelve isolates were collected during sampling in 1985, 1991, 1992, 1993, 1995 and 1996.

Table 2. Different susceptibility of cultivars of subterranean clover to races of *P. clandestina* (R: resistant, severity rating 0–2.5; S: susceptible, severity rating 2.6–5.0)

Race	Differential cultivar					
	Woogenellup	Larisa	Trikkala	Leura	Meteora	Seaton Park
0	S	R	R	R	R	R
1	S	S	S	S	R	R
2	S	R	R	R	S	R
3	S	S	S	S	S	R
4	S	S	S	S	S	S

Isolates collected in 1985–1993 were tested with Woogenellup, Larisa, Trikkala and Meteora, whereas isolates collected in 1995–1996 were tested with Woogenellup, Leura, Meteora and Seaton Park.

of *P. clandestina* on subterranean clover conducted in 1991–1996. One additional isolate was collected in 1985 from Western Australia (Table 1). At each location, 1–5 isolates were sampled. All isolates were obtained from roots of infected subterranean clover plants using the methods described in Flett (1994). Isolates were maintained on lima bean agar (LBA) (20% (w/v) canned lima bean (Ward McKenzie Pty. Ltd., Altona, Australia) blended and strained through six layers of cheesecloth to remove most solid matter, 2% (w/v) agar, with the final pH being adjusted to 6.0) at 20 °C with a 12 h photoperiod (light intensity 70 $\mu Em^{-2}s^{-1}$).

Race determination

Isolates of *P. clandestina* were evaluated for their virulence spectrum using subterranean clover differentials (Table 2). Four differential cultivars (Woogenellup, Larisa, Trikkala and Meteora) chosen according to their response to races of the pathogen were used to test isolates collected during 1985–1993. To allow for the detection of race 4, another set of differentials (Woogenellup, Leura, Meteora and Seaton Park) was used to examine the isolates collected in 1995–1996. Isolates of race 3 collected in 1993 were re-tested using the second set of differentials. Larisa, Leura and Trikkala have the same resistance (Purwantara et al., 1995, 1996a). Seeds of the differentials were planted in potting mix consisting of coarse sand and pine bark artificially infested with a particular isolate following the procedure of Flett et al. (1993) in a glasshouse or growth cabinet maintained at 20 ± 2 °C. Three pots (8 cm diameter) were used as replicates with 10 seeds per pot. After seedlings reached the cotyledon stage (1 week after germination), and then at weekly intervals, pots were immersed in tap water for 2 h to induce infection. Three weeks after sowing, seedlings were scored for disease severity using a 0–5 rating system (0: roots healthy, 5: roots completely rotted or plant dead) (Purwantara et al., 1996a). Seedlings with average disease severity $\leq$2.5 were rated as resistant, and seedlings with average disease severity $>$2.5 were rated as susceptible. Additionally, the races of the pathogen were determined using bioassays as described by Purwantara et al. (1996b) involving the planting of differential cultivars in soil samples containing the pathogen in small planting trays and scoring the disease severity on growing seedlings. The latter method was used to confirm the results of the former method.

DNA isolation

For DNA isolation, isolates were grown in 10 ml of 20% clarified V_8 juice (Campbell's Soup Co., Lemnos, Australia) in McCartney bottles for 14 days. The culture medium was removed by filtration through Whatman filter paper and the mycelium was washed twice with sterile distilled water and blotted with filter paper to remove excess water. Alternatively, the fungus was grown on LBA in Petri dishes for 14 days and the mycelium was separated from the agar by cutting out the colony and melting the LBA in a microwave oven. The mycelium was washed with sterile distilled water three times, blotted dry with filter paper, transferred to a 1.5 ml microcentrifuge tube and stored at −20 °C until used for DNA extraction. DNA was extracted from the frozen mycelium (Lee and Taylor, 1990). The concentration of DNA was determined using a DyNA Quant 200 fluorometer (Hoefer Scientific Instruments, San Fransisco, CA, USA). The DNA was dissolved in milliQ water to a final concentration of 20 ng μl^{-1} and stored at −20 °C.

RAPD analysis

Five ten-base oligonucleotide primers (Operon Technologies Inc., Alameda, CA, USA) were selected from a total of 20 primers screened and used to determine the genotype of each isolate (Table 3). Primers were selected for their ability to generate reproducible

Table 3. Primers used for RAPD analysis of *P. clandestina*, total number of amplified DNA bands and number of polymorphic DNA bands obtained with each primer

Primer[a]	Base sequence of primer (5′ to 3′)	No. of amplified bands[b]	No. of polymorphic bands
OPA-13	CAGCACCCAC	7	0
OPH-03	AGACGTCCAC	6	0
OPH-12	ACGCGCATGT	4	0
OPR-15	GGACAACGAG	5	2
OPT-07	GGCAGGCTGT	8	0
Total		30	2

[a]Coding according to Operon Technologies Inc., Alameda, CA, USA.
[b]Only bands consistently amplified were incorporated.

and well-separated DNA bands after two separate experiments of each primer. Amplification reactions were performed in a total volume of 25 µl, containing 50 mM KCl, 10 mM Tris.HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM primer, 2 Units of *Taq* polymerase (Biotech International, WA, Australia) and 50 ng of DNA template. Positive and negative controls were included in all experiments. Amplification was performed with a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT, USA) programmed as follows: 5 min initial denaturation at 94 °C; denaturation at 94 °C for 1 min, primer annealing at 37 °C for 1 min, extension at 72 °C for 2 min, repeated for 40 cycles; final extension at 72 °C for 10 min. Amplified products were size-fractionated on a 1.5% agarose gel in Tris Borate EDTA (TBE) buffer at 120 V for approximately 6 h. A 100-base-pair ladder (Pharmacia Biotech, USA) was used as a DNA size marker. Gels were stained in an ethidium bromide solution for 20 min, destained in deionised water for 30 min and photographed under UV light.

Data analysis

Presence or absence of amplified bands was scored for all strongly amplified bands. A total of 30 bands from the five primers was combined to derive a multilocus RAPD genotype. Each band of a certain length was assumed to represent a single genetic locus. Isolates that were identical for the 30 loci investigated were considered to have the same multilocus RAPD genotype. A measure of genotypic diversity was calculated on the basis of the frequency of different multilocus RAPD genotypes collected from each state. Genotypic diversity in each state was measured according to Stoddart and Taylor (1988) using the formula:

$$G = \frac{1}{\sum_{x=0}^{N}[(fx) \cdot (x/N)^2]},$$

where N is the sample size and fx is the number of different multilocus RAPD genotypes observed x times in each state.

Results

The 112 isolates of *P. clandestina* collected from four states in southern Australia were classified into five races on the basis of their virulence on a set of differential cultivars. Even though race 4 was not detectable using the pre-1995 differential set, later tests using Seaton Park as one of the differential cultivars confirmed that the pre-1995 isolates of race 3 did not include race 4. Race 0 was found in all states except New South Wales, race 1 was found in all states, races 2 and 3 were found only in Victoria and New South Wales, respectively, and race 4 was found in Victoria and Western Australia (Table 1, Figure 1). Races 1, 2, 3 and 4 were detected for the first time in 1991, 1992, 1993 and 1996, respectively.

RAPD analysis of the 112 isolates using five primers detected two polymorphic bands from a total of 30 scored DNA bands (Table 3, Figure 2). These two polymorphisms allowed four multilocus RAPD genotypes to be distinguished. Individual RAPD genotypes contained from two to four races. All four RAPD genotypes occurred in all states except New South Wales, in which only genotypes 1, 2 and 4 were found (Table 4). RAPD genotypes 1 and 2 were the most common (81–90% of isolates tested) in all states except Western Australia, where RAPD genotype 3 was common (29% of the isolates). Isolates of the widely distributed race 1 had all four RAPD genotypes in South Australia, Victoria and Western Australia. Isolates of races 2 and 3 occurred only once in multilocus RAPD genotype 2 in Victoria and in genotype 3 in New South Wales, respectively. On the other hand, isolates of different races shared identical RAPD genotypes. Of the three races found in Western Australia, races 0 and 1 had RAPD genotypes 1, 2, 3 and 4 whereas race 4 exhibited RAPD genotype 4 (Table 4). There was no relationship between the year of isolation of different isolates and their RAPD genotype. The number of polymorphic bands (2/30)

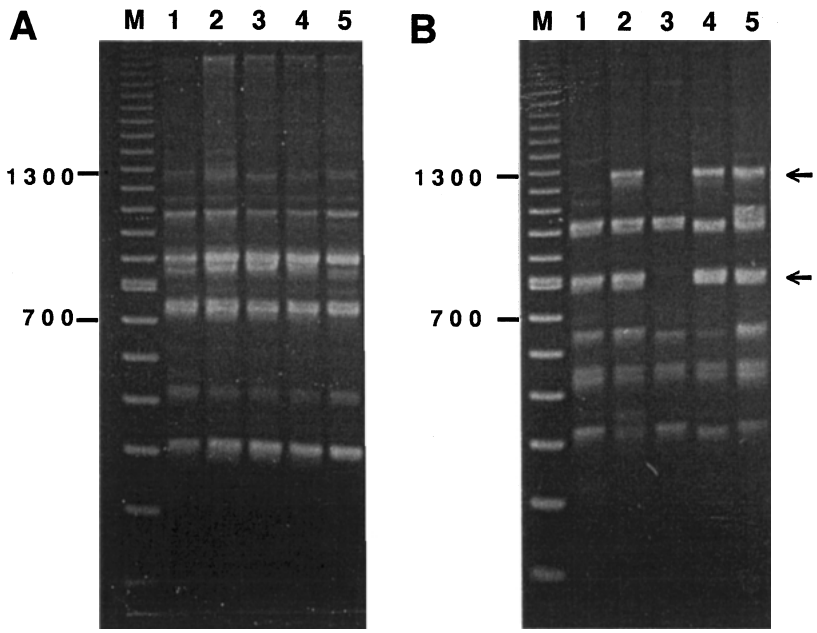

Figure 2. Examples of RAPD patterns generated from isolates of *P. clandestina* using primers OPH 12 (A) and OPR 15 (B). On each gel, lanes are, from left to right: molecular weight markers and five isolates of *P. clandestina*. The arrows show polymorphic bands consistently present on amplification with primer OPR 15 (approx. 800 and 1300 bp). Molecular weights (bp) are indicated on the left-hand side of the gel.

was low. Genotypic diversity in the different populations of *P. clandestina* studied ranged from 1.7 to 3.6, which was 43–90% of the maximum possible with only two polymorphic loci. Genotypic diversity was slightly greater in Western Australia where the sample size was larger.

Discussion

Isolates of *P. clandestina* collected from four states in southern Australia were racially diverse. Several new races have been detected since 1991, with the newly reported race 4 being detected in two states in 1996. Using RAPD markers, low levels of genotypic diversity were identified among 112 isolates collected from 1985 to 1996. Races 0 and 1 of *P. clandestina* were distributed in all subterranean clover-growing states and had all four RAPD genotypes. RAPD genotype 3 was more common in samples from Western Australia than in South Australia and Victoria, and was absent from New South Wales.

Although *P. clandestina* was reported for the first time in Australia in 1982, it is suspected that this pathogen has been associated with decline in subterranean clover pastures since the early 1950s (Greenhalgh, 1992). Initially only race 0 was isolated (Greenhalgh, 1992). Race 1 was detected in Victoria on the moderately resistant cultivar Bacchus Marsh in 1990 (Flett, 1994) and races 2, 3 and 4 were subsequently detected in different areas in 1992, 1993 and 1996, respectively. The occurrence of new races can be attributed partly to the large-scale cultivation of resistant cultivars. Our data show that the same races can occur within different RAPD genotypes, indicating either that new races may have evolved independently in different genotypes, or that there is a lot of recombination. Possibly the different races evolved independently through mutation in identical or different genetic backgrounds in different areas, and were subsequently subjected to selection when different resistance genotypes were deployed in new cultivars. This has been shown in *P. sojae* (Drenth et al., 1996; Förster et al., 1994), and in *P. infestans* (Goodwin, 1997; Goodwin et al., 1992, 1995). Once a new race occurs at a particular site, it may spread to other areas via oospores or hyphae carried with soil or plant debris on seed, especially as seed of subterranean clover is

Table 4. Distribution of *P. clandestina* RAPD genotypes by state and races found within each multilocus RAPD genotype

State	RAPD genotype	No. of isolates[a]	Race	G^b
New South Wales	1	11 (73)	1, 3	
	2	2 (13)	1	
	4	2 (13)	1	
Total	3	15	2	1.7
South Australia	1	4 (25)	0, 1	
	2	9 (56)	0, 1	
	3	2 (13)	1	
	4	1 (6)	1	
Total	4	16	2	2.5
Victoria	1	25 (64)	0, 1, 4	
	2	10 (26)	0, 1, 2	
	3	1 (3)	0	
	4	3 (8)	0, 1	
Total	4	39	4	2.1
Western Australia	1	14 (33)	0, 1	
	2	11 (26)	0, 1	
	3	12 (29)	0, 1	
	4	5 (12)	0, 1, 4	
Total	4	42	3	3.6

[a]Percentage of isolates calculated within each state in parentheses.
[b]Genotypic diversity (Stoddart and Taylor, 1988).

vacuumed from the soil surface along with dust and plant debris.

The low number of polymorphic markers may suggest that *P. clandestina* is of recent origin on subterranean clover in southern Australia, either because of recent introduction from the centre of evolution of the pathogen or because of adaptions of native species of *Phytophthora* to the introduced host. The possibility also exists of outcrossing among existing races in the field, as *P. clandestina* is homothallic and readily produces gametangia and oospores in infected tissue (Taylor et al., 1985c; Purwantara et al., 1998). A low percentage (<5%) of outcrossing *in vitro* has been reported to occur in *P. sojae*, another homothallic *Phytophthora* species (Bhat and Schmitthenner, 1993; Förster et al., 1994; Whisson et al., 1994). The occurrence of all four RAPD genotypes in approximately equal frequencies may indicate outcrossing of *P. clandestina* in Western Australia. However, more thorough study with more isolates and more primers is required to distinguish the above possibilities.

Acknowledgements

This work was partially funded by the Rural Industry Research and Development Corporation, the International Wool Secretariat, the Cooperative Research Centre for Tropical Plant Pathology and the School of Botany, La Trobe University. We wish to thank Professor R.W. Parish, School of Botany, La Trobe University for supporting this study, Ms G. Wagels for technical support and all participants of the national subterranean clover survey including Drs M.J. Barbetti and G.M. Murray. The first author was a recipient of a postgraduate fellowship of the Australian Agency for International Development (AusAID).

References

Barbetti MJ (1994) Root rots of subterranean clover: A Western Australian perspective. In: Flett SP and Guppy W (eds) Technical workshop proceedings of Phytophthora root rot in subterranean clover 1994 (pp 18–19), Agriculture Victoria, Tatura, Australia

Bhat RG and Schmitthenner AF (1993) Genetic crosses between physiologic races of *Phytophthora sojae*. Experimental Mycology 17: 122–129

Botstein D, White RL, Skolnick M and Davis RW (1980) Construction of a genetic linkage map in man using Restriction Fragment Length Polymorphisms. American Journal of Human Genetics 32: 314–331

Dear BS, Murray GM and Flett SP (1994) Phytophthora root rot in subterranean clover. Agnote New South Wales Agric 9: 1–4

Drenth A, Whisson SC, Maclean DJ, Irwin JAG, Obst NR and Ryley MJ (1996) The evolution of races of *Phytophthora sojae* in Australia. Phytopathology 86: 163–169

Erwin DC and Ribeiro OK (1998) Phytophthora diseases worldwide. American Phytopathological Society, St. Paul

Flett SP (1994) Studies on *Phytophthora clandestina*, the cause of tap root rot in subterranean clover. I. Evidence for physiological specialisation in *Phytophthora clandestina*. Australian Journal of Experimental Agriculture 34: 1125–1129

Flett SP, Guppy W and Cook T (1994) Update on Phytophthora root rot research. In: Flett SP and Guppy W (eds) Technical workshop proceedings of Phytophthora root rot in subterranean clover 1994 (pp 1–3) Agriculture Victoria, Tatura, Australia

Flett SP, Taylor PA and Wakley V (1993) A method for the greenhouse evaluation of subterranean clover (*Trifolium subterraneum*) breeding lines for *Phytophthora clandestina* root rot. Proceedings of the 17th International Grassland Congress, Session 22: 70

Förster H, Tyler BM and Coffey MD (1994) *Phytophthora sojae* races have arisen by clonal evolution and by rare outcrosses. Molecular Plant-Microbe Interactions 7: 780–791

Goodwin SB (1997) The population genetics of *Phytophthora*. Phytopathology 87: 462–473

Goodwin SB, Spielman LJ, Matuszak JM, Bergeron SN and Fry WE (1992) Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82: 955–961

Goodwin SB, Sujkowski LS and Fry WE (1995) Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85: 669–676

Greenhalgh FC (1992) Studies on the aetiology and significance of root diseases of subterranean clover, and the histopathology and control of Phytophthora root rot. PhD thesis. La Trobe University, Melbourne

Greenhalgh FC and Clarke RG (1985) Use of fungicides to study significance and etiology of root rot of subterranean clover in dryland pastures of Victoria. In: Parker CA, Moore KJ, Wong PTW, Rovira AD and Kollmorgen JF (eds) Ecology and management of soilborne plant pathogens (pp 234–236) American Phytopathological Society, St. Paul

Greenhalgh FC, de Boer RF, Merriman PR, Hepworth G and Keane PJ (1994) Control of Phytophthora root rot of irrigated subterranean clover with potassium phosphonate in Victoria, Australia. Plant Pathology 43: 1009–1019

Greenhalgh FC and Taylor PA (1985) *Phytophthora clandestina*, cause of severe taproot rot of subterranean clover in Victoria, Australia. Plant Disease 69: 1002–1004

Lee SB and Taylor JW (1990) Isolation of DNA from fungal mycelia and single spores. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds) PCR protocols: a guide to methods and applications (pp 282–287) Academic Press, London

McDermott JM and McDonald BA (1993) Gene flow in plant pathosystems. Annual Review of Phytopathology 31: 353–373

Purwantara A, Flett SP and Keane PJ (1995) Variation in pathogenicity and virulence of *Phytophthora clandestina* on four cultivars of subterranean clover. Australian Journal of Experimental Agriculture 35: 717–724

Purwantara A, Flett SP and Keane PJ (1996a) Resistance of 19 cultivars of subterranean clover to 4 races of *Phytophthora clandestina*. Euphytica 91: 351–358

Purwantara A, Flett SP and Keane PJ (1996b) Bioassay and baiting methods for determining occurrence of races of *Phytophthora clandestina* in soil. Australian Journal of Experimental Agriculture 36: 815–822

Purwantara A, Flett SP and Keane PJ (1998) Colonization of roots of subterranean clover cultivars by virulent and avirulent races of *Phytophthora clandestina*. Plant Pathology 47: 67–72

Smiley RW, Taylor PA, Clarke RG, Greenhalgh FC and Trutmann P (1986) Simulated soil and plant management effects on root rots of subterranean clover. Australian Journal of Agricultural Research 37: 633–645

Stoddart JA and Taylor JF (1988) Genotypic diversity: Estimation and prediction in samples. Genetics 118: 705–711

Taylor PA (1984) An unusual *Phytophthora* associated with root rot of subterranean clover. Plant Disease 68: 450

Taylor PA, Barbetti MJ and Wong DH (1985a) Occurrence of *Phytophthora clandestina* in Western Australia. Plant Protection Quarterly 1: 57–58

Taylor PA, Clarke RG, Kelly K and Smiley R (1985b) Root rot of irrigated subterranean clover in northern Victoria: significance and prospects for control. In: Parker CA, Moore KJ, Wong PTW, Rovira AD and Kollmorgen JF (eds) Ecology and management of soilborne plant pathogens (pp 271–273) American Phytopathological Society, St. Paul

Taylor PA and Greenhalgh FC (1987) Significance, causes and control of root rots of subterranean clover. In: Wheeler JL, Pearson CJ and Robards GE (eds) Temperate pastures: their production, use and management (pp 249–251) Australian Wool Corporation/Commonwealth Scientific and Industrial Research Organisation, Melbourne

Taylor PA, Pascoe IG and Greenhalgh FC (1985c) *Phytophthora clandestina* sp. nov. in roots of subterranean clover. Mycotaxon 22: 77–85

Whisson SC, Drenth A, Maclean DJ and Irwin JAG (1994) Evidence for outcrossing in *Phytophthora sojae* and linkage of a DNA marker to two avirulence genes. Current Genetics 27: 77–82

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey S (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531–6536