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Soilborne root disease pathogen complexes drive widespread decline of subterranean clover pastures across diverse climatic zones

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Abstract. Subterranean clover (*Trifolium subterraneum* L.) is an important pasture legume in many regions of Australia, and elsewhere. A survey was undertaken in 2014 to define the levels of soilborne disease and associated pathogens in annual subterranean clover pastures across southern Australia. Most of the 202 samples processed had very severe levels of taproot rot disease (disease index 60–80%) and extremely severe lateral root rot disease (disease index 80–100%). A complex of soilborne root pathogens including *Aphanomyces trifolii*, *Phytophthora clandestina*, and one or more of *Pythium*, *Rhizoctonia* and *Fusarium* spp. was found responsible for severe pre- and post-emergence damping-off and root disease. This is the first study to highlight the high incidence of *A. trifolii* across southern Australian pastures and the first to highlight the existence of natural synergistic associations in the field between *Rhizoctonia* and *Pythium* spp., *Pythium* and *Fusarium* spp., *Pythium* spp. and *A. trifolii*, and *P. clandestina* and *A. trifolii*. Nodulation was generally poor, mainly only in the 20–40% nodulation index range. There was no relationship between rainfall zone and tap or lateral root disease level, with root disease equally severe in lower (330 mm) and higher (1000 mm) rainfall zones. This dispels the previous belief that severe root disease in subterranean clover is an issue only in higher rainfall zones. Although overall the relationship between tap and lateral root disease was relatively weak, these two root-disease components were strongly positively expressed within each pathogen's presence grouping, providing explanation for variability in this relationship across different field situations where soilborne root disease is a major problem. Most producers underestimated the levels and effect of root disease in their pastures. This study established that tap and lateral root diseases are widespread and severe, having devastating impact on the feed gap during autumn–early winter across southern Australia. Severe root disease was independent of the highly variable complex of soilborne pathogens associated with diseased roots, geographic location and rainfall zone. It is evident that soilborne root diseases are the primary factor responsible for widespread decline in subterranean clover productivity of pastures across southern Australia. Implications for disease management and options for extension are discussed.

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Introduction

Subterranean clover (*Trifolium subterraneum* L.) is a critical component of pasture grazing systems worldwide, particularly regions with Mediterranean-type climates in Africa, Asia, Australia, Europe, North America, and South America (Nichols *et al*. 2014). In southern Australia alone, it covers ~29 Mha (Hill and Donald 1998) and is the foundation for nutritious feed for livestock and an important source of nitrogen for cereal crops. In Australia in 2012, an estimated ~100 000 agricultural businesses included pastures on their properties (Australian Bureau of Statistics 2012). However, various soilborne pathogens attack root systems, resulting in severe herbage and seed-production losses (Barbetti *et al*. 1996). There is a well-established negative relationship between the plant root and shoot size and the level of taproot and/or lateral root disease under both artificial (e.g. Wong *et al*. 1984, 1985, 1986; Barbetti *et al*. 1987*a*, 1987*b*) and field (e.g. Barbetti and MacNish 1978, 1984; Simpson *et al*. 2011) conditions. Further, livestock producers across southern Australia, historically and currently, have reported severe decline of pastures and their failure to persist, leading to serious reductions in livestock-carrying capacity and whole-farm profitability. This decline manifests as a decrease in desirable species and an increase in weedy species (Barbetti and Jones 2011). Although soilborne pathogens cause most severe pre- and post-emergence damping-off and root disease in seedlings (Wong *et al*. 1984; Barbetti *et al*. 1986*b*; Barbetti *et al*. 2007), mature swards can also be seriously affected (O'Rourke *et al*. 2009), greatly reducing pasture productivity throughout the season (Barbetti *et al*. 1996, 2007).

Crucially, livestock producers inherently face critical feed shortage across autumn–winter, coinciding with the main soilborne pathogen attack and consequent damage from extensive pre- and post-emergence damping-off and seedling root disease (Barbetti *et al*. 2007). Several specific soilborne pathogens have been highlighted as the major pathogens posing a serious threat to annual subterranean pastures to the extent that reseeding is required (Barbetti *et al*. 2007). These include *Phytophthora clandestina*, various *Pythium* species and particularly *P. irregulare*, *Aphanomyces trifolii*, *Rhizoctonia solani*, and one or more *Fusarium* species such as *F. avenaceum* (Barbetti *et al*. 2007; Nichols *et al*. 2014; You *et al*. 2005, 2006, 2016). The impact of soilborne pathogens can be mitigated in several ways including fungicide treatments (Barbetti *et al*. 1987*a*, 1987*b*), cultural practices (Barbetti and MacNish 1984; Barbetti 1991; O'Rourke *et al*. 2012), and, most importantly, by deploying effective host resistance (Barbetti *et al*. 1986*b*, 2007; You *et al*. 2006). Of these, the last offers the most cost-effective and successful means of reducing damping-off and root disease (Barbetti *et al*. 1986*b*, 2007; Nichols *et al*. 2014). Whereas many cultivars developed up until the mid-1990s were known to have some level of resistance to at least one of the more important soilborne pathogens, cessation of support for routine disease screening in breeding programs has meant that, generally, little is known about the disease resistances of cultivars developed subsequently (Nichols *et al*. 2014).

There is a paucity of knowledge on the current situation regarding the extent, severity and impact of soilborne root disease and on the identity of prevailing soilborne pathogen populations across the agro-geographical regions of southern Australia. This makes disease control by utilising host resistance, especially deployment of more recent cultivars, largely ineffective as a means to manage losses in productivity and persistence of subterranean clover pastures from soilborne pathogen complexes. Disease surveys are necessary not only to establish the geographic distribution and severity of soilborne root diseases and their associated pathogen complexes, but also to determine any potential or actual breakdown in host resistances to root diseases in subterranean clover. Previous smaller surveys and/or investigations of damping-off and root disease of subterranean clover in southern Australia (e.g. Wong *et al*. 1985; Simpson *et al*. 2011) were of limited value because of the difficulty of obtaining information concerning the prevalence and severity of diseases over large areas. Soilborne root diseases of subterranean clover clearly need to be reappraised over much larger areas (across a greater diversity of soil types and rainfall zones) in order to provide a reliable estimate of disease losses and associated pathogen complexes, and to improve targeting of future research, advisory, and educational strategies. For these reasons, a comprehensive survey was undertaken in 2014 to define: (*i*) the levels of soilborne disease in annual subterranean clover pastures across the different agro-geographical regions of southern Australia, (*ii*) the predominant pathogens associated soilborne disease, and (*iii*) any influence of environment and farmer practices on soilborne disease incidence and severity. We highlight the extent and severity of soilborne root disease of pastures across southern Australia and how it is independent of pathogen complex, agro-geographic location, and environment. Implications for disease-management and options for extension are discussed.

Materials and methods

Survey sites

In southern Australia, self-regenerating annual pastures such as subterranean clover are grown from May (late autumn) to November (late spring). Free sampling kits (630 kits, two kits per package) were distributed to livestock producers within farming districts of New South Wales (NSW, 206), Victoria (108), South Australia (SA, 100) and Western Australia (WA, 216). Sites sampled across southern Australia are shown in Fig. 1. All sites sampled were non-irrigated, dryland subterranean

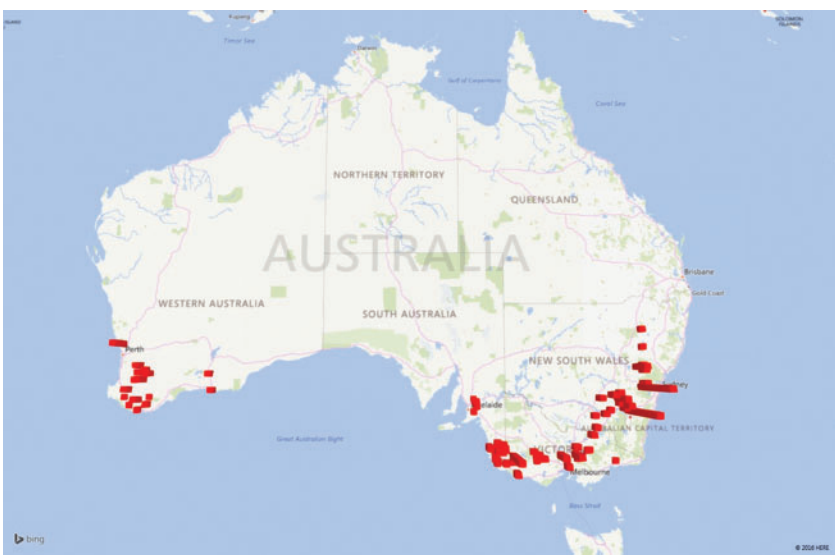

Fig. 1. Locations (red squares) of 145 sites sampled across southern Australia. 'Taller' site indicators indicate greater numbers of samples in similar locations.

clover pastures. Producers were asked to submit at least five samples (up to two with photos of plants), 900 mL for the plants and 250 mL for the soil, inside sealed plastic containers (Décor Tellfresh). In total, 295 individual annual pasture plant and related soil samples were received (a return rate of 46%), and 202 samples had sufficient subterranean clover seedlings to be assessed (Fig. 2*a, b, g*). All plant samples and soils were placed and maintained in a temperature-controlled (~25°C) PC2 quarantine glasshouse, and where possible the subterranean clover varieties were identified. Further, the majority of survey pasture samples also came with the requested digital photos, which were used to assist in identifying the variety (or varieties) and to help to assess the condition of the swards. Subterranean clover seedlings considered variants or natural crossbreds and of genetic interest to breeders were transplanted into large pots and the seed was harvested. A survey questionnaire requested information on paddock history over the last 2 years (a valuable source of grower information and district practice), and farmer perceptions of the extent of root disease in subterranean clover pastures, cultivars present, years since pasture was last renovated, and grazing practices. Individual producers were not identified in this study and their individual results and exact paddock locations remain confidential.

Assessments of survey samples

All samples from outside WA were sent via pre-paid postal packages; those in WA were sampled in person. Upon arrival in Perth, all samples, where possible, were assessed for the level of root rot disease, the presence of identifiable pathogens, and extent of nodulation, and subterranean clover variety or strain was identified. Plant roots were thoroughly washed under running tapwater to remove all soil and were then floated in shallow trays of deionised water. Taproots and lateral roots of each individual plant were visually scored independently for disease severity by using a modified 0–5 scoring system described and used earlier (Wong *et al*. 1984). The scores were: 0, root healthy, no discoloration; 1, <25% of root brown, no significant lesions; 2, 25–<50% of root brown, lesions towards base of taproot; 3, 50–<75% root brown, lesions mid taproot; 4, ≥75% root affected, significant lesions towards crown; 5, plant dead and/or root system completely rotted off. The number of plants in each tap and lateral root disease-severity category was recorded. The average percentage root-disease indices (%DI), based on the above disease ratings, were then calculated via the method described by McKinney (1923):

$$\%\mathrm{DI} = \frac{(a\times 0)+(b\times 1)+(c\times 2)+(d\times 3)+(e\times 4)+(f\times 5)}{(a+b+c+d+e+f)\times g}\times 100$$

where a, b, c, d, e and f represent the number of plants with taproot or lateral root disease scores of 0, 1, 2, 3, 4 and 5 for root ratings, and g represents the highest rating of tap or lateral root disease.

Nodulation was assessed by using a modified rating scheme from Corbin *et al*. (1977). The scoring system contained six

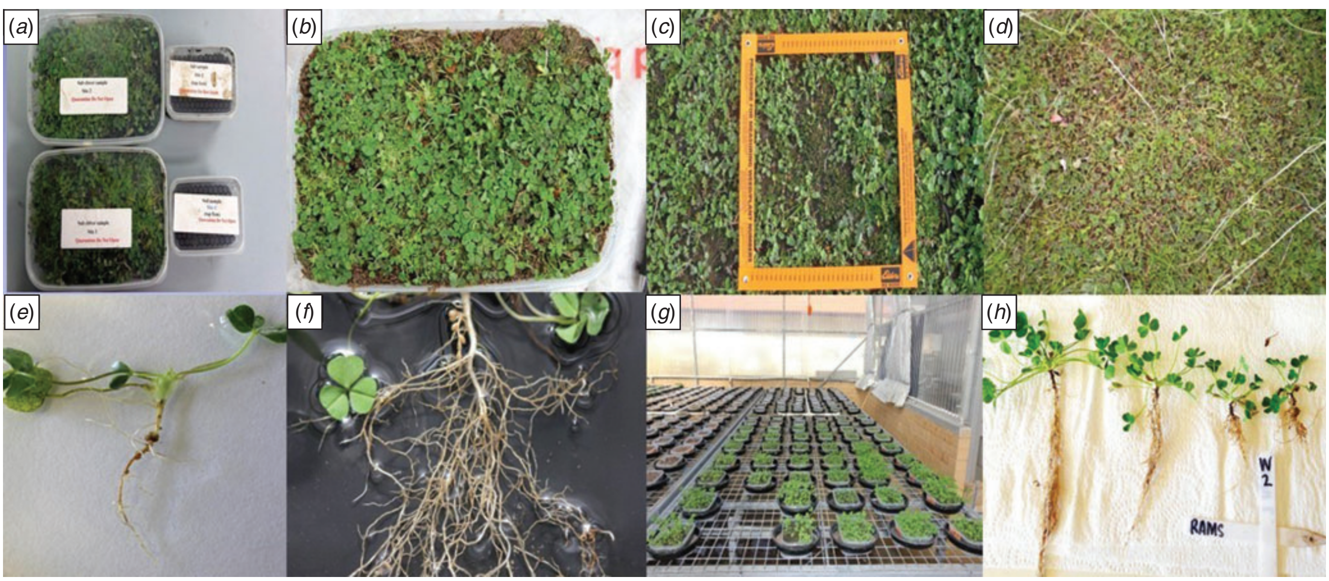

Fig. 2. (*a*) Free sampling kits (630 in total, two per package) distributed to livestock producers in New South Wales, Victoria, South Australia and Western Australia; 295 individual annual pasture plant and related soil samples were received (return rate of 46%) and 202 samples had sufficient seedlings to be assessed. (*b*) Typical samples as scored for the incidence of root rot disease, for plant density and composition, and extent of nodulation; seedling roots were assessed for type of pathogen(s) present, variety of subterranean clover was identified where possible, and, where possible, surviving subterranean clover plants were transplanted and then harvested for seed. (*c*) Example of pasture sampled in the field showing high levels of tap and lateral root disease. (*d*) Typical pasture showing individual subterranean clover plants within the sward displaying reddening and/or purpling of leaves, indicative of stressed plants because of significant root disease. (*e*) Typical severe taproot rot disease as commonly observed on plants across southern Australia during the early plant growth stages; seedlings affected by severe root rot such as shown often subsequently die, especially following sudden or heavy grazing. (*f*) Healthy seedling root system—one of the few observed in the survey samples. (*g*) Plant samples on arrival placed inside PC2 controlled-temperature quarantine facility for assessment. (*h*) Typical increasing root disease severity from left to right and associated minimisation of plant size in subterranean clover pastures.

nodulation categories based on nodule numbers and positioning: 0, no nodules on the crown or elsewhere; 1, no nodules on the crown with a few (1–10) elsewhere; 2, a few crown nodules but no nodules elsewhere; 3, many crown nodules (>10) but no nodules elsewhere; 4, many crown nodules with a few nodules elsewhere; 5, many crown nodules with many nodules elsewhere. The average percentage-nodulation indices, based on the above nodulation ratings, were then calculated as described above using the method described by McKinney (1923).

Root systems of each sample were floated in sterile distilled water and examined at 12, 24, 48 and 72 h for the presence of visually observable morphological characteristics consistent with *A. trifolii*, *P. clandestina*, *Pythium*, *Rhizoctonia* or *Fusarium* spp. (using 10×, 20× and 100× Olympus light microscope). The occurrence and typical symptom(s) of each pathogen on the floated roots were recorded and used for calculating the relative contributions of each pathogen towards the root-rot disease index recorded at each site. Earlier studies had been undertaken to corroborate the reliability of this methodology by extensive comparisons made with identifications following isolation of pathogens onto selective agar for these five pathogens (M. P. You, unpubl. data). Commercial DNA-based soil 'Predicta B' assays, developed by the South Australian Research and Development Institute (SARDI), Adelaide (Ophel-Keller *et al.* 2008), were also undertaken to confirm that these major fungal and oomycete pathogens that affect subterranean clover roots could be reliably identified by using light microscopy, methodology successfully utilised previously (e.g. You *et al.* 2016).

Subterranean clover cultivar (varietal or strain) identification of plants samples received was undertaken by using morphological markers and the identification guides of Nichols *et al.* (1996) and Gladstones and Collins (1984) and in conjunction with digital photographs submitted by producers.

Data analyses

GENSTAT® (14th edition, VSN International, Hemel Hempstead, UK) was used to test the significance of correlation coefficients between the tap and lateral root disease-assessment parameters measured and with other assessment data.

Results

Field symptoms

Of the 202 sampling kits (Fig. 2*a, b, g*) assessed, pasture swards varied from 'adequate' to 'poor' to 'severely affected' in terms of overall appearance of swards. The latter two categories generally showed clear stress symptoms of red or purpling discoloration of leaves as a consequence of soilborne root disease (Fig. 2*c, d*). When plants were removed from the samples, most showed typical severe root-disease symptoms (see Fig. 2*e*), and across the whole survey, only a single sample submitted (Fig. 2*f*) had healthy roots. The more severe the root disease, the smaller the plant size (see example in Fig. 2*h*).

Root disease

Data for root rot disease showed that most of farmer pasture fields not only had a very severe level of tap root rot, with a taproot rot disease index of 60–80% (Figs 3, 4), they also had extremely severe lateral root rot disease, with a disease index of 80–100% (Figs 3, 4). The consistent combination of severe tap and lateral root disease was at a level known to have serious adverse impact upon the autumn–early winter feed gap, as evidenced by very small size of plants received with severe root disease. Severe taproot and lateral root disease occurred consistently across all states, viz. NSW (Fig. 4*a, b*), SA (Fig. 4*c, d*), Victoria (Fig. 4*e, f*) and WA (Fig. 4*g, h*). Most producers greatly underestimated and underreported their levels of root disease in the survey, with 22% of producers unaware of any root disease present in their pastures (data not shown).

Pathogens

Tests for the presence of identifiable pathogens revealed a range of major soilborne pathogens widely present across the samples, as would be expected in association with the severe tap and lateral root disease noted above. Known major pathogens of subterranean clover pastures, *P. clandestina*, *A. trifolii* and *Pythium*, *Rhizoctonia* and *Fusarium* spp., were all present at high incidence across southern Australia. *Phytophthora clandestina* was found in ~25% of samples from WA and 20% of samples from Victoria, but there was a lower incidence in SA and least in NSW (Fig. 5*a*). A high incidence of *Pythium* spp. was observed on roots of samples from all states, particularly SA and NSW (Fig. 5*b*). There was a very high incidence of *Rhizoctonia* spp. across all states, particularly in the eastern states compared with WA (Fig. 5*c*). In relation to *A. trifolii*, the highest incidence was in WA, at ~25% of samples, followed by Victoria at ~20% and then a much lower incidence in NSW and SA (Fig. 5*d*). The highest incidence of *Fusarium* spp. was in NSW and WA, at 25–30%, followed by Victoria and SA (Fig. 5*e*). More than two-thirds of the samples had multiple pathogens present (Fig. 5*f*). Overall, there was a very high incidence of *Rhizoctonia* spp., followed by *Pythium* spp., and then lower overall incidence for each of *Fusarium* spp., *A. trifolii* and *P. clandestina* (Fig. 5*g*). On a national basis, 48% of samples had two different pathogens in combination, 32% had only a single pathogen, 17% had three different pathogens in combination, 1.5% had four different pathogens in combination, 1.5% had no detectable pathogens, but no samples contained all five different pathogens in combination (Fig. 5*h*). Combinations including *Rhizoctonia* species were the most common associations, particularly combinations of

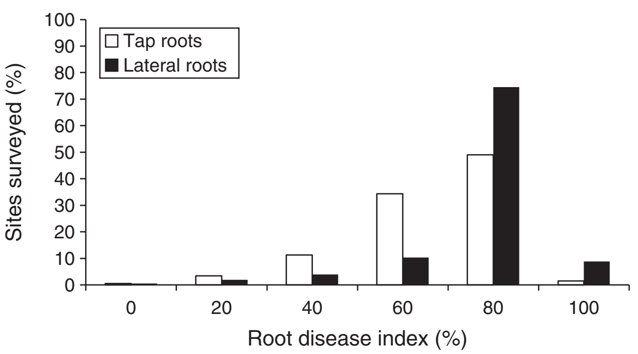

Fig. 3. Overall incidence of tap and lateral root disease, assessed on a visual rating scale, and taproot and lateral root percentage disease indices computed, for 202 sites sampled across southern Australia.

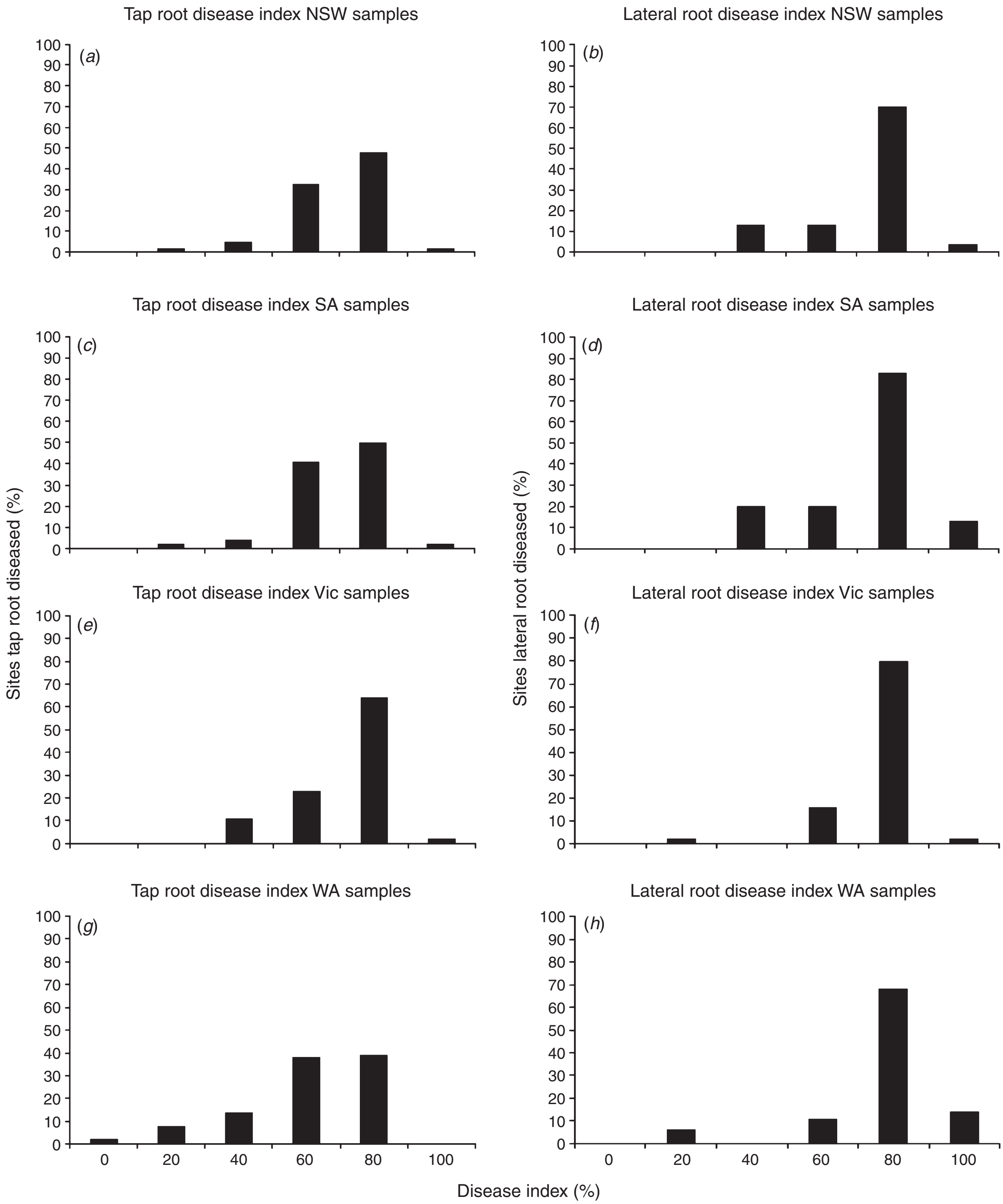

Fig. 4. Taproot and lateral root disease indices (0–100%) for: (*a*, *b*) 46 pasture samples from New South Wales, (*c*, *d*) 46 pasture samples from South Australia, (*e*, *f*) 44 pasture samples from Victoria, (*g*, *h*) 66 pasture samples from Western Australia.

two with any of the other pathogens, and largely reflected the overall higher incidence of *Rhizoctonia* species; however, some synergistic associations were clear (Fig. 6). For example, the total incidence of the *Rhizoctonia* + *Pythium* spp. combination, and in each case the *Pythium* + *Fusarium* spp., *Pythium* spp. + *A. trifolii*, and *P. clandestina* + *A. trifolii* combinations, exceeded the individual incidence for each species occurring on its own.

Rainfall

There was no relationship between rainfall zone and either tap ($R^2 = 0.007$) or lateral root ($R^2 = 0.020$) disease level (data now

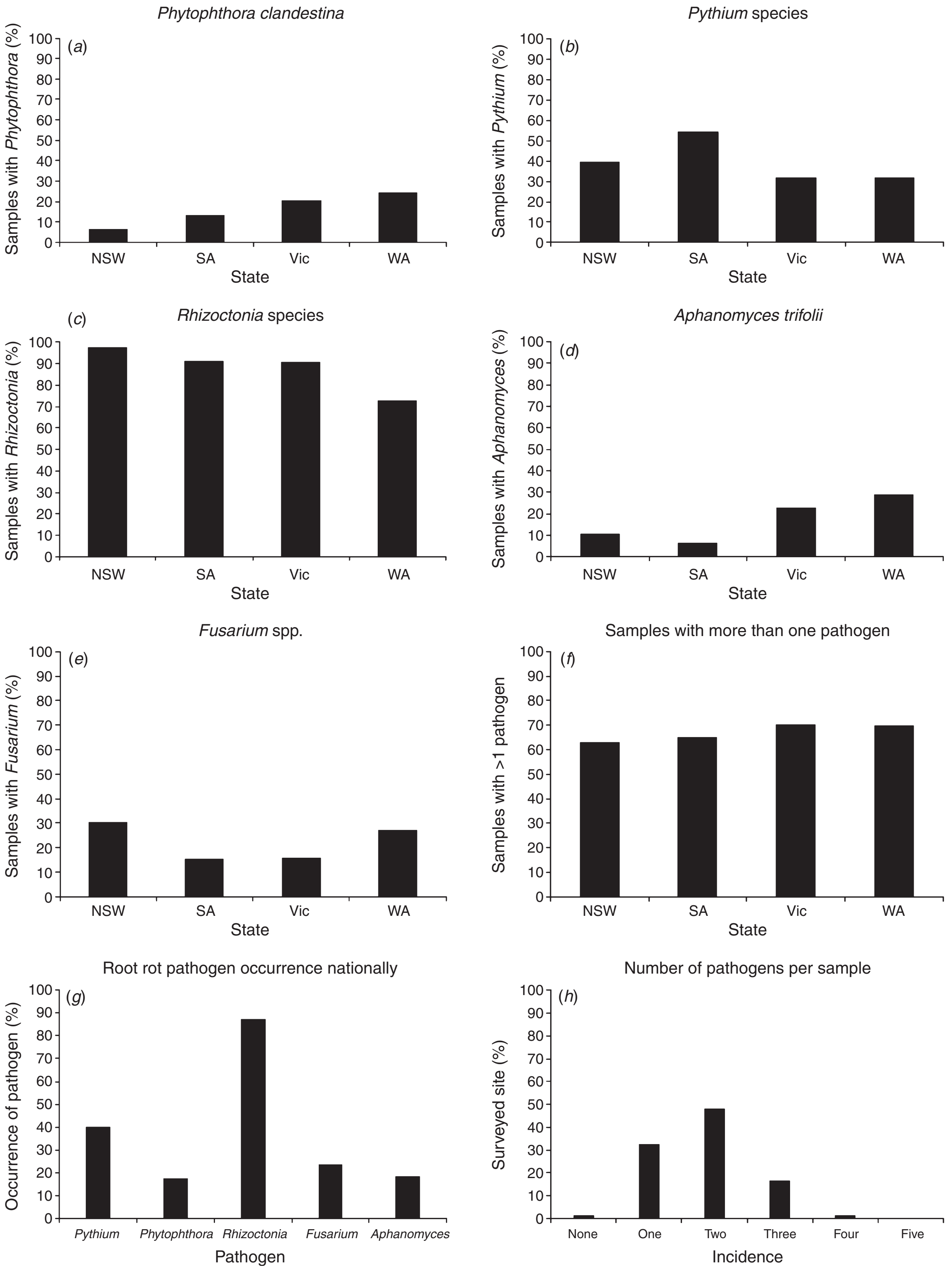

Fig. 5. Incidence of the different pathogens across 202 samples on a state basis and Australia-wide: (*a*) *Phytophthora clandestina*, (*b*) *Pythium* spp., (*c*), *Rhizoctonia* spp., (*d*) *Aphanomyces trifolii*, (*e*), *Fusarium* spp., (*f*) where more than one pathogen was present in the same sample, (*g*) total incidence of pathogens compared nationally, (*h*) percentage of samples nationally with different numbers of pathogens.

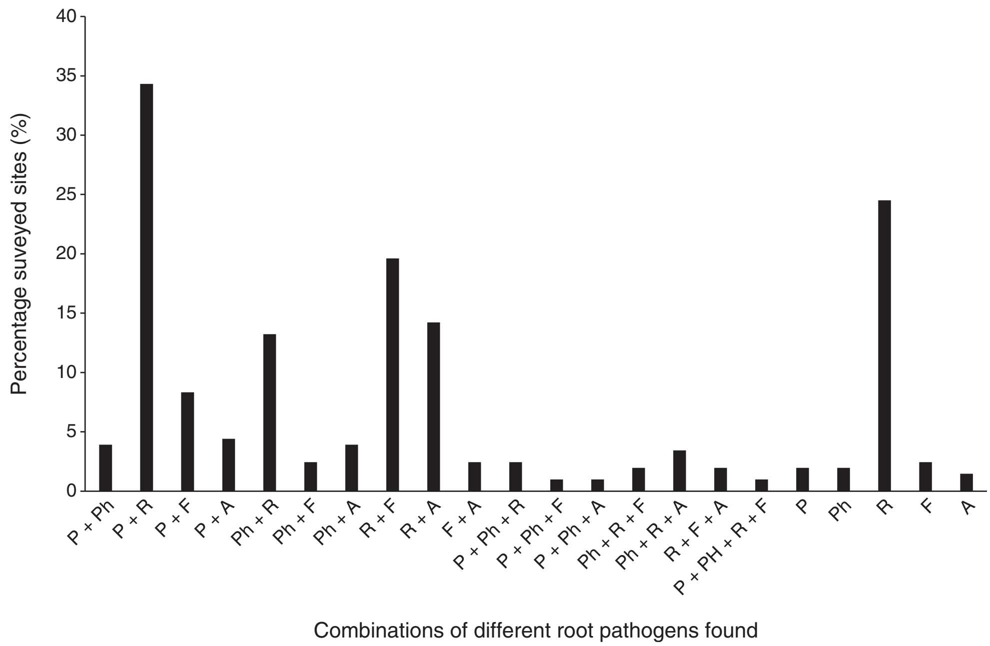

Fig. 6. Incidence of surveyed sites nationally showing different pathogen combinations. A, *Aphanomyces trifolii*; F, *Fusarium* spp.; P, *Pythium* spp.; Ph, *Phytophthora clandestina*; and R, *Rhizoctonia* spp.

shown). Taproot and/or lateral root disease was an equally severe disorder in lower (≤450 mm) and higher (≥800 mm) rainfall zones; for example, severe *Phytophthora* taproot rot was identified across all rainfall zones in the survey groups (Fig. 1). Percentage of total survey sites sampled across lower (≤450 mm), intermediate (455–750 mm) and higher (≥800 mm) rainfall zones, respectively, were 3.6%, 22.4% and 5.6% for NSW; 5.6%, 16.0% and 0.8% for SA; 4.0%, 15.6% and 3.2% for Victoria; and 15.2%, 10.4% and 1.6% for WA.

Grazing and nodulation

The extent of *Rhizobium* nodulation was generally low. Across NSW, SA, Victoria and WA, greatest nodulation was in only the 20–40% nodulation index range (data not shown). This was expected because failure of roots to nodulate adequately is a commonly observed condition where root disease is severe. There was little or no suggestion that grazing had any significant influence on the level of either tap or lateral root disease (data not shown).

Taproot v. *lateral root disease*

There was, overall across all samples, a weak relationship ($R^2 = 0.22$) between tap and lateral root disease, (Fig. 7*a*). However, the relationship between the two different root disease components was much more significant when examined within each individual pathogen's grouping, with R^2 values ≥0.79 (Fig. 7*b–f*).

Paddock history and variety

In all states, many producers reported low or declining legume content of their pastures over several years, especially in WA, SA and Victoria. Overall, the most frequent subterranean clover varieties found across the pastures samples were Trikkala (in 16% of survey samples), Seaton Park (12%), Dinninup and Leura (8%), Dalkeith and Mt Barker (5%), Woogenellup and Dwalganup (4%), Nungarin, Geraldton and Esperance (or Daliak), Yarloop, Bacchus Marsh and Bindoon (all 3%), Coolamon, Goulburn and Riverina (all 1%), and Gosse, Losa, Denmark, Nungarin, Campeda and Urana (all <1%). For 13% of samples, the variety could not be determined.

There was often low adoption of newer varieties; for example, in SA, <15% of the paddocks had been renovated in the last 15 years. The most common known varieties reported by producers in SA and subsequently confirmed were Trikkala, Dinninup, Yarloop and Mt Barker (data not shown), the last released in the 1930s. Few producers in Victoria (28%) and WA (31%) have resown a paddock to one of the newer clover varieties in the last 15 years. By contrast, in NSW, 65% of the paddocks had been renovated in the last 5 years, mostly with newer midseason varieties such as Coolamon, Bindoon, Leura and Gosse but also with older subterranean clover varieties (Trikkala, Seaton Park and Dalkeith). When these paddocks were renovated, >90% of producers indicated that they inoculated seed with rhizobia. Within the four states, there was minimal reported investment in fertiliser application or weed-management sprays over the last 2 years (data not shown), likely a consequence of locations outside NSW generally being long-term subterranean clover pastures infrequently or never cropped. Most producers (70%) applied <130 kg single superphosphate, and some applied as little as 40–60 kg. A very broad range of soil textures occurred across the samples (e.g. sandy and clay loams, red ironstone, red and black earth, gravel loams, jarrah sands, sand over clay duplex, karri loams, granite over sandy loams, black clay and even basalts), and these soils would vary in fertility, acidity and many other characteristics. Varietal identifications

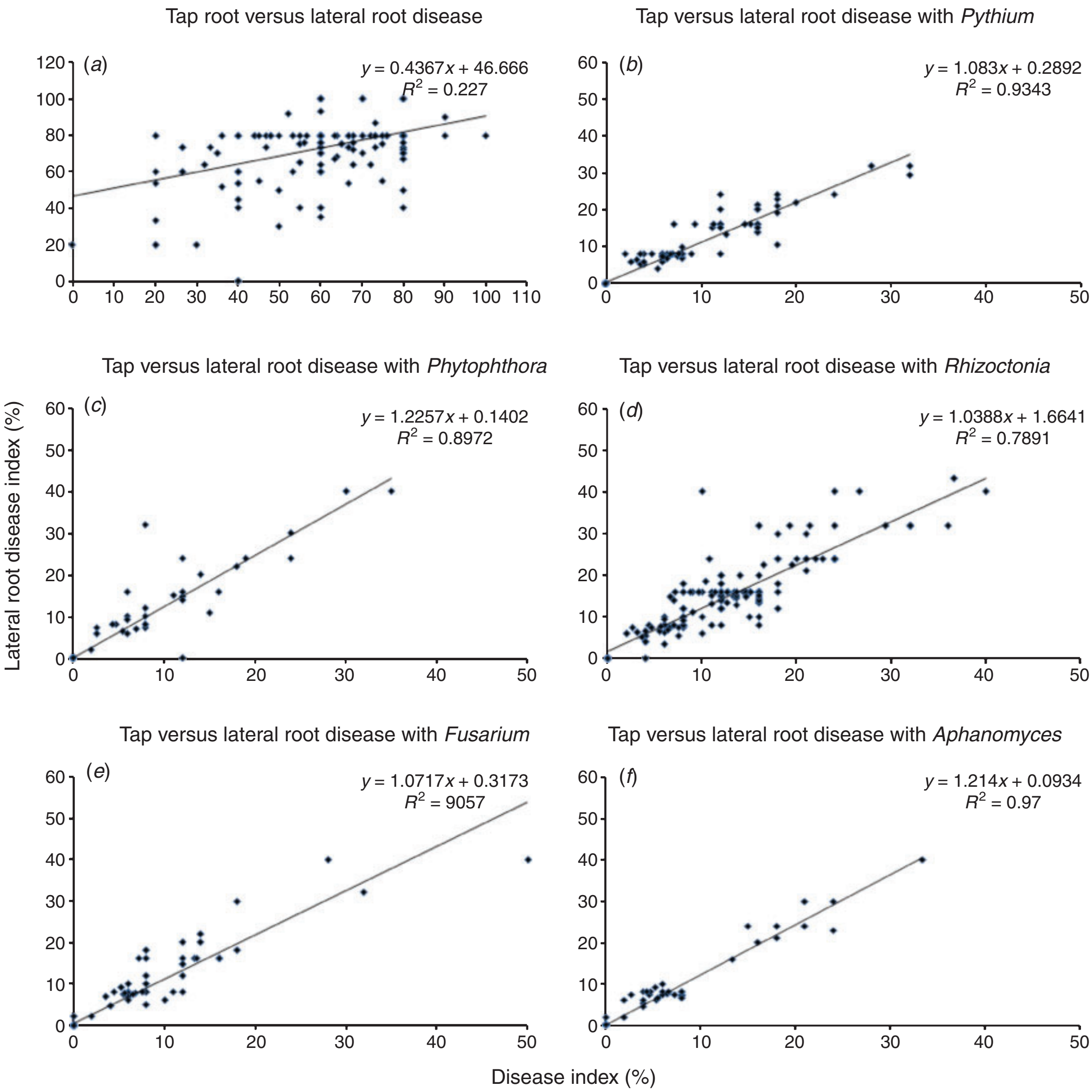

Fig. 7. (*a*) Overall relationship (across all the pathogens) between tap and lateral root disease. Strong relationship between tap and lateral root disease in the presence of (*b*) *Pythium* spp., (*c*) *Phytophthora clandestina*, (*d*) *Rhizoctonia* spp., (*e*) *Fusarium* spp., (*f*) *Aphanomyces trifolii*; where taproot disease increases so does lateral root disease in the presence of the pathogen.

reported by producers showed that the majority (52%) were not able to identify or recall the subterranean clover cultivar growing on their properties. Some 10% of pastures had been sown 40–50 years ago to one subterranean clover variety and had never been renovated or reseeded since (data not shown).

Discussion

This is the first comprehensive study to demonstrate and quantify the severity of soilborne disease in subterranean clover pastures throughout the four states of southern Australia. Of the 202 samples assessed, most not only have a very severe level of taproot rot (disease index of 60–80%), they also have extremely severe lateral root rot disease (disease index 80–100%). It is notable that although the overall relationship between tap and lateral disease was not strong, the relationship was strongly positively expressed within each different pathogen's grouping. As such, this is the first study to highlight the reason for variability in the relationship between tap and lateral root disease as occurs across different field situations where soilborne root disease is a major problem, a consequence of variable pathogen components within the pathogen complexes.

The level of severe root disease found greatly aggravates the challenges producers already face with the autumn–early winter feed-gap period in southern Australia. For subterranean clover pastures, seedling survival and density is a key component of overall plant productivity, and severe seedling root disease adversely impacts forage production from these pastures (Johnstone and Barbetti 1987; Barbetti *et al*. 2005, 2007). It also reduces seed production, persistence (as seed reserves are exhausted), nitrogen fixation and regeneration capacity for subsequent years. It is of concern that despite root disease being very severe, so few producers and experienced agronomists were aware of its occurrence or severity within subterranean clover pastures. Although the expectation was that producers would underestimate and underreport their levels of root disease in the survey, the level of underestimation by producers is of concern. Aboveground symptoms of damping-off and seedling root disease in subterranean clover did vary across different survey samples, likely due to varying components of the different pathogens and possibly environmental (Barbetti and MacNish 1983) and soil nutritional influences, making identification of aboveground disease symptoms challenging. However, this survey and subsequent feedback to participants have focused attention on and heightened awareness of root disease in subterranean clover pastures and its role in pasture decline. Despite this, an urgent need remains to provide similar guidance to other producers and to agronomists and extension personnel.

Tests for the presence of identifiable pathogens revealed a range of major soilborne pathogens widely present, and as would be expected in association with such severe tap and lateral root disease. *Phytophthora clandestina*, *A. trifolii* and *Pythium*, *Rhizoctonia* and *Fusarium* spp. all were found to be widespread across southern Australia. Of these pathogens, *P. clandestina* is considered the most important and most damaging. It was widespread but not universally associated with all diseased samples. It was found in nearly 25% of samples from WA and ~20% of samples from Victoria, but had a lower incidence in SA and least in NSW. This lower incidence of *P. clandestina* observed in NSW is likely due to the crop–pasture rotations, and/or the effects of cultivation *per se*.

There was a high incidence of *Pythium*, *Rhizoctonia* and *Fusarium* spp. across all states. There was also a high incidence of *A. trifolii*, and this is the first study to highlight the incidence of this recently defined and significant contributor to severe root disease of subterranean clover pastures across southern Australian (You *et al*. 2016). Root rot of clover clearly involves a complex of fungi interacting not only with each other but also with the biotic and abiotic environment surrounding them; however, it was noteworthy in the present survey that natural synergistic associations existed in the field between some pathogens. For example, the total incidence of each of *Rhizoctonia* + *Pythium* spp., *Pythium* + *Fusarium* spp., *Pythium* spp. + *A. trifolii* and *P. clandestina* + *A. trifolii* combinations exceeded the individual incidence for each species occurring on its own. This suggests that some of these pathogens have a higher incidence in the presence of one or more other pathogens; this is the first time such preference for 'company' pathogens has been observed for soilborne pathogens within annual pasture systems. That these soilborne pathogens operate as a complex and probably have a preference to operate in that way has historically been known and demonstrated under controlled conditions (e.g. Wong *et al*. 1984, 1986; Barbetti *et al*. 1986*a*) but not previously confirmed under natural field conditions, although widely assumed to be the case (e.g. Barbetti and MacNish 1978). This perhaps explains, for the first time, that whereas several different fungi are normally associated with diseased roots, no single pathogen has been able to reproduce the range of different field disease symptoms observed in different locations (Barbetti *et al*. 2007).

There was no relationship between rainfall zone and either tap or lateral root disease levels. This was generally unexpected, because O'Rourke *et al*. (2009) found a positive correlation in WA between rainfall and taproot disease. Similarly, You *et al*. (2006) showed that the majority and most diverse of *P. clandestina* races occurred in the higher rainfall (700–1000 mm) zone. Together, these earlier findings reinforced the long-held belief that the most severe root disease occurs in higher rainfall zones (Gillespie 1983). Such a conclusion was not surprising, because key oomycete pathogens such as *P. clandestina*, *A. trifolii* and various *Pythium* spp. are all strongly favoured by wet soil conditions (Barbetti *et al*. 2007). However, there can be exceptions to this, as occurred in the present study. Other exceptions where disease severity was independent of rainfall zone include the study of You *et al*. (2006), which also found that *P. clandestina* race 173 occurred across all rainfall zones ≥300 mm and race 177 in all zones ≥400 mm. An important outcome of the present study has been to highlight that this very severe root disease extends throughout high to low rainfall zones of southern Australia and is the major contributor to the widespread decline observed throughout this entire region. This situation is likely fostered by the wide range of pathogens operating as a disease complex, with varying pathogen components prevailing and equally effective across different rainfall zones, dispelling the previous belief that severe root disease in subterranean clover is an issue only in higher rainfall zones.

Smiley *et al*. (1986) demonstrated that removal of seedling leaves to simulate grazing accentuated root rot severity in subterranean clover, suggesting that both the timing and frequency of grazing will affect disease severity. However, there was no evidence to suggest that grazing had any influence on the level of either taproot or lateral root disease. This outcome likely arises because the intensity and timing of grazing could not be accurately assessed qualitatively from the survey data sheets, with some producers reporting grazing as 'lightly grazed', 'strategically grazing', 'rotationally grazed, or 'sometimes grazed'. These different 'forms' of grazing management will have exerted different influences on the levels of root rot disease in the samples submitted. Grazing is known to exacerbate the effects of root disease, and the recovery of subterranean clover pastures from root rot following grazing can be extremely poor (M. J. Barbetti, unpubl. data). Hence, it is likely that the timing and extent of grazing could be manipulated in ways to reduce the impact of disease, especially at the early seedling stages, and further investigation is warranted.

Nodulation levels recorded on the seedlings in the present study (nodulation indices of 20–40%) were far below that considered ideal (Roughley *et al*. 1970). Even despite the high percentage (>90%) of pasture being renovated in NSW with *Rhizobium*-inoculated seed, nodulation was still poor. Under normal growing conditions, subterranean clover plants would be expected to nodulate by 6–10 weeks after germination (Roughley *et al*. 1970). Jones and Curnow (1986) also found poor nodulation of subterranean clover in Victoria where root rot was present, with only 40% of plants effectively nodulated at 8–10 weeks after germination. Similarly, in WA, a very strong negative correlation exists between root disease and level of nodulation (M. J. Barbetti, unpubl. data). Possible reasons for the negative interaction between root rot and nodulation include a reduction in root growth from disease, resulting in less root area for signalling of nodulation for the nodule-forming bacteria and/or fewer lateral roots to form nodules, and/or possible competition between the pathogens and rhizobia in the soil and rhizosphere. In relation to the last point, Hosseini *et al.* (2014) proposed that legume pathogens may have evolved mechanisms to recognise the chemical signals that *Rhizobium* use to establish symbiosis, and this helps in pathogenesis. This is another area warranting further investigation.

Most producers were unaware of the subterranean clover varieties sown or remaining in their paddocks, and this represents a significant challenge for all in trying to manage root disease. Further, discussions with consultants and agronomists at field days across southern Australia over the last 3 years indicate that almost none were in a position to be able to confidently advise producers on the varieties in the their pastures. Naturalised variants of subterranean clover cultivars were also found locally (from natural crossing events and/or mutations) and a proportion of divergent types were noted in pasture samples submitted, these being particularly difficult to identify in the field.

An important finding of the present study was that occasional subterranean clover stands remained productive over 40–50 years. This enhances the already existing evidence that natural selection for disease resistance to root pathogens has been taking place in at least some situations in southern Australia. These persisting isolated and apparently more productive, older clover cultivars (and/or their naturalised strains) appear to show general field tolerance against soilborne diseases that reduces the disease impact. To date, no study has examined the level of disease resistance *in situ* in populations of subterranean clover in southern Australia, despite this offering a rapid alternative means for improving the overall success of subterranean clover, as has previously been proposed by Gladstones and Collins (1983). These particular paddocks and samples identified in the present study offer a valuable resource for identifying both improved disease resistance against individual soilborne pathogens (or combinations), and general field tolerance against soilborne diseases. Both are likely to have developed naturally because subterranean clovers pastures were first established many decades ago.

Subterranean clover is known for high fertility demand (phosphorous and sulfur), and root rot disease is often less pathogenic on vigorously growing plants (O'Rourke *et al.* 2012). It is likely that the reported levels of nutrition applied by producers in each year of the survey as 'maintenance' were often suboptimal, resulting from need to reduce farm expenses. There is previous evidence that low soil fertility contributes to loss of subterranean clover from root-rot affected pastures (Hochman *et al.* 1990). Other recent studies across southern Australia have noted large responses in growth of subterranean clover from supply of plant nutrients additional to those currently available on farms or being applied by producers (M. P. You, M. J., Barbetti, unpubl. data).

Except in NSW, where phase farming systems often require the pasture to be resown following each cropping phase, many of the pasture-renewal benefits offered by newer cultivars have not been realised on-farm in the southern states. Economic considerations have to be borne in mind in renovating older, more susceptible pastures (the main barrier to adoption is often the perceived cost). However, the losses and associated costs from root rot diseases clearly have not been recognised by most producers, consultants or agricultural industry bodies. Yet, root disease poses a significant and insidious economic loss of production and carrying capacity and now appears to be the overriding driver of the widespread and ongoing pasture decline across southern Australia.

In the present study, the severity of the disease was generally independent of pathogen complex composition, geographic location and annual rainfall. Immediate options for management include deployment of available host resistances to prevailing pathogens in different geographic areas, application of fungicides, and manipulation of on-farm cultural practices known to be effective (e.g. cultivation for reducing high disease levels, reduced grazing pressure to allow recovery of diseased seedlings). Differences in soil nutrient content have also been shown to influence root rot disease (O'Rourke *et al.* 2012). Lower incidence of *P. clandestina* was found in samples from NSW in the crop–pasture zones where producers were inoculating their seed with *Rhizobium* when resowing after a cereal phase. Smiley *et al.* (1986) demonstrated that root rots could be reduced by treatment of legume seeds with *Rhizobium* or cultivation *per se*. However, for long-term sustainable management across southern Australia where composition of the pathogen complex is highly variable, there is a need to identify general field tolerance in subterranean clover germplasm to a wide cross-section of the pathogen complex as the basis for developing new cultivars with general field tolerance for building durable resistance. The existence of such field tolerance in subterranean clover genotypes has already been confirmed (M. J. Barbetti, unpubl. data). Knowledge and expertise to confidently identify root disease, understand disease-management options, and develop improved disease resistance for all future subterranean clover cultivars are critical gaps needing attention amongst consultants and researchers. This, combined with the fact that most producers (especially in SA, Victoria and WA) were not able to identify accurately or recall the cultivar(s) they are currently growing, may hinder progress in developing effective management strategies. There is also evidence of an overall increase in the proportion of high-oestrogenic and hardseeded clovers such as Dwalganup and Dinninup in formally 'oestrogenically safe' pastures.

Clearly, severe root disease is not limited to particular high-rainfall zones as previously understood, but rather is now a serious problem and challenge across all agro-geographical zones where subterranean clover is grown. The subterranean clover pastures have an assured place in our farming systems; however, recent dry years combined with severe levels of root rot disease have shown the fragility of the pasture farming systems to predicted climate change. The present survey highlights the poor nodulation of subterranean clover plants, not only in permanent pastures but also in pastures within the crop–pasture rotation zones of NSW. Although root disease, poor nodulation and suboptimal nutrition all may be factors contributing to decline in subterranean clover productivity and persistence in pastures across southern Australia, the critical driver is clearly root disease. Not only are plants with root disease inherently unproductive, but severe root disease prevents adequate nodulation or uptake of water and nutrients. Further, there is a strong negative correlation between extent of nodulation and the severity of either taproot or lateral root disease (M. P. You and M. J. Barbetti, unpubl. data). There is need for a comprehensive industry extension plan covering all key aspects of root disease and its management through workshops and winter field days in each state. This must be underlain by identification of host resistance to the individual soilborne pathogens and, in particular, identification of general field tolerance within subterranean clover genotypes. Finally, this study has established a valuable benchmark for current prevalence and distribution of root disease.

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