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PRATYLENCHUS AND *RADOPHOLUS* SPECIES IN AGRICULTURAL SOILS AND NATIVE VEGETATION IN SOUTHERN AUSTRALIA

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Summary

RILEY, I. T. & WOUTS, W. M. (2001) *Pratylenchus* and *Radopholus* species in agricultural soils and native vegetation in southern Australia. *Trans. R. Soc. S. Aust.* **125**(2), 147-153, 30 November, 2001.

Pratylenchus species were found in 105 and *Radopholus* species in five of 284 samples taken from agricultural soils and native vegetation in areas of southern Australia. *Pratylenchus crenatus* (2 samples), *P. neglectus* (80), *P. penetrans* (3), *P. scribneri* (1), *P. teres* (10), *P. thornei* (13), *Radopholus nativus* (4) and *R. crenatus* (1) were identified. *Pratylenchus teres* has not previously been recorded in Australia and its widespread occurrence in agricultural soils in Western Australia may have important implications for crop production. Morphometrics and diagnostic features for *P. teres* are presented to facilitate its distinction from the morphologically similar *P. thornei*.

KEY WORDS: Nematoda, *Pratylenchus*, *Radopholus*, distribution, species diversity, *Pratylenchus teres*.

Introduction

Pratylenchus Filipjev, 1936 consists of migratory endoparasitic nematodes that feed in the roots of plants and are important pests of dryland agriculture in southern Australia. *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941 and *P. thornei* Sher & Allen, 1953 have been identified as important pest species in south-eastern Australia and have been the subject of much research since the late 1980s (Vanstone 1991[1]; Taheri *et al.* 1994; Farsi *et al.* 1995; Potter *et al.* 1998; Vanstone *et al.* 1998; Nicol *et al.* 1999; Taylor *et al.* 1999; Hollaway *et al.* 2000). In response to the findings of this research, interest developed in determining the significance of *Pratylenchus* in Western Australia (WA). This prompted an extensive survey of the wheat growing areas of that State (Riley & Kelly in press). This survey revealed that potentially yield-limiting populations of *P. neglectus* and *P. thornei* occurred in much of the WA wheatbelt. In addition, the study found an unexpectedly high level of *Pratylenchus* species diversity. Although *P. neglectus* was most commonly detected, populations identified as *P. brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941, *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941, *P. scribneri* Steiner in Sherbakoff & Stanley, 1943, *P. thornei*, *P. zeae* Graham, 1951 and an undescribed species similar to *P. thornei* were also found. Concurrently with this survey, *Radopholus nativus* Sher, 1986 was found in 10 of 300 diagnostic samples with migratory endoparasitic nematodes (Riley & Kelly 2001), further highlighting the diversity of migratory endoparasites in cropping areas of WA.

The species diversity in WA has significant ramifications because, until now, all efforts to establish resistance of crop species and cultivars grown in southern Australia have been limited to *P. neglectus* and *P. thornei* (Taylor *et al.* 2000; Hollaway *et al.* 2000). Also DNA based quantification of root lesion nematodes in cropping soils, provided initially by the South Australian Research and Development Institute (SARDI) and now by C-Qnetec Diagnostics (a division of Aventis CropScience) is restricted to *P. neglectus* and *P. thornei*. The work of Taylor, Hollaway and their coworkers has already shown that resistance to either *P. neglectus* or *P. thornei* does not always provide resistance to the other (Taylor *et al.* 2000; Hollaway *et al.* 2000). This means that successful management of *P. neglectus* and *P. thornei* could be undermined by a shift to predominance of other *Pratylenchus* species for which the crops grown are not resistant. It is, therefore, important that in population monitoring all *Pratylenchus* species occurring in cultivated fields and native vegetation in agricultural areas are identified, either by conventional diagnosis or DNA tests, so that effective options can be determined for sustainable management.

Taxonomists examined only a limited quantity of material from the earlier survey in WA (Riley & Kelly in press). Combined with the limited number and nature of surveys for *Pratylenchus* in south-eastern Australia, this means that the diversity of species of *Pratylenchus* in southern Australia is largely unknown. For the present study soil and root samples were therefore collected in areas of southern

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[1] VANSTONE, V. A. (1991) The role of fungi and the root lesion nematode, *Pratylenchus neglectus*, in damaging wheat roots in South Australia. PhD thesis University of Adelaide (unpub.).

Australia for the extraction of *Pratylenchus* spp. and their identification by detailed morphological examination and morphometrical comparison, and to provide additional information on geographical distribution. The results are presented and discussed below.

Materials and Methods

Soil and root samples were obtained in dryland cropping areas of the southern States of mainland Australia in September and October, 1999. In South Australia (SA), 173 samples were collected from 49 sites and in WA, 102 samples from 38 sites. Sites were generally cultivated fields with adjacent native vegetation. Thirty five per cent of the samples from SA and 48% from WA were collected from cultivated fields. Samples from cultivated soils were composites of about six subsamples of roots and soil to 100 mm deep and samples from native vegetation were mostly collected adjacent to single plants. Sites in SA were selected along public access routes providing reasonable coverage of the main wheat growing regions viz., Murray Mallee, Mid North, Yorke Peninsula and Eyre Peninsula. In WA, a proportion of the sites visited had been identified previously as potentially having species other than *P. neglectus* and *P. thornei*; other samples were collected in areas where the greatest species diversity was known to occur. A further nine samples from eight sites from cropping areas in Victoria (Vic.) were provided by G. Hollaway (Agriculture Vic.).

Nematodes were extracted from soil by wet sieving (45 µm) and sugar flotation (Wouts & Sher 1971) and from roots in a misting cabinet (Southey 1986). Nematodes were heat killed, fixed in formalin and mounted in glycerol for microscopic examination (Wouts & Sher 1971).

Results

Pratylenchus species were found in 105 samples and included *P. crenatus* Loof, 1960, *P. neglectus*, *P. penetrans*, *P. scribneri*, *P. teres* Khan & Singh, 1975 and *P. thornei* (Table 1). Some populations could not be identified to species level because of lack of adults or obscured characters. Although some *Pratylenchus* species were found in native vegetation, most were present in cultivated soils associated with field crops, pasture or weeds. In SA, where native vegetation was more thoroughly sampled, three of the four species collected were also found in these less disturbed habitats.

Pratylenchus crenatus was found in only two samples both from wheat fields near Westmere and Willaura, Vic. These localities are in a 600-700 mm rainfall zone, a zone not sampled in WA and SA.

Pratylenchus neglectus was the most common species in SA, being found in 95% of the *Pratylenchus* populations sampled in that State. Although *P. neglectus* is considered to be the most common species in cropping areas of WA (Riley & Kelly in press), our sampling purposefully focused on areas where this species was known to be less common, so *P. neglectus* was found in only 30% of *Pratylenchus* populations sampled in WA. *Pratylenchus neglectus* was found in most crops including some that are considered poor or non hosts viz., field pea, lupin and vetch (Taylor *et al.* 2000). *Pratylenchus neglectus* was also found in Vic.

Pratylenchus penetrans was found in native vegetation at one site in SA and in a narrow-leafed lupin crop (*Lupinus angustifolius* L.) and associated weedy brassica in WA.

Pratylenchus scribneri was found in only one sample of barley roots from SA, but there were few specimens and the identification is somewhat uncertain.

Pratylenchus teres was found only in WA where it was the most common of the species collected (40% of populations). It was found in association with a broad range of plant species viz., canola, native plants, oat, pasture plants, various weeds and wheat. Given that this is a new record for Australia, measurements are provided (Table 2) for comparison with earlier descriptions and diagrams to show (Fig. 1) some difference from *P. thornei*, the species it most closely resembles.

Pratylenchus thornei was found in the three States, mostly in cropping soils but also in native vegetation in SA. Notably, it was collected in association with field pea and lentil, both crops considered to be resistant (Hollaway *et al.* 2000). This may represent carryover from the previous season. *Pratylenchus thornei* was found in a relatively minor proportion (7%) of *Pratylenchus* populations in SA, where samples were collected more randomly. In WA, about 24% of samples had *P. thornei* but this is likely to reflect the different sampling criteria.

Mixed populations of *P. neglectus* and *P. thornei* were found in 6 samples (4 sites) from SA and 2 samples from WA. Therefore more than half the *P. thornei* populations detected occurred in conjunction with *P. neglectus*. Apart from the uncertain record of *P. scribneri*, which was associated with *P. neglectus*, none of the other species was found in mixed populations.

Heterodera avenae Wollenweber, 1924 males were also extracted from wheat and barley root systems from 12 sites in SA. In all cases, they occurred in association with *P. neglectus* and in one case with a mixed population of *P. neglectus* and *P. thornei*. *Heterodera avenae* was not found in WA. This is consistent with the finding of Riley & Kelly (in

TABLE 1. *Species of* Pratylenchus *found in association with crops, pasture, weeds and native vegetation in southern states of mainland Australia, indicating the number of samples and associated plants for each species.*

Pratylenchus species	Australian State					
	South Australia		Western Australia		Victoria	
	Samples	Plants	Samples	Plants	Samples	Plants
P. crenatus	0		0		2	wheat
P. neglectus	71	barley, canola, lupin, native, oat, pea, vetch, wheat	8	mixed pasture, oat, weeds, wheat	1	wheat
P. penetrans	1	native	2	lupin, weedy brassica	0	
P. scribneri	?1[2]	barley	0		0	
P. teres	0		10	canola, native, oat, pasture, weeds, wheat	0	
P. thornei	5	native, pea, wheat	6	lupin, oat, wheat, weedy brassica	2	lentil, wheat
Pratylenchus sp.	3	native, vetch	2	wheat, weeds	0	
Total samples with *Praylenchus*[1]	75		25		5	

[1]Populations of mixed *Pratylenchus* spp. were found in some samples. [2]Identity uncertain.

TABLE 2. *Morphometrics of* Pratylenchus teres. (Measurements in µm).

	Western Australia	Khan & Singh, 1975			van den Berg & Quénéhervé 2000
		Paratypes	Amritsar	Solan	
	n = 10 Mean ± SD (Range)	n = 5 Mean (Range)	n = 17 Mean (Range)	n = 4 Mean (Range)	n = 8 Mean ± SD (Range)
Total length (L)	580 ± 40 (490-620)	410 (400-420)	550 (420-630)	550 (520-600)	504 ± 18.2 (472-531)
Width of body	25 ± 1.9 (21-28)	-	-	-	18, 17
Length of stylet	16.6 ± 0.54 (15.5-17.0)	17 (17-18)	16 (16-18)	17 (17-18)	18 ± 0.4 (17-18)
Height of stylet base	2.2 ± 0.23 (2.0-2.5)	-	-	-	2
Width of stylet base	4.6 ± 0.44 (4.0-5.5)	-	-	-	3.2
Width of first body annule	9.7 ± 0.46 (9.0-10.5)	-	-	-	-
Distance of dorsal gland opening from stylet base	2.8 ± 0.51 (2.0-3.5)	-	-	-	3
Length of oesophagus	86 ± 6.6 (79-98)	-	-	-	-
Distance of secretory-excretory pore from anterior end	89 ± 4.5 (79-95)	-	-	-	84, 85
Distance the oesophageal glands overlap the intestine	44 ± 20.4 (11-73)	-	-	-	-
Length of posterior uterine branch	18.6 ± 3.3 (14-24)	-	-	-	36 ± 7.5 (33-40)
Length of tail	38 ± 2.4 (34-42)	-	-	-	34.5 ± 2.8 (31-39)
Length of the clear part of the tail	4 ± 0.7 (3-5)	-	-	-	-
Body width at the position of the vulva	23 ± 1.8 (20-26)	-	-	-	-
Body width at the position of the anus	16 ± 1.5 (14-18)	-	-	-	-
a	24 ± 2.6 (21-30)	21.7 (21.1-23.3)	30.8 (22.1-39.9)	29.5 (28.8-30.7)	30 ± 0.9 (29-31)
b	6.8 ± 0.46 (6.2-7.4)	4.1 (4.1-4.2)	4.6 (3.5-5.6)	4.6 (3.9-5.5)	4
c	15.5 ± 1.29 (12.2-16.7)	14 (14-16)	18.2 (11.5-27.0)	16.5 (14.8-17.9)	14.5 ± 1 (13-16)
c´	2.4 ± 0.24 (2.1-2.9)	-	-	-	3
V	74 ± 2.2 (68-76)	73 (70-77)	70 (69-78)	73 (72-75)	72 ± 2 (69-74)

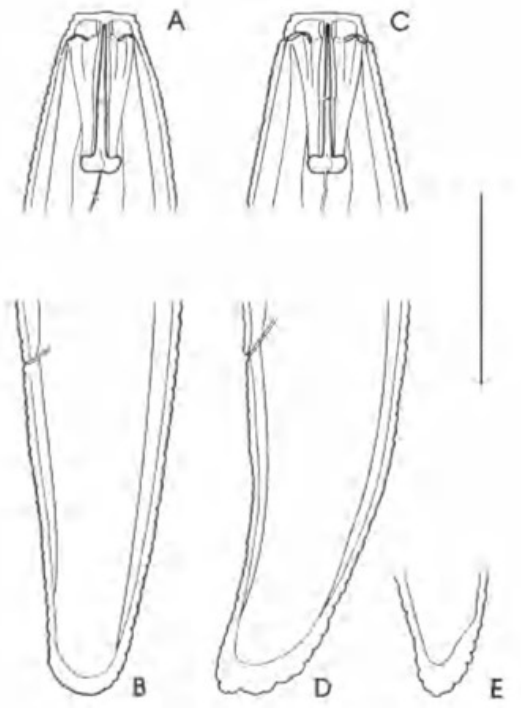

Fig. 1. A, B. *Pratylenchus thornei*. A. Anterior end. B. Posterior end. C-E. *Pratylenchus teres*. C. Anterior end. D. Posterior end. E. Tail terminus variation. Scale bar = 20 µm.

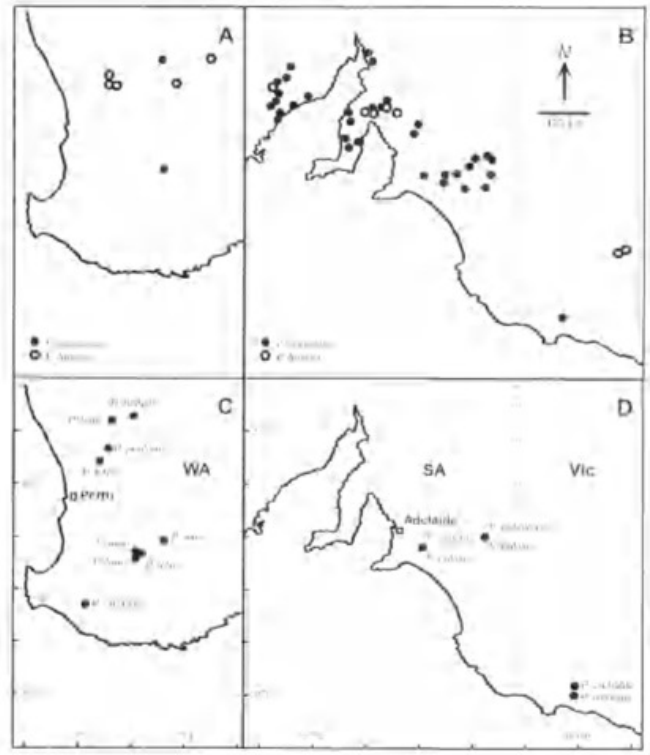

Fig. 2. Distribution of *Pratylenchus* and *Radopholus* species collected in southern Australia. A, B. *Pratylenchus neglectus* and *Pratylenchus thornei*. A. In WA. B. In SA and Vic. C, D. Other species. C. In WA. D. In SA and Vic.

press) that *H. avenae* is not common in that State.

Radopholus nativus and *Radopholus crenatus* Colbran, 1971 were found in native vegetation; two samples each of *R. nativus* in SA and WA and one sample of *R. crenatus* in WA. One *R. nativus* population from SA occurred in association with *P. neglectus*. Although *R. nativus* was not found in cropping soils, as reported by Riley & Kelly (2001), a small number of *Radopholus* juveniles was found at the same site near Wyalkatchem that they had investigated. This site was dominated by capeweed (*Arctotheca calendula* (L.) Levyns) and a small proportion of grasses (such as *Lolium rigidum* Gaudin, *Hordeum leporinum* Link and *Bromus* sp.) in 1999. It appears that capeweed and these grasses are not hosts for either *P. neglectus* nor *R. nativus*, which were absent or scarce in the eleven samples collected at the site. This observation is consistent with the findings of Vanstone & Russ (2001a, b), who have shown the plants species found at this site to be largely resistant to *P. neglectus*.

Figure 2 shows the geographic distribution of the *Pratylenchus* spp. and *Radopholus* spp. collected. In WA, species other than *P. neglectus* occurred toward the west and south where annual rainfall is higher. In SA and Vic., *P. neglectus* was widespread and, although less common, *P. thornei* occurred throughout most of the area sampled. The other species present in eastern SA were mostly in native vegetation. *Pratylenchus crenatus* occurred in cropping soils of a high rainfall area of Vic.

Discussion

This study confirms the diversity of *Pratylenchus* species in WA cropping soils (Riley & Kelly in press) and the relative lack of diversity in SA (Nicol 1996[2]). A predominance of *P. neglectus* and/or *P. thornei* in cereal soils is consistent with that in other countries with climates similar to southern Australia, for example South Africa (Jordaan *et al.* 1992) and Italy (Palmisano 1992). In Portugal, however, *P. penetrans* and *P. crenatus* were more common in cereals and other crops than *P. neglectus* and *P. thornei* (de O. Abrantes 1987). Similarly, in other climatic zones, other *Pratylenchus* species have become predominant in cereal crops, for example *P. scribneri* is predominant in Arkansas, USA (Robbins *et al.* 1989) and *P. penetrans* in Prince Edward Island, Canada (Kimpinski *et al.* 1989).

While *P. neglectus* and *P. thornei* may be the most common species in cereal producing areas of

[2] Nicol, J. M. (1996) The distribution, pathogenicity and population dynamics of *Pratylenchus thornei* on wheat in South Australia. PhD thesis University of Adelaide (unpub.).

Australia and similar areas worldwide, some authors report wide species diversity as seen in WA. For instance, Jordaan *et al.* (1992) found *P. brachyurus*, *P. penetrans* and *P. zeae* along with *P. neglectus* and *P. thornei* in wheat fields in winter rainfall areas in South Africa and Potter & Townshend (1973) found *P. crenatus*, *P. neglectus* (most common), *P. penetrans* and *P. pratensis* (de Man, 1880) Filipjev, 1936 in cereal soils in Ontario, Canada. In moister, more agriculturally diverse environments species diversity can be even greater; nine species were reported from field soils of eastern Germany (Decker & Dowe 1974) and 14 species in eastern Canada (Townshend *et al.* 1978).

Pratylenchus teres has not previously been recorded in Australia. However, in the earlier survey of WA, Riley & Kelly (in press) found an unidentified species similar to *P. thornei* with affinities to *P. teres* or *P. fallax* (M. Hodda, pers. comm., 1998), which was probably the *P. teres* as identified in this study. *Pratylenchus teres* identified here closely fits the original description of the species (Khan & Singh 1975) and material from the French West Indies recently described by van den Berg and Quénéhervé (2000) (Table 2). The WA specimens seem to be somewhat longer, but fit within the range for the stylet length and the a and c values. The b value is considerably higher (6.2-7.4 v. 4.1-5.5) but this may be due to distortion of the oesophageal region in several of our specimens which may have moved the base of the oesophagus somewhat anteriad resulting in measuring inaccuracies. The greatest discrepancy seems to be the length of the posterior uterine sac which in the original description as well as by van den Berg and Quénéhervé, is reported as about twice as long as in our material. *Pratylenchus teres* closely resembles *P. thornei* in body, stylet and tail length, the shape of the lip region and the stylet knobs and the position of the vulva (Fig. 1). *Pratylenchus teres*, therefore, could be confused with *P. thornei*, especially in areas where the latter is common. The annulated tail, the main character separating the two species, is quite variable and specimens with only a light crenation on the tail could be identified as *P. thornei* with slight markings on the tail terminus, a characteristic not uncommon in that species. Generally though when material is plentiful, the difference between the two species is obvious with *P. teres* having a more pointed crenate tail. It was further observed that the lip region of *P. teres* is about one micron wider than the lip region of *P. thornei*. This character may be difficult to measure but in direct comparison is immediately apparent. Also the lip region of *P. teres* is more set off and the cephalic framework extension shorter than in *P. thornei*.

Although not all the species previously found in dryland cropping soils of WA (Riley & Kelly, in press) were collected, the addition of *P. teres* to the list is significant. As *P. teres* was the most common species collected in WA and occurred in a variety of crops and native vegetation, it should be given priority for further investigation. As indicated above, work on *Pratylenchus* in southern Australia has concentrated on *P. neglectus* and *P. thornei* with differences in host range, distribution and impact being found. It is likely that *P. teres* will differ from both of these and crop management strategies designed to control *P. neglectus* and *P. thornei* may be undermined by *P. teres*. Since its description from mustard in 1975 there have been relatively few reports of *P. teres* and studies of its biology or agricultural significance. There is, therefore, no information from which to predict its importance in WA.

The detection of *P. penetrans* in WA is notable because the lupin roots were exceptionally heavily infested at this site and the preceding wheat crop had also been heavily infested (S. Kelly, pers. comm. 2000). Narrow-leafed lupin is considered to be resistant to *P. neglectus* (V. Vanstone, pers. comm. 2000), the only lupin/*Pratylenchus* combination assessed, so it appears that this resistance is not general for all *Pratylenchus* spp. The occurrence of *P. penetrans* in lupin, wheat and brassicas is also important as it indicates that it may not be easily controlled by crop rotation (especially if its host range includes the common cereal, legume and brassica crops). *Pratylenchus penetrans* has been recorded widely in all Australian States, largely in higher rainfall areas and/or associated with perennial crops (McLeod *et al.* 1994) but also in association with lupin in Queensland (Qld) and brassicas in various States.

Pratylenchus scribneri has been identified recently in samples from cropping soil in WA (Riley & Kelly in press), but earlier records in Australia are now considered to be *Pratylenchus jordanensis* Hashim, 1984 (McLeod *et al.* 1994). Further collections of *P. scribneri* in Australia are required to confirm its presence.

Pratylenchus crenatus was found only in a high rainfall area of Victoria, which lies outside the main cropping areas of southern Australia. It has been recorded in other Australian States in high rainfall areas and mostly in association with perennials (McLeod *et al.* 1994). With the marked increase in annual cropping associated with the relative decline in returns from grazing enterprises in such areas, it is possible that *P. crenatus*, along with other *Pratylenchus* species, will emerge as important pests.

The collection of *R. nativus* from native vegetation in SA is also noteworthy. This species has been recognised as a potential agricultural pest in WA and,

although less common than some *Pratylenchus* spp., it is found widely distributed (Riley & Kelly 2001). If particular factors, such as high frequency of lupin cropping, are confirmed to contribute to the dominance of *R. nativus* over *Pratylenchus* under certain circumstance in WA, a search based on this information may also find *R. nativus* in agricultural soils in SA.

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