Pratylenchus thornei: The Relationship Between Presowing Nematode Density and Yield Loss in Wheat and Barley

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ABSTRACT

The root lesion nematode *Pratylenchus thornei* causes economic losses in wheat and barley internationally through both reduced grain yield and grain quality. This study investigated the relationships between the presowing *P. thornei* density and grain yield and the postharvest nematode densities. Four field experiments were conducted at the same site between 2010 and 2014. A range of presowing *P. thornei* densities was established in the first year by growing three cereal cultivars that ranged from resistant to susceptible. In the following year, plots were sown with the five same cereal cultivars. A linear relationship was observed between the natural log of the presowing *P. thornei* density and grain yield across all seasons. The results showed that grain yield losses varied between cultivars and seasons. The importance of season was significant, with this study conducted over several seasons, and it highlighted the variability in yield losses between seasons, which will

need further investigation. The greatest yield losses observed were 25 to 28% when the maximum presowing *P. thornei* densities ranged between 150 and 250 *P. thornei* g of soil^{−1}. An analysis of the relationship between the presowing and postharvest nematode densities revealed that increased presowing nematode densities resulted in decreased multiplication rates in all seasons and in all cultivars. Nematode multiplication rates also varied between seasons. These results explain why it is difficult to predict nematode levels based on cropping history, and additionally, they highlight the importance of growing resistant cultivars to maintain low levels of *P. thornei* to minimize risk of yield losses.

Keywords: disease control and pest management, cereal resistance, equilibrium point, nematology, population biology, *Pratylenchus thornei*, root lesion nematode, yield loss

The root lesion nematode *Pratylenchus thornei* causes worldwide grain yield losses in wheat (*Triticum aestivum*) (Mokrini et al. 2016; Ortiz-Monasterio and Nicol 2004; Smiley et al. 2005; Thompson et al. 2017). These nematodes are migratory polycyclic endoparasites that feed on and within the roots of plants, affecting root function and crop performance (Moens and Perry 2009; Thompson et al. 2012; Vanstone et al. 2008). In Australia, *P. thornei* has been estimated to cost the wheat and barley (*Hordeum vulgare*) industries \$50 and \$8 million per annum, respectively (Murray and Brennan 2009, 2010).

Reported yield losses caused by *P. thornei* are variable across the grain-growing regions of Australia. In northern Australia, yield loss caused by *P. thornei* in wheat crops has been well established, with losses of up to 65% reported (Mills and Sheppard 1998; Thompson et al. 1999). However, most studies have measured differences in the grain yield of wheat cultivars exposed to high densities of *P. thornei* to select for tolerance to the nematode (Thompson 2008; Thompson et al. 1993, 1995, 1999, 2008, 2012). In other regions of Australia, there are limited reports of yield loss caused by *P. thornei* in wheat, and where losses have been reported, they are generally less than those reported in northern Australia (Vanstone et al. 2008).

A range of approaches has been used to assess yield loss caused by root lesion nematodes in field crops. Several studies selected

fields with high nematode densities and applied nematicides, such as aldicarb, or biocides, such as methylbromide, to suppress the nematodes in selected plots or blocks, and then, they used the yield difference between the treated and untreated areas to determine the losses caused by nematodes (Doyle et al. 1987; Eastwood et al. 1994; Smiley 2009; Smiley et al. 2014; Taylor and McKay 1993; Taylor et al. 1999; Thompson et al. 1993, 1995, 2012). Nematicides are rarely used as research tools because of their high mammalian toxicity. In drier years, it has also been hypothesized that nematicides may only reduce nematode densities in the upper soil layer and not have any influence at depth (Thompson et al. 1999), thus underestimating potential yield losses.

An alternative method to assess yield losses is to measure the grain yield of the test cultivars grown in the presence of high and low presowing nematode densities of the target nematode that were established in the previous season by growing resistant and susceptible crops (Nicol et al. 1999; Owen et al. 2014; Reen et al. 2014; Smiley and Machado 2009). Both Owen et al. (2014) and Reen et al. (2014) used this method to examine the relationship between the presowing *P. thornei* densities and grain yield of wheat, with yield losses of 0.44 and 0.09 t/ha reported with increases of 0.5, ln(presowing *P. thornei*/kilogram of soil + 500) and ln(presowing *P. thornei*/kilogram of soil + 1), respectively. The combination of these methods has been previously reported in sugar beet. The relationships between yield losses and nematode densities were extensively studied in a range of crops by Seinhorst (1965, 1967, 1972), and the method of growing resistant and susceptible crops in rotation to differentiate nematode densities within field experiments was successfully used with sugar beet cyst nematode (*Heterodera schachtii*) (Kenter et al. 2014 and Schmidt et al. 1993). These alternative methods have the advantage of manipulating nematode densities throughout the soil profile; however, it is important that the crops or cultivars selected have minimal differential effects on residual soil water and nutrition (e.g., nitrogen), which will confound the measurement of the nematode

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effect on yield (Angus et al. 2015; Armstrong et al. 2019; Hannah and O’Leary 1995; Owen et al. 2014; Reen et al. 2014).

Resistance to nematodes is defined as the ability of a plant to prevent nematode reproduction compared with tolerance, which is defined as the ability of a plant to grow and yield well in the presence of nematodes (Cook and Evans 1987). Resistance levels to *P. thornei* vary between crops and cultivars (Dababat et al. 2019; Fanning et al. 2018; Hollaway et al. 2000; Mokriani et al. 2018; Sheedy et al. 2015). Resistance to nematodes is usually assessed by quantifying initial (*Pi*) and final (*Pf*) nematode densities in a field plot or pot in a controlled environment and ranking cultivars based on their nematode multiplication rate or reproduction ratios (*Pf/Pi*) (Fanning et al. 2018; Hollaway et al. 2000; Keil et al. 2009; Rodda et al. 2016; Sheedy and Thompson 2009; Taylor et al. 2000) or by ranking based on final nematode densities (Hollaway 2002; Schmidt et al. 2005; Sheedy et al. 2015).

A range of methods has been used to assess the resistance of field crops to *Pratylenchus* species, with most studies conducted under controlled environmental conditions (Keil et al. 2009; Sheedy and Thompson 2009; Sheedy et al. 2015), using field based approaches (Ballard et al. 2006; Dababat et al. 2019; Fanning et al. 2018; Hollaway et al. 2000; Taylor et al. 2000), and combining data collected under both controlled environment and field conditions (Rodda et al. 2016; Thompson et al. 1999). Usually, field studies are completed using a single naturally occurring nematode density that can be variable (Ballard et al. 2006; Hollaway et al. 2000; Taylor et al. 2000). Fanning et al. (2018) in their study assessed field resistance in the presence of high and low initial nematode densities that were established in the previous year using cereals with contrasting resistance/susceptibility to the target nematode and found that higher multiplication was associated with the lower initial nematode densities. This finding is consistent with the findings of Ferris (1985) who, in the studies of the nematode *Meloidogyne incognita*, also found higher multiplication rates with lower initial nematode densities.

Nematode densities can be quantified by extracting nematodes from soil samples and visually identifying and quantifying them (Sheedy and Thompson 2009; Sheedy et al. 2015; Smiley et al. 2014) or by measuring DNA concentration in soil using quantitative PCR (qPCR) tests (Ophel-Keller et al. 2008; Sheedy et al. 2015). In Australia, growers have access to a commercial PREDICTAB soil test (Ophel-Keller et al. 2008). The results from these tests can be used to inform the choice of management strategies to minimize yield losses.

To interpret results based on nematode counts or DNA concentration in soil, it is important to understand the relationship between nematode density and risk of yield loss. Growers can then make informed decisions on appropriate management strategies to implement, and plant breeders and research agencies can allocate resources to develop new resistant cultivars or other appropriate management strategies. However, to develop relationships between preplanting nematode density and potential yield loss, it is important that more than two initial nematode densities (i.e., high and low) are used (unless the relationship is known to be linear). Only then can the effect of an initial nematode density on yield loss be predicted.

This study used a 2-year approach to experimental design. In the first year, a range of nematode densities were established by sowing crops that had differential resistance/susceptibility to *P. thornei* but

were unlikely to have differential effects on potentially confounding variables such as soil, water, and nutrition. In the second year, five test cultivars were sown into the established range of nematode densities with the aims to

- I. Determine the relationship between presowing *P. thornei* densities determined through a DNA-based test (PREDICTAB) and grain yield for a set of commercial wheat and barley cultivars,
- II. Explore the consistency of the relationship between presowing *P. thornei* densities and grain yield for the set of cultivars across several growing seasons, and
- III. Measure the multiplication of *P. thornei* for the set of cultivars in the presence of differing presowing nematode densities.

MATERIALS AND METHODS

Field site. Four 2-year field experiments were conducted within the same field at Banyena, Victoria, Australia (−36.5721, 142.7940) during the years 2010 to 2014. The field site was selected because of the low to medium density of *P. thornei* (5 to 20 *P. thornei* g soil^{−1}) and low or nondetectable densities of inoculum of other regionally important soilborne pathogens (*Heterodera avenae*, *Fusarium pseudograminearum*, *Fusarium culmorum*, *Rhizoctonia solani*, and *Gaeumannomyces graminis* var. *tritici*) determined using the PREDICTAB soil test delivered by the South Australian Research and Development Institute (Adelaide, Australia). The site has a gray cracking clay soil type, and the long-term (1889 to 2014) average rainfall per annum for the closest weather station (Rupanyup; 16 km from the experimental site) was 421 mm (Table 1) (Queensland Government 2019). The site was managed with crop rotations of vetch and fallow to maintain ideal densities of *P. thornei* (5 to 20 *P. thornei* g of soil^{−1}) before field experiments while limiting buildup of nontarget soilborne pathogens. Pathogen populations were monitored following each crop and before each experiment using the PREDICTAB soil test (SARDI).

Field experiments. In the first year (setup year) of each experiment, *P. thornei* densities were manipulated using three cereal cultivars with contrasting resistance/susceptibility to *P. thornei* to establish a range of presowing nematode densities (Table 2) (Fanning et al. 2018; Hollaway et al. 2000). In the second year (experimental year), the experiments were sown to five cereal cultivars, which also varied in their resistance/susceptibility to *P. thornei* (Table 2) (Fanning et al. 2018; Hollaway et al. 2000).

Experiments were planted following rain in late May to June (winter) (Table 3) at a rate of 70 kg of seed ha^{−1}. Experimental plot sizes were 8.5 × 1.4 m composed of six rows spaced at 150 mm. Fertilizer application is reported in Table 3. Foliar diseases were controlled using a foliar fungicide application between Zadoks growth stage Z31 to Z39 in each year. Weeds were controlled using preemergent and postsowing herbicides, consistent with best grower practice. Harvest occurred following crop maturity during December (summer) (Table 3) using a plot harvester, with a weight recorded for each plot and converted to metric tons per hectare for data analysis.

Experimental design. Each experiment consisted of six replicates of 15 unique treatments, resulting from the two-way factorial treatment combination of setup year cultivars (three levels) and

TABLE 1. Summary of the monthly and annual rainfall (millimeters) for the experimental years 2010 to 2014 and the long-term mean (1889 to 2014) at Rupanyup post office (16 km from the experimental site) (Queensland Government 2019)

Year	January	February	March	April	May	June	July	August	September	October	November	December	Total
2010	9	40	51	51	22	28	42	105	60	42	126	148	723
2011	201	47	20	10	23	18	55	44	14	36	42	48	558
2012	5	10	40	10	39	44	53	49	46	14	13	34	359
2013	0	19	1	10	36	60	61	74	44	51	18	12	386
2014	9	4	12	62	39	37	43	12	20	15	25	15	292
Mean ^a	23	23	24	30	41	46	44	45	43	43	31	28	421

^a Long-term mean from 1889 to 2014.

experimental year cultivars (five levels). In the 2011 and 2012 experimental years, the treatments were randomized according to a randomized complete block design. In the 2013 experimental year, treatments were randomized according to a split plot design, whereby the experimental year cultivars were randomized to main plots and the setup year cultivars were randomized to subplots within main plots. A similar split plot randomization of treatments was used in the 2014 experimental year; however, setup year cultivars were randomized to main plots, whereas the experimental year cultivars were randomized to subplots within main plots (the reverse of the 2013 randomization). Only data collected in the second year of experimentation (experimental year) were analyzed in this study.

Nematode quantification. In the experimental year, soil samples were taken from each plot 1 to 3 months prior to sowing and again, 1 to 3 months postharvest to measure initial (*Pi*) and final (*Pf*) *P. thornei* densities, respectively. Each soil sample consisted of 20 cores (150-mm deep and 15-mm diameter) taken using an Accucore sampler (Spurr Soil Probes). They were sampled evenly from across each plot, with four cores taken from five locations in each plot. The cores from each plot were pooled, resulting in 300 to 500 g of soil in each sample. Soil samples were submitted to the PREDICTAB service at the South Australian Research and Development Institute (Adelaide, Australia) for quantification of *P. thornei* densities using qPCR (Ophel-Keller et al. 2008). Quantification of the *P. thornei* densities used specific ribosomal DNA (TaqMan) probe sequences from the whole dried soil samples, with DNA standards used to convert the qPCR results to the number of *P. thornei* per gram of dried soil.

Plant assessments. In the experimental year, 20 plants were removed from a subset of plots at anthesis, with the roots of the plants washed and visually examined for symptoms of root disease. This process was conducted for the cultivars Catalina (2011 to

TABLE 2. Cereal cultivars and their reported resistance to *Pratylenchus thornei* that was planted during the setup or experimental years (2010 to 2014) to explore the relationships between yield, host resistance, and populations of *P. thornei*

	Cultivar resistance ^a	Setup year
Setup year cultivar (crop)		
Tamaroi (durum ^b)	Resistant	2010–2012
Yawa (durum)	Resistant	2013
Chara (wheat)	Moderately resistant	2010–2013
Yitpi (wheat)	Susceptible	2010–2013
Experimental year cultivar (crop)		
Catalina (wheat)	Moderately resistant	2011–2014
Chara (wheat)	Moderately resistant	2011–2014
Hindmarsh (barley)	Moderately resistant	2011–2014
Derrimut (wheat)	Susceptible	2011–2014
Yitpi (wheat)	Susceptible	2011–2014

^a Varietal ranking based on data from Fanning et al. (2018).

^b *Triticum turgidum* subsp. *durum*.

TABLE 3. Summary of the fertilizer application and sowing and harvest dates for each experiment conducted to explore the relationships between yield, host resistance, and populations of *Pratylenchus thornei*

Experimental year	Sowing fertilizer ^a (kg ha ⁻¹)		Sowing date ^a	Harvest date ^a
	MAP ^b	Urea		
2011	100	100	31/5	8/12
2012	100	100	18/6	10/12
2013	70	100 ^c	19/6	15/12
2014	70	100	12/6	1/12

^a These details refer to the experimental year.

^b Monoammonium phosphate (MAP) consists of nitrogen:phosphorus:potassium:sulfur:zinc of 10:22:0:1.5:1.

^c Equally split application of fertilizer at sowing and 3 months postsowing.

2014), Derrimut (2011 to 2014), and Hindmarsh (2012 to 2014) in three replicates of all three setup nematode densities.

Statistical analysis. *Yield loss relationships.* Relationships describing the rate of change in grain yield as presowing *P. thornei* densities (*Pi*) increased were estimated for each of the experimental year cultivars in each of the experimental years using regression implemented in a linear mixed model framework. Presowing *P. thornei* densities were natural log transformed [$\ln(Pi + 1)$] prior to analysis to linearize the relationship between the dependent and independent variables while also reducing the potential excess leverage exerted by large observed *Pi*.

A continuous covariate consisting of the transformed presowing *P. thornei* densities measured on a plot basis in each experimental year was fitted as a fixed effect in the model along with terms to account for the experimental year, experimental year cultivar, and the interaction between experimental year and experimental year cultivar. Additional interactions were also fitted between the transformed presowing *P. thornei* densities and experimental year, experimental year cultivar, and their interaction. Together, these terms enabled the estimation of differential yield potentials and rates of change for each experimental year cultivar in each experimental year, where the yield potential of an experimental year cultivar is the predicted yield of a cultivar in a particular experimental year in the absence of *P. thornei*. Terms to account for the structure of the experimental design in each experiment were included in the model as random effects while using the methods of Gilmour et al. (1997) to account for natural or extraneous sources of variation in the model that arose during each experiment.

The yield potential and rate of yield change for each experimental year cultivar in each experiment were estimated from the model as empirical best linear unbiased estimators. All variance components were estimated using residual maximum likelihood (Patterson and Thompson 1971) implemented through the ASReml-R package (Butler et al. 2009) in the R statistical computing environment (R Core Team 2019); the significance of fixed effects was tested at the 5% significance level.

Cultivar resistance. Empirical observation of the experimental data suggested that a logarithmic relationship existed between the final (*Pf*) and initial (*Pi*) nematode densities with form

$$Pf = e^{\alpha} Pi^{\beta}$$

where α and β are the intercept and slope, respectively, from a linear regression of natural log ($\ln$) *Pf* on $\ln Pi$. In this equation, β has a direction interpretation with respect to how much the slope curves (i.e., $\beta = 1$ would give a straight line, implying a constant multiplication rate, whereas $\beta < 1$ implies that the multiplication rate is lower for higher *Pi*). The multiplier e^{α} grows exponentially with α , and the larger the α , the faster the *Pi* multiplies. If $\alpha < 0$, it implies a resistant cultivar where the resulting *Pf* will be lower than *Pi* on average.

To estimate the parameters α and β , an initial linear mixed model was fitted to the data regressing natural log-transformed *Pf* densities on natural log-transformed *Pi* densities, including the main effects of experimental year cultivar, experimental year, and all interactions. Terms in the treatment structure were included in the model as fixed effects, and terms to account for the structure of the experimental design in each experimental year were included in the model as random effects. A backward step regression was applied to derive a final model, with interaction terms in the fixed part of the model tested using Wald-type tests in a two-stage process. Where an interaction term was significant, indicating that at least one coefficient was significantly different from zero, individual coefficients were subsequently tested for inclusion. Models were fitted with residual maximum likelihood (Patterson and Thompson 1971) using the R software package ASReml (Butler et al. 2009; R Core Team 2019).

The equilibrium density ($\widehat{EQ}$) of an experimental year cultivar in an experimental year is defined as the value of *Pi* at which *Pf* and *Pi* are equal. The equilibrium density could be described as the

population maintenance line, and it biologically implies that cultivars are maintaining nematode densities over the period measured. The estimated equilibrium density ($\widehat{EQ}_{ij}$) was derived for each experimental year cultivar i in each experimental year j using the formula

$$\widehat{EQ}_{ij} = \exp\left(\frac{\hat{\alpha}_{ij}}{1 - \hat{\beta}_{ij}}\right)$$

where $\hat{\alpha}_{ij}$ and $\hat{\beta}_{ij}$ are the regression coefficients.

RESULTS

Experimental establishment. The main symptoms of root disease observed were caused by root lesion nematodes in all experimental years except for 2012, where take all (*G. graminis* var. *tritici*) was observed in a limited number of plants at low severity.

Differential initial nematode densities for each of the experimental years were successfully established (Fig. 1) as expected based on the relative resistance/susceptibility (Table 2) of the three setup cereal cultivars grown during the prior setup year.

Yield loss relationships. For grain yield, a significant interaction between experimental year, experimental year cultivar, and presowing nematode density was observed ($P = 0.024$). This interaction indicated differential rates of change in yield per unit increase in presowing *P. thornei* density between experimental year cultivars across experimental years. However, on testing the significance of the relationship between the presowing *P. thornei* density and grain yield for each experimental year cultivar in each experimental year, it was found that not every experimental year cultivar demonstrated significant yield losses in every experimental year within the study (Table 4). The experimental year cultivars Derrimut and Yitpi were the two cultivars of the five within the study that demonstrated a loss in grain yield with increased presowing *P. thornei* densities (Table 4). These yield losses were observed in both cultivars during the 2011 season, but they were only in Derrimut during the 2012 season. The predicted yield losses associated with increasing presowing *P. thornei* densities and the maximum yield losses estimated to be 28, 27, and 28% for Yitpi during 2011 and Derrimut during 2011 and 2012, respectively, are presented in Table 5. The seasonal variability of the yield losses

Fig. 1. The range of initial *Pratylenchus thornei* densities (*P. thornei* grams of soil⁻¹) established (minimum, maximum, median, and upper and lower quartiles) in the previous year using the setup cereal cultivars Tamaroi or Yawa (resistant), Catalina (moderately resistant), and Yitpi (susceptible) at the experiment site in Banyena, Victoria.

observed for the cultivar Derrimut is presented in Figure 2. In some seasons, the other three experimental year cultivars tested (cultivars Catalina, Chara, and Hindmarsh) had significant increases in grain yield associated with increases in presowing *P. thornei* densities (Table 4). These yield increases were observed from 2012 to 2014 for Catalina, in 2014 for Hindmarsh, and during 2013 and 2014 for Chara (Table 4). The predicted yield potential of the experimental year cultivars differed significantly from zero ($P < 0.001$) in all 4 experimental years.

Cultivar resistance. *P. thornei* multiplication (Pf/Pi) varied significantly between the experimental years (calculated from Fig. 3 and Table 6). This resulted in a significant ($P < 0.001$) interaction between the experimental year and experimental year cultivar. The effect of season on nematode multiplication is graphically represented (Fig. 3), where the relationship between presowing and postharvest *P. thornei* densities for the experimental year cultivar Derrimut is shown. These data highlight the differences between seasons and show that there was a narrower range of presowing nematode densities in the experimental years 2011 and 2012 compared with 2013 and 2014. The equilibrium point is reached during 2014 for Derrimut, whereas during 2011 to 2013, the equilibrium point is outside the data range.

The regression coefficients and associated errors together with the estimated equilibrium points ($Pi = Pf$) calculated from the regression coefficients are presented for the relationship between presowing and postharvest *P. thornei* densities (Table 7). The estimated equilibrium points for the cultivars Derrimut and Yitpi in the experimental years 2011 to 2013 were outside the range of data observed, which implies that the equilibrium point was greater than

TABLE 4. The effect of increasing presowing densities of *Pratylenchus thornei* on grain yield^a

Experimental year cultivar and experimental year	Rate of change (slope)	<i>P</i> value (rate of change)	Yield potential ^b
Catalina			
2011	0.028	0.626	3.76
2012	0.111	0.055	1.72
2013	0.116	0.044^c	2.41
2014	0.062	0.007	0.45
Chara			
2011	-0.017	0.719	4.35
2012	0.060	0.363	2.35
2013	0.213	0.002	1.87
2014	0.049	0.018	0.40
Hindmarsh			
2011	0.071	0.214	4.54
2012	0.029	0.681	4.19
2013	0.099	0.057	3.87
2014	0.099	0.000	0.95
Derrimut			
2011	-0.207	0.000	4.08
2012	-0.187	0.001	3.36
2013	0.078	0.136	2.53
2014	0.002	0.943	0.86
Yitpi			
2011	-0.215	0.000	4.28
2012	-0.101	0.137	2.83
2013	0.013	0.810	2.23
2014	0.002	0.930	0.79

^a The slope [(tons hectare⁻¹) (ln initial *P. thornei* grams of soil⁻¹)⁻¹], constant (yield potential; tons hectare⁻¹), and significance of the relationship are presented for each of the five experimental cereal cultivars for each year between 2011 and 2014 at Banyena, Victoria.

^b The yield potential (tons hectare⁻¹) provides a prediction of the yield potential in the absence of *P. thornei*, but it should be used with caution because no presowing nematode densities were zero. The yield potential was significantly different from zero ($P < 0.001$) of each experimental year cultivar in each experiment.

^c Bold text indicates a rate of slope change significantly ($P < 0.05$) different from zero.

for the other cultivars Catalina, Hindmarsh, and Chara where the equilibrium point could be estimated. A graphical representation of regression solutions from the 2013 experiment data is presented (Fig. 4), highlighting distinct differences in the relationship between presowing and postharvest *P. thornei* densities for the cultivars evaluated, with the cultivars Catalina, Hindmarsh, and Chara having a significantly decreased $\hat{\alpha}$ estimate compared with the cultivars Derrimut and Yitpi. The results show that cultivars Derrimut and Yitpi have not reached the equilibrium point within the range of observed experimental data.

The relationship between presowing and postharvest nematode densities shows that the multiplication rate decreased as the presowing nematode densities increased (Figs. 3 and 4). The results in Figure 3 demonstrate how the change in multiplication rate varied between experimental years, and those in Figure 4 show the variation between cultivars in a single experimental year.

DISCUSSION

We used a unique approach to assess the relationship between presowing densities of *P. thornei* and grain yield or nematode

multiplication rates. The relationship between the natural log of presowing *P. thornei* densities determined using a DNA-based soil test (PREDICTAB) and grain yield was found to be linear for each of the cultivars in each of the seasons within the study. The effect of season was found to be a major driver in determining the extent of the yield losses measured, with grain yield losses observed in only two of the five cultivars and in only two of the four seasons. Analysis of *P. thornei* multiplication data demonstrated that an increase in presowing nematode densities resulted in a decreased nematode multiplication rate in all seasons in all cultivars, despite variation between seasons. In the susceptible cultivars Derrimut and Yitpi, an equilibrium point could only be calculated in one of the four experimental years, demonstrating the ability of these cultivars to support increased densities of *P. thornei* to high levels in the field.

Validity of study. Unlike previous studies (Nicol et al. 1999; Smiley and Machado 2009), this study relied on wheat to establish a range of presowing *P. thornei* densities. The use of similar crop types ensured that the setup year cultivars did not result in other pathogen changes within the soil, with soil testing and inspection of plant roots conducted with each experiment to confirm that no other soilborne pathogens of importance were detected.

TABLE 5. Predicted grain yield (tons hectare⁻¹) and percentage yield loss (shown in parentheses) in the experimental cereal cultivar Derrimut during 2011 and 2012 and cultivar Yitpi during 2011 at a range of presowing nematode densities (*Pratylenchus thornei* grams of soil⁻¹) using the equations presented in Table 4

Experimental year cultivar	Experimental year	Grain yield (t ha ⁻¹) and yield loss (%) at specified presowing <i>P. thornei</i> densities (<i>P. thornei</i> g of soil ⁻¹)									
		0	5	10	20	30	50	100	150	200	250
Derrimut	2011	— ^a	—	3.58 (12)	3.45 (15)	3.37 (17)	3.26 (20)	3.12 (23)	3.04 (25)	2.98 (27)	—
Derrimut	2012	—	3.03 (10)	2.92 (13)	2.80 (17)	2.72 (19)	2.63 (22)	2.50 (26)	2.43 (28)	—	—
Yitpi	2011	4.28 (0)	3.89 (9)	3.76 (12)	3.62 (15)	3.54 (17)	3.43 (20)	3.29 (23)	3.20 (25)	3.14 (27)	3.09 (28)

^a These presowing nematode densities are not presented because they are outside the range of observed experimental data.

Fig. 2. The effect of increasing presowing *Pratylenchus thornei* density [$\ln(P. thornei \text{ grams of soil}^{-1}) + 1$] on grain yield (tons hectare⁻¹) for the wheat cultivar Derrimut grown at Banyena, Victoria from 2011 to 2014.

A range of presowing nematode densities was established in each of the experimental years rather than two distinct strata of presowing nematode densities corresponding with the setup year cultivars. Conducting the experiments over several seasons highlighted differences between the maximum and minimum presowing nematode densities, with presowing nematode densities for each cultivar in each experimental year being different. Despite these differences, the relationships between the presowing *P. thornei* densities and grain yield loss or final nematode densities could be estimated robustly.

Grain yield losses. Yield losses were observed in the experimental year cultivars Derrimut and Yitpi during 2011 and only in Derrimut during 2012, with the maximum yield losses observed estimated to be 27, 28, and 28%, respectively. These yield losses are similar to the 27% yield loss reported for the cultivar Machete in South Australia by Nicol et al. (1999). Nicol et al. (1999) also reported higher yield losses of 38% for the cultivar Spear. Eastwood et al. (1994) examined yield losses caused by *P. thornei* in four locations across Victoria during 1993, with losses in the cultivar Meering varying from 0 to 44%, demonstrating the potential range of losses that can be observed in one season for one cultivar. Interestingly, the yield losses observed by Eastwood et al. (1994) and Nicol et al. (1999) were at significantly lower *Pi* values than this study, with the maximum *Pi* values observed being 15.5 and 22.0 *P. thornei* g of soil⁻¹, respectively. We used a DNA-based soil test for nematode quantification, whereas the earlier two studies both used a manual extraction technique, which could account for lower nematode densities observed. In Queensland, grain yield losses varied between 26 and 76%, with *Pi* values of 8.1 and 3.5 *P. thornei* g of soil⁻¹, respectively, which is consistent with the higher yield losses reported in northern Australia (Owen et al. 2014; Reen et al. 2014).

Yield losses reported internationally along with the variability in yield losses between seasons are consistent with those reported in this study, although at lower *P. thornei* densities than in this study (Ortiz-Monasterio and Nicol 2004; Smiley 2009). This may be a consequence of the different nematode quantification techniques or may suggest that a threshold level exists, whereby no further yield losses are incurred past a particular nematode density. Alternatively, these differences in yield losses could be because of a number of other factors, including but not limited to a changing farming system, location, soil type, and rainfall, which have previously been reported to affect nematode multiplication and may, therefore, also

TABLE 6. The minimum and maximum presowing (*Pi*) and postharvest (*Pf*) densities of *Pratylenchus thornei* (*P. thornei* grams of soil⁻¹) in the experimental year of each experiment for each experimental year cultivar

Time of density measurement and experimental year cultivar	Experimental year			
	2011	2012	2013	2014
<i>Pi</i> density (range)				
Catalina	1.7–90.2	2.0–76.8	19.0–182.0	3.2–133
Chara	0.7–77.0	2.5–33.2	20.0–167.0	4–197.1
Hindmarsh	2.6–136.8	3.2–44.8	12.0–163.0	4.1–151.3
Derrimut	0.4–53.6	1.5–59.6	15.0–188.4	4.9–184.2
Yitpi	0.3–139.8	2.6–37.6	12.0–171.0	5.8–288.6
<i>Pf</i> density (range)				
Catalina	4.4–51.9	5.6–27.5	19.3–67.6	3.7–44.5
Chara	6.1–83.8	6.5–66.3	21.2–86.2	2.1–64.4
Hindmarsh	3.2–81.2	3.1–45.0	14.8–80.2	3.2–41.3
Derrimut	11.4–218.1	9.6–178.1	83.3–229.7	10.5–113
Yitpi	18.1–259.6	19.0–129.2	56.0–263.8	6.8–109.9

Fig. 3. The relationship between presowing (*Pi*) and postharvest (*Pf*) *Pratylenchus thornei* densities (*P. thornei* grams of soil⁻¹) in the wheat cultivar Derrimut from 2011 to 2014 at Banyena, Victoria. The equilibrium line (*Pi* = *Pf*) is indicated to show when the nematode multiplication rate (*Pf/Pi*) is equal to one.

affect yield losses (Cadet et al. 2004; Castillo et al. 1995; Fanning et al. 2018; Farsi et al. 1995; Govaerts et al. 2006; Kincaid 1946; Thompson et al. 2015a).

Grain yield losses were measured in only two of the four experimental years in the experimental year cultivar Derrimut and only one of the four experimental years in the experimental year cultivar Yitpi. Few studies have repeatedly assessed grain yield loss caused by *P. thornei* over consecutive years. To the best of our knowledge, Ortiz-Monasterio and Nicol (2004) and Smiley (2009) are the only others to measure grain yield losses and the variation between years or seasons. Smiley (2009) used a nematicide to establish high and low nematode densities, with 23 and 40% yield losses measured between 2 years in one cultivar, and in other experiments, Smiley (2009) measured from 9 to 29% and from 6 to 15% yield losses within 3 years at two different locations. Ortiz-Monasterio and Nicol (2004) observed yield losses in 2 consecutive years varying between 12 and 37% but only in plots that had reduced irrigation compared with in another experimental year when they observed no yield differences between treatments when using a full irrigation system. The results of this study together with those of Ortiz-Monasterio and Nicol (2004) and Smiley (2009) highlight the importance of conducting yield loss experiments over multiple years in the same location because the yield losses caused by *P. thornei* seem highly variable across years.

No yield losses caused by *P. thornei* were observed in the three cultivars Catalina, Chara, and Hindmarsh. Unexpectedly, these cultivars demonstrated a significant positive relationship between presowing *P. thornei* densities and grain yield, resulting in increased grain yield as nematode densities increased in some seasons. Supplementary data collected from the experiments did not provide a definitive explanation of these yield increases. However, through observation of the experiments during crop maturity, it was evident

that field plots with higher nematode densities remained greener for longer and may have been able to capitalize on later rainfall compared with plots with lower nematode densities, which matured slightly earlier. These results highlight the inherent dangers in extending the yield loss knowledge to growers and advisors without providing the variation between years and cultivars. The differences in the degree of yield losses measured between experimental year cultivars within this study highlight the variation in tolerance between cultivars and the importance of screening for tolerance to nematodes. If tolerant cultivars that do not display yield losses when planted in high nematode densities can be identified, there would be less reliance on nematode resistance and reducing nematode densities.

Cultivar resistance. The relationship between the presowing (P_i) and postharvest (P_f) *P. thornei* density demonstrated that, as the P_i increased, the *P. thornei* multiplication rate (P_f/P_i) decreased in all experimental year cultivars in all experimental years. This relationship has been reported by Ferris (1985) and Ortiz-Monasterio and Nicol (2004) for *M. incognita* and *P. thornei*, respectively. Ferris (1985) suggested that the resource availability for nematodes is decreased at higher nematode densities, and therefore, there is a decreased multiplication rate at higher P_i values. In contrast to these studies, an earlier study by Nicol et al. (1999) reported that the cultivar Warigal, susceptible to *P. thornei*, displayed a quadratic relationship compared with the resistant cultivars, which displayed a linear relationship. However, their study had limited data at higher P_i values, which may have affected the relationships reported. We found that the relationship between P_i and P_f was similar for all cultivars, which has management implications. The cultivation of resistant cultivars is an effective method to control *P. thornei* (Fanning et al. 2018), and the relationship reported here indicates that it will be difficult to reduce nematode densities at lower P_i values. These relationships further highlight the importance of regularly screening cultivars to ensure that the resistance is known to prevent these increases in nematode numbers.

The equilibrium point, defined as the value of P_i at which P_f and P_i are equal, could be calculated for the susceptible cultivars Derrimut and Yitpi in only 1 of the 4 experimental years. This was a result of the putative equilibrium point being above the maximum P_i for the other 3 experimental years, with the experimental year that the equilibrium point could be estimated for coinciding with the lowest rainfall and grain yields. In comparison, an equilibrium point could be estimated in all years for the moderately resistant cultivars, having lower equilibrium points than the susceptible cultivars as expected. Previously, Nicol et al. (1999) compared the relationship between P_i and P_f for *P. thornei* in relation to the equilibrium point for the cereal line cultivars GS50a (resistant) and AUS4930 (resistant) versus Warigal (susceptible). The equilibrium points reported by Nicol et al. (1999) were 8.9, 9.2, and 60.0 *P. thornei* g of soil⁻¹ for genotypes GS50a, AUS4930, and Warigal, respectively. These equilibrium points are significantly lower than those estimated in our study. However, similar to our study, the resistant cultivars had significantly lower equilibrium points when compared with susceptible cultivars. These results show that, irrespective of season, susceptible cultivars have the ability to support significantly higher nematode densities.

Nematode multiplication varied across seasons. Few studies have carried out similar experiments over several seasons in field conditions. Previously, Seinhorst (1967) reported that multiplication rates varied between years in *Pratylenchus crenatus*, and Fanning et al. (2018) reported multiplication rates variation between years in *Pratylenchus neglectus* and *P. thornei*. This variation is as expected with nematode multiplication rates previously reported to be affected by changes in rainfall, soil temperature, pH, moisture, and nutrition (Cadet et al. 2004; Castillo et al. 1995, 1996; Kincaid 1946; Mai and Kable 1968; Mizukubo and Adachi 1997; Thompson et al. 2015b). The changes in multiplication rate reported here and previously are important from

TABLE 7. The estimates of α and β values for the regression solution used to determine the postharvest density of *Pratylenchus thornei* [postharvest nematode density ($P. thornei$ grams of soil⁻¹; P_f) = $e^{\alpha} \times$ presowing nematode density (P_i^{β})]^a

Experimental cultivar and experimental year	$\hat{\alpha}$	SE ($\hat{\alpha}$)	$\hat{\beta}$	SE ($\hat{\beta}$)	Equilibrium point ($\bar{EQ}$)
Catalina					
2011	2.13	0.156	0.297	0.051	20.7
2012	1.30	0.133	0.461	0.043	11.1
2013	2.64	0.252	0.214	0.059	28.7
2014	1.10	0.175	0.461	0.043	7.7
Chara					
2011	2.00	0.134	0.419	0.037	31.3
2012	1.35	0.109	0.584	0.029	25.7
2013	2.36	0.220	0.336	0.049	35
2014	0.70	0.141	0.584	0.029	5.4
Hindmarsh					
2011	1.71	0.177	0.615	0.052	84.9
2012	0.81	0.135	0.779	0.045	39.1
2013	1.48	0.244	0.532	0.058	23.6
2014	-0.11	0.180	0.779	0.045	0.6
Derrimut					
2011	3.05	0.137	0.419	0.037	NA ^b
2012	2.46	0.106	0.584	0.029	NA
2013	3.59	0.215	0.336	0.049	NA
2014	1.73	0.145	0.584	0.029	64
Yitpi					
2011	3.44	0.133	0.419	0.037	NA
2012	2.52	0.106	0.584	0.029	NA
2013	3.51	0.210	0.336	0.049	NA
2014	1.38	0.146	0.584	0.029	27.6

^a The standard errors (SEs) associated with each of the parameter estimates for each experimental year cultivar in each experimental year are also presented. Based on these parameter estimates, the equilibrium point ($P_f = P_i$) was estimated for each experimental year cultivar in each experimental year.

^b NA means that the equilibrium point is outside the range of data in the experimental year.

a management perspective. Growers will need to measure nematode densities regularly because the multiplication rates will change depending on the season; additionally, a resistant cultivar may not be reducing nematode densities, or a susceptible cultivar may not be increasing nematode densities to the extent expected. Unfortunately, it means that a prediction of final *P. thornei* densities based on a presowing nematode density and cultivar resistance cannot be achieved with these results. However, the trends estimated from this study can enable the determination of when nematode testing will be most beneficial in fields.

Methods used and comparison with other methods—improvements. Historically, the study of yield losses owing to root lesion nematodes has primarily been conducted with the use of nematicides or fumigation (Doyle et al. 1987; Eastwood et al. 1994; Smiley 2009; Smiley et al. 2014; Taylor and McKay 1993; Taylor et al. 1999; Thompson et al. 1993, 1995, 2012). However, numerous issues surrounding the use of these treatments have been reported, including limited efficacy and toxicity. Eastwood et al. (1994) suggested that the nematicide aldicarb only reduced *P. thornei* densities down to 30 cm, but >50% of *P. thornei* are found deeper than 30 cm, suggesting only partial control of nematodes through the soil profile. Fumigation has also been reported to cause toxicity in plants, making quantifying yield losses difficult because of the confounding effect of the treatment (Jones 1968; Nicol et al. 1999). Nematicides have high toxicity to mammals, and they are difficult to apply, resulting in resistant cultivars being adopted by growers for nematode control. Currently, there are no nematicides registered for use in cereal crops in Australia (Vanstone et al. 2008).

With the complexities inherent in nematicide research, other methods of quantifying yield losses have been adopted. Nicol et al.

(1999) used a range of crop types (linseed [*Linum usitatissimum*], canola [*Brassica napus*], barley, oat [*Avena sativa*], and wheat) and fallow to establish presowing nematode densities and related these to grain yield loss through regression. The same method of regression was used by Owen et al. (2014) and Reen et al. (2014), who also planted a range of crops to establish presowing nematode densities over a number of seasons. Although Nicol et al. (1999) reported that there was no indication that they affected grain yield, using different crop types could result in differences in soil moisture and nitrogen, which also affect grain yields (Angus et al. 2015; Armstrong et al. 2019; Hannah and O’Leary 1995). We overcame these potential issues by planting different wheat cultivars with contrasting resistance and susceptibility, thus reducing any potential soil moisture and nitrogen differences.

The designs still induced a range of minimum and maximum presowing nematode densities across the setup year cultivars, meaning that the range of presowing nematode densities for each experimental year cultivar was different. The range of presowing nematode densities meant that the regression relationship between presowing *P. thornei* densities and grain yield could be determined and provides an ideal approach for determining yield losses caused by root lesion nematodes (Table 7). Further research is required to understand the effects of environment before growers can predict yield losses in a given season. However, currently this method allows growers to predict a range of potential yield losses at presowing nematode densities in their fields, allowing them to make informed decisions around the presowing *P. thornei* densities in which they will start control methods to prevent further grain yield losses.

Conclusion. The relationship between presowing *P. thornei* densities, determined through a DNA-based soil test (PREDICTAB), and grain yield was established for a set of commercial wheat

Fig. 4. The relationship between presowing (P_i) and postharvest (P_f) *Pratylenchus thornei* densities (*P. thornei* grams of soil⁻¹) for the cereal cultivars Catalina, Hindmarsh, Chara, Yitpi, and Derrimut during the 2013 experimental year at Banyena, Victoria. The equilibrium line ($P_i = P_f$) is indicated to show when the nematode multiplication rate (P_f/P_i) is equal to one.

and barley cultivars. A linear relationship was observed between the natural log of the presowing *P. thornei* density and grain yield across all seasons. Grain yield losses varied between cultivars and seasons, with the greatest yield losses observed (25 to 28%) during 2011 and 2012. Although these results cannot be used to definitively estimate yield losses in a given season, they do indicate potential risk of yield loss from a given presowing nematode density. Further research is required to define the seasonal effects on grain yield loss.

The relationship between presowing and postharvest *P. thornei* densities was determined, demonstrating that increasing presowing nematode densities resulted in decreased multiplication rates in all seasons and in all cultivars. Although multiplication varied from one season to the next, making it difficult to predict postharvest nematode densities, there was consistent ranking of cultivars based on their known resistance/susceptibility rating. These results emphasize that growers need to measure nematode densities regularly because multiplication rates will change depending on the season; cultivars may not be increasing or decreasing nematode densities to the extent expected.

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