Resistance of Wheat Genotypes to Root-Lesion Nematode (*Pratylenchus thornei*) Can be Used to Predict Final Nematode Population Densities, Crop Greenness, and Grain Yield in the Field

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ABSTRACT

The root-lesion nematode *Pratylenchus thornei* is a major pathogen of wheat (*Triticum aestivum*) in many regions globally. Resistance of wheat genotypes to *P. thornei* can be determined from final nematode population densities in glasshouse experiments but combining results across multiple experiments presents challenges. Here, we use a factor analytic method for multiexperiment analysis of final population densities of *P. thornei* for 1,096 unique wheat genotypes in 22 glasshouse experiments. The resistance to *P. thornei* of the genotypes was effectively represented by a two-factor model with rotation of the axes to a principal components solution. Principal axes 1 and 2 (PA1 and PA2) accounted for 79 and 11% of the genetic variance, respectively, over all experiments. Final population densities of *P. thornei* as empirical best linear unbiased predictors (PA[1+2]-eBLUPs) from the combined glasshouse experiments were highly predictive ($P < 0.001$) of final nematode population densities in the soil profile, crop canopy greenness (normalized difference

vegetation index), and grain yield of wheat genotypes in *P. thornei*-infested fields in the Australian subtropical grain region. Nine categories of resistance ratings for wheat genotypes from resistant to very susceptible were based on subdivision of the range of PA(1+2)-eBLUPs for use in growers' sowing guides. Nine genotypes were nominated as references for future resistance experiments. Most (62%) Australian wheat genotypes were in the most susceptible three categories (susceptible, susceptible to very susceptible, and very susceptible). However, resistant germplasm characterized in this study could be used in plant breeding to considerably improve the overall resistance of Australian wheat crops.

Keywords: disease control and pest management, genetics and resistance, MET analysis, nematode population densities predictions, nematology, root-lesion nematodes, wheat genotype resistance, wheat yield prediction

The root-lesion nematode *Pratylenchus thornei* is a major pathogen of wheat (*Triticum aestivum*) in many regions of the world (Smiley and Nicol 2009), particularly subtropical and warm temperate zones such as the subtropical or northern grain region of eastern Australia (Thompson et al. 2008; Webb et al. 1997). This region, which lies between latitude 20°S in Queensland and latitude 32°S in New South Wales, is characterized by rain-fed agriculture dependent on dryland farming practices, with a range of broad-acre crop species grown throughout the year. Major cool-season crops, termed winter crops, are wheat, barley (*Hordeum vulgare*), and chickpea (*Cicer arietinum*), while major warm-season crops, termed summer crops, are sorghum (*Sorghum bicolor*) and mung bean (*Vigna radiata*), with a number of other cereal, pulse, and oilseed crop species grown to lesser extents. Deep cracking clay soils (vertisols) are favored for cropping in this region because of their high water-holding capacities to store fallow rainfall to supplement rainfall during the next crop phase (Webb et al. 1997).

P. thornei is polyphagous, being hosted by many cereal and pulse crop species, including wheat and chickpea; which, in other

respects, are valuable rotational crops for each other. Some wheat genotypes can lose up to 65% yield (Thompson et al. 1999) and some chickpea genotypes up to 25% yield (Reen et al. 2014) from high population densities of *P. thornei* in the soil profile at sowing. It has been estimated that *P. thornei* costs 4.3% of the total wheat production/year in the eastern Australian subtropical grain region, with this loss valued at AU\$38 million (Murray and Brennan 2009). Other hosts of *P. thornei* among crop species grown in this region include winter crops barley, triticale (*xTriticosecale*), durum (*T. turgidum* subsp. *durum*), and faba bean (*Vicia faba*), and summer crops mung bean, black gram (*Vigna mungo*), and soybean (*Glycine max*) (Owen et al. 2012, 2014).

Management of *P. thornei* for wheat production depends on crop sequences that include moderately resistant crop species such as sorghum (Owen et al. 2014; Thompson et al. 2008, 2012a), which is the major summer crop species in the subtropical grain region of Australia. However, sorghum cannot be grown profitably across the entire region. Fixed rotations are difficult to implement due to variable rainfall patterns in this region, and flexible crop sequencing (termed opportunity cropping) is often practiced. *P. thornei* survives well in the clay soils of the region and, following growth of a susceptible wheat cultivar, approximately 3 years of fallow or resistant rotational crops are needed to reduce high nematode population densities to below the threshold for damage (Owen et al. 2014; Whish et al. 2017), which is approximately 2,000 nematodes/kg of soil for intolerant wheat cultivars (Thompson et al. 2008). Protection of the major at-risk crops through breeding resistant and tolerant genotypes is essential for the long-term management of *P. thornei* in this region.

Tolerance and resistance to nematodes are treated as separately measured characteristics in nematology. Tolerance is defined as the capacity of plant genotypes to grow and yield with little loss in the

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presence of high initial nematode population densities, and resistance is defined as the capacity of plant genotypes to limit nematode reproduction in the roots (Roberts 2002; Trudgill 1991). Although tolerance and resistance can be measured independently they are not necessarily independent characteristics. Roberts (2002) indicated that a resistant plant genotype may not only have low nematode population densities in its roots but also, as a consequence of the low population density, can have maximum plant growth at least equal to a tolerant genotype. In field experiments, *P. thornei* was shown to decrease the uptake by wheat plants of soil nutrients (Thompson et al. 2012a) and water (Whish et al. 2014) under both drier and wetter seasonal conditions than the long-term average. Previously, Wallace (1987) suggested that genetic factors that confer on plants the abilities to withstand stresses such as water and nutrient deficiencies would function in a nonspecific way to confer tolerance to nematode attack. On the other hand, genetic factors for resistance to nematodes are specific and, in fact, certain wheat genotypes resistant to *P. thornei* are susceptible to *P. neglectus* (Farsi et al. 1995; Thompson 2008). Similarly, other wheat genotypes resistant to cereal cyst nematode (*Heterodera avenae*) are susceptible to *P. thornei* (Nombela and Romero 2001; Romero and Nombela 1999; Thompson 2008).

Resistance to nematodes is measured by the increase in nematode population densities during plant growth. Resistance of crop genotypes to root-lesion nematodes can be assessed in the field or glasshouse, with the more resistant genotypes resulting in lower final population densities (P_f) of nematodes than the more susceptible genotypes. The advantage of glasshouse methods is that the initial population density (P_i) of a single nematode species can be made constant for all genotypes to be tested in an experiment whereas, under field conditions, the P_i of the site for the target nematode species has to be determined from soil sampling (De Waele and Elsen 2002). Spatial variability of nematode population densities in a field can be a confounding factor that may require the P_i of every plot in a field experiment to be determined (Fanning et al. 2018), thereby considerably increasing the resources needed. Furthermore, better environmental control is possible under glasshouse conditions, resulting in a greater proportion of experiments successfully completed with less variability between experiments. Another issue for assessing crop resistance to *P. thornei* in field experiments on vertisols is that the nematodes can be distributed down the soil profile to approximately 90 cm of depth with various vertical distribution patterns (Owen et al. 2014; Thompson et al. 1999), further complicating the determination of nematode population densities in the field.

Glasshouse-based methods have been used to characterize crop genotypes in replicated experiments for resistance to *P. thornei*, including advanced lines in wheat breeding programs (Thompson et al. 1999) and cultivars for growers' sowing guides (Lush 2018; Matthews et al. 2017). These methods have also been used as a single plant test for selecting resistant genotypes within segregating populations for breeding programs. These glasshouse methods were calibrated during initial development with field results by comparing final *P. thornei* population densities associated with four selected wheat genotypes grown in both the glasshouse and field (Thompson et al. 2015a,b). Over time, the glasshouse methods have been modified to provide conditions conducive for both plant growth and nematode reproduction so that differences in the P_f among genotypes are controlled by genetic difference rather than environmental factors.

Resistance of a wheat genotype in the glasshouse method is assessed after 16 weeks of plant growth by the final nematode population density in the roots and soil, because *P. thornei* can migrate between the roots and soil. Reference or check genotypes that cover a range of resistance and susceptibility are included in each experiment for comparison with new genotypes to be tested and to gauge nematode reproduction in an experiment. This method allows ranking of genotypes on their resistance in any single experiment but presents difficulties in comparing results across

multiple unbalanced experiments in which many of the genotypes under consideration differ from experiment to experiment. This lack of balance arises from the ongoing need to characterize the resistance of a changing suite of advanced plant breeding lines prior to their potential release to growers. To combine results across experiments, we previously used the average reproduction factor (RF) = P_f/P_i . The range of average RF was then divided into nine arithmetically equal categories to produce resistance ratings of cultivars for sowing guides. Nine categories are used in Australian sowing guides, ranging from resistant (R) to very susceptible (VS) (Lush 2018), known as an alpha rating scale to distinguish it from previous methods of assigning numbers 1 to 9 to these ordinal categories. Despite standardized experimental conditions, nematode reproduction rates can vary among experiments. This results in higher average RF values and susceptibility ratings of genotypes present in experiments with higher P_i but absent from experiments with lower P_i , and vice versa. Clearly, a more rigorous statistical approach is required in order to compare the resistance to *P. thornei* of large numbers of wheat genotypes assessed across multiple glasshouse experiments.

In a previous study with chickpea, a factor analytic (FA) approach was used for a combined analysis of 10 glasshouse experiments that assessed resistance to *P. thornei* of 531 genotypes of chickpea, related wild species (*C. reticulatum*, *C. echinospermum*, and *C. bijugum*), and interspecific hybrids (Thompson et al. 2011). This FA approach was based on methods developed for multi-environment trial (MET) analysis of crop yields in regional field trials (Smith et al. 2001). In the context of glasshouse studies, experiments are dealt with similarly to environments in MET analyses. Genotype-experiment (G×E) interaction effects are modeled by an FA regression model in a mixed-model framework. This is in order to accommodate heterogeneity of genetic variance across experiments and heterogeneity of covariance and, hence, correlation between experiments, while adjusting for spatial trends. These data sets from multiple glasshouse experiments on genotypes conducted over a period of years are typically unbalanced with respect to the occurrence of genotypes among experiments, and can involve large numbers of both genotypes and experiments. This is similar to the large numbers of genotypes and environments usually encountered in MET data (Cullis et al. 2010). The dimensionality of the data defined by the number of experiments can be reduced by FA models to a number of underlying factors. Best linear unbiased predictors (BLUPs) (Piepho 1998; Robinson 1991) of nematode population density for the genotypes are obtained from the variance parameter estimates. This FA approach has been shown to provide improved prediction of genotype performance relative to traditional variance component models and is also superior to conducting separate analysis of individual experiments (Kelly et al. 2007).

In this article, we apply FA methods for a combined analysis of 22 replicated glasshouse experiments conducted from 1996 to 2015 that were designed to characterize the resistance to *P. thornei* of a large number of genetically fixed genotypes of wheat. We further show that this approach to combining glasshouse resistance experiments provides a single strong predictor of relative P_f of *P. thornei*, crop growth, and grain yield associated with specific wheat genotypes grown in *P. thornei*-infested fields. This provides a validated new method for ranking cereal genotypes on resistance to *P. thornei* for wheat improvement purposes and for growers' sowing guides.

MATERIALS AND METHODS

Glasshouse experiments. The data analyzed are from a series of experiments that we have conducted annually to characterize resistance to *P. thornei* of advanced wheat lines for plant breeding programs and wheat cultivars for growers' sowing guides. Data from 22 glasshouse experiments on resistance to *P. thornei* of 1,096 unique wheat genotypes were used for the combined analysis.

Genotypes of other crops were present in some of these experiments; namely durum, barley, and triticale. Canary grass (*Phalaris canariensis* ‘Moroccan’) as a resistant crop control and an unplanted soil control were also included in some experiments. The number of genotypes, experimental designs, and any differences in experimental procedures for the experiments in each year are given in Table 1.

Glasshouse experimental methods. In each experiment, single plants of each genotype were grown for 16 weeks in three replicate pots of soil, a black Vertosol (Isbell 1996) of the Irving Series (Thompson and Beckmann 1959) containing 78% clay from Wellcamp (latitude 27.55°S, longitude 151.87°E, elevation approximately 500 m, near Toowoomba, QLD, Australia). In experiments from 1996 to 2012, the soil was pasteurized at 70°C for 30 min to kill nematodes (Thompson 1990a) and fungal pathogens such as *Fusarium pseudograminearum* (Thompson 1990b) that might damage the plants and thereby limit *Pratylenchus thornei* reproduction. In later experiments (2013 to 2015), the temperature of pasteurization was increased to 85°C to ensure control of *Pythium* spp. originating from oospores (PennState Extension 2017). Each pot of soil was inoculated at sowing with a suspension of *P. thornei* at a rate of 10,000 nematodes/kg of soil (oven dry equivalent) extracted from open-pot cultures on wheat (O’Reilly and Thompson 1993). The strain of *P. thornei* was originally isolated from Formartin, QLD, Australia (latitude 27.46°S, longitude 151.43°E, elevation 364 m). In the experiments conducted from 1996 to 1997, methods for pot culture with top watering, as described by Thompson and Haak (1997), were used. In experiments conducted from 1999 to 2015, methods for pot culture with bottom watering, as described by Sheedy and Thompson (2009), were used. A summary of conditions for each experiment is given in Table 1. Experiments were laid out

on the glasshouse benches as three randomized complete blocks which had row-column spatial arrangements (Williams 1986), except in the first two experiments.

Water supply to the pots was ceased at approximately 15 weeks after sowing so that the soil dried down to approximately 45% moisture content in preparation for sampling at 16 weeks after sowing. This moisture content expedited processing of this soil type for nematode extraction. The soil and roots from each pot were placed in a tray where they were cut and broken manually into pieces of <1 cm, before thorough mixing. Soil moisture content was determined by drying a 150-g subsample at 105°C in a forced-draft oven for 48 h. Nematodes were extracted from 150-g subsamples of soil in Whitehead trays (Whitehead and Hemming 1965) for 48 h at 22°C and concentrated using a 20-µm aperture sieve into approximately 15 ml of water. *P. thornei* individuals were counted in a Peters 1-ml gridded slide (Chalex Corporation, Portland, OR, U.S.A.) under a compound microscope and population densities were expressed as number per kilogram of soil on an oven dry weight basis.

Field experiments. P_t of *P. thornei* in the soil profile following the growth of wheat genotypes in field experiments and canopy greenness and grain yield were used to validate the glasshouse assessments of resistance or susceptibility of wheat genotypes.

Two field experiments to assess advanced wheat breeding lines in comparison with existing cultivars were conducted in 2001 on a Black Vertosol of the Bongeen soil type (Harris et al. 1999) containing 60% clay near Macalister, Australia (latitude 27.03°S, longitude 151.07°E, elevation 337 m). Experiment 1 had 23 late-maturity wheat genotypes while experiment 2 had 52 main-maturity wheat genotypes, each with three replications. Both experiments were laid out in the field in three blocks as a row-column design

TABLE 1. Aspects of methods used for glasshouse experiments to assess wheat genotypes for resistance to *Pratylenchus thornei*

Exp ^b	Genotypes ^c	Experimental design ^a		Water ^d	Nutrient ^e	Temp ^f	Nematodes ^g	Mode ^h
		Rows	Columns					
1996	111	RB	RB	Top	Solution	No	2,500	In soil
1997	90	RB	RB	Top	Solution	No	2,500	In soil
1999	125	RC	RC	Bottom	Solution	Yes	10,000	Suspension
2000	126	RC	RC	Bottom	Solution	Yes	10,000	Suspension
2001	118	RC	RC	Bottom	Solution	Yes	10,000	Suspension
2002	129	10	39	Bottom	Solution	Yes	10,000	Suspension
2003	154	11	42	Bottom	Osmocote	Yes	10,000	Suspension
2005	143	18	24	Bottom	Osmocote	Yes	10,000	Suspension
2006	139	18	24	Bottom	Osmocote	Yes	10,000	Suspension
2007	228	29	24	Bottom	Osmocote	Yes	10,000	Suspension
2008	27	9	9	Bottom	Osmocote	Yes	10,000	Suspension
2009C	45	9	15	Bottom	Osmocote	Yes	10,000	Suspension
2009N	117	13	27	Bottom	Osmocote	Yes	10,000	Suspension
2010C	156	36	13	Bottom	Osmocote	Yes	10,000	Suspension
2010N	156	36	13	Bottom	Osmocote	Yes	10,000	Suspension
2011C	110	11	30	Bottom	Osmocote	Yes	10,000	Suspension
2011N	156	39	12	Bottom	Osmocote	Yes	10,000	Suspension
2012C	110	33	10	Bottom	Osmocote	Yes	10,000	Suspension
2012N	143	39	11	Bottom	Osmocote	Yes	10,000	Suspension
2013	144	36	12	Bottom	Osmocote	Yes	10,000	Suspension
2014	110	33	10	Bottom	Osmocote	Yes	10,000	Suspension
2015	120	36	10	Bottom	Osmocote	Yes	10,000	Suspension

^a Experimental design (number of rows and number of columns). All experiments had three replicates laid out as randomized complete blocks. RB = randomized block design only and RC = additional row column design but exact positions not available for the combined analysis of experiments.

^b Experiment (year conducted). C = preponderance of released cultivars in the experiment and N = preponderance of breeders’ advanced lines in the experiment.

^c Number of genotypes.

^d Watering system. Details of methods used with top-watered pots for 1996 to 1998 and with bottom-watered pots for 1999 to 2015 have been described by Thompson and Haak (1997) and Sheedy and Thompson (2009), respectively. For top watering, the soil was brought to 56% moisture content as required whereas, for bottom watering, the soil was held at a constant 2 cm of water tension.

^e Nutrient application. Solution = nutrients added from solutions to provide NO₃-N at 200 mg/kg of soil, P at 25 mg/kg of soil, K at 88 mg/kg of soil, S at 36 mg/kg of soil, Ca at 285 mg/kg of soil, and Zn at 5 mg/kg of soil. Osmocote = 1 g of Osmocote native gardens plus micronutrients (17:1.6:8.7 N-P-K) slow-release fertilizer pellets (Scotts Australia Pty Ltd., Baulkham Hills, Australia).

^f Under-bench control of soil temperature at 22°C.

^g Inoculum (nematodes per kilogram of soil).

^h Inoculum mode.

(Williams 1986), with plots being 8 m long by seven drill rows on 25-cm spacing. The plots were fertilized with N at 55 kg/ha as urea before sowing, and with Starter Z (Granulock Z, Incitec Pivot) at 40 kg/ha supplying N at 4.4 kg/ha, P at 8.7 kg/ha, S at 1.6 kg/ha, and Zn at 0.4 kg/ha applied with the seed at sowing. Seeding rate was adjusted based on grain weight and germination percentage of each genotype to sow 100 viable seeds/m². The cropping history of the land immediately prior to the experiments was wheat cultivar Hybrid Mercury, double cropped to black gram cultivar Regur (*V. mungo*), then clean fallowed until the wheat experiments were sown 14 months later. During the course of the wheat growing season, symptoms of damage from root-lesion nematodes were noted on some of the *P. thornei*-intolerant cultivars in the experiments. Therefore, after grain was harvested by machine, the three replicate plots of a subset of genotypes (11 in experiment 1 and 19 in experiment 2) were soil sampled to assess P_f of root-lesion nematodes. Deep soil samples were taken with a vehicle-mounted hydraulic soil corer using push tubes of 45 mm in diameter. Four positions were sampled per plot from the middle rows at approximately even intervals along the harvested length of 6 m of each plot. The four soil cores were subdivided and composited into one bulk in each of the following layers—0 to 15, 15 to 30, 30 to 45, 45 to 60, 60 to 90, 90 to 120, and 120 to 150 cm of depth—and placed in polythene bags. The soil was broken into pieces manually and mixed thoroughly, a 100-g subsample was oven dried to determine soil water content, and another 150-g subsample was extracted in Whitehead trays to determine nematode population densities. *P. thornei* was identified (Fortuner 1977) and counted as described above.

A field experiment to assess normalized difference vegetation index (NDVI) as a measure of the tolerance of wheat genotypes to *P. thornei* conducted in 2013 (Robinson et al. 2019) was also used to validate the glasshouse resistance experiments. This experiment was located on a Black Vertosol of the Waco series (Beckmann and Thompson 1960) with 70% clay, near Formartin. The land was managed in a 4-year rotation of sorghum, long fallow, bulk wheat, wheat experimental plots, then long fallow back to bulk sorghum, as described previously (Thompson et al. 1999). The partially resistant wheat line QT8343 and the susceptible wheat cultivar Kennedy were grown in a three-replicate strip design as the first wheat crop in the 4-year rotation to generate low and high *P. thornei* population densities, respectively. All test wheat cultivars were sown into both low (*P. thornei* at 2,570 individuals/kg of soil at 0 to 90 cm from

back-transformation of $\ln[x + 1]$ mean, where $\ln$ = natural logarithm or $\log_e$ where e is Euler's number, following QT8343) and high (9,090 individuals/kg of soil at 0 to 90 cm from back-transformation of $\ln[x + 1]$ mean following cultivar Kennedy) population densities in the following year (Robinson et al. 2019). Other field procedures were similar to those described for Macalister, except the rate of urea applied before sowing supplied N at 100 kg/ha to each crop.

After grain harvest in November, the soil was sampled by taking two cores/plot with a hydraulically driven push tube to 90 cm of depth at one-third intervals along the plot, and subdivided and composited in intervals of 0 to 30, 30 to 60, and 60 to 90 cm. Nematode population densities and soil water contents were determined as described above for the Macalister experiments.

Statistical analysis of glasshouse experiments. A consideration for the estimation of parameters in a multiexperiment model is the concurrence of genotypes; that is, the number of genotypes in common between pairs of experiments (Table 2). Most pairs of glasshouse experiments had suitable numbers of concurrent genotypes, except for the experiment 2009N, which had only two genotypes in common with eight other experiments (Table 2). The data on final *P. thornei* population densities were transformed by natural logarithms to ensure homogeneity of variance over the range of fitted values. The multiexperiment analysis modeled $\ln(P. thornei \text{ nematodes/kg of soil} + 1)$ in a linear mixed-model framework, following the approach of Smith et al. (2001). A fixed term was included for experiment effects and a random term was included for replicate effects for all experiments. An FA variance structure was fitted to the G×E effects, allowing for a different genetic variance for each experiment, and heterogeneous covariance (and hence correlation) between each pair of experiments. Spatial location of the pots in the two-dimensional arrangement on the glasshouse benches was fitted through a spatial correlation process across rows and columns (where significant for experiments from 2002 to 2015) following the approach of Gilmour et al. (1997). Random terms for row and column effects were included through the spatial modeling process, where significant. A crop-type factor was included in the analysis as a fixed effect to account for wheat, durum, barley, triticale, canary grass, and unplanted control.

The FA model was extended by including higher order terms in the model, until at least 90% of the genetic variance over all experiments was explained. The effective number of dimensions of the FA model was then tested with the Akaike information criterion as used by Beeck et al. (2010) and by assessing the percentage of

TABLE 2. Concurrence of genotypes between experiments (Exp) testing for resistance to *Pratylenchus thornei* in 22 glasshouse experiments conducted from 1996 to 2015^a

Exp	1996	1997	1999	2000	2001	2002	2003	2005	2006	2007	2008	2009C	2009N	2010C	2010N	2011C	2011N	2012C	2012N	2013	2014	2015
1996	111	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1997	22	90	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1999	33	24	125	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2000	29	20	54	126	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2001	27	21	45	63	118	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2002	34	27	39	47	69	129	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2003	29	21	32	34	38	56	154	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2005	21	15	27	31	33	36	43	143	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2006	24	15	28	32	34	38	41	87	139	...	...	...	...	...	...	...	...	...	...	...	...	...
2007	24	15	28	31	33	38	37	66	98	228	...	...	...	...	...	...	...	...	...	...	...	...
2008	4	4	4	4	4	5	7	11	19	20	27	...	...	...	...	...	...	...	...	...	...	...
2009C	17	12	20	21	25	30	30	27	29	33	9	45	...	...	...	...	...	...	...	...	...	...
2009N	2	2	2	2	2	2	3	4	5	9	4	3	117	...	...	...	...	...	...	...	...	...
2010C	30	15	31	36	40	59	41	44	50	53	14	40	7	156	...	...	...	...	...	...	...	...
2010N	4	4	5	4	5	6	6	5	6	8	4	5	59	11	156	...	...	...	...	...	...	...
2011C	15	6	16	17	21	31	22	25	31	37	11	28	9	96	20	110	...	...	...	...	...	...
2011N	5	5	5	4	5	5	14	4	5	6	4	4	24	11	70	11	156	...	...	...	...	...
2012C	6	5	6	6	7	23	12	8	6	9	7	11	15	79	31	75	24	110	...	...	...	...
2012N	4	4	5	4	5	5	8	4	5	5	4	4	8	11	41	11	66	11	143	...	...	...
2013	7	5	6	6	7	21	8	8	6	8	7	10	12	60	32	53	36	75	53	144	...	...
2014	7	5	7	5	6	13	5	4	5	5	4	5	2	27	14	20	21	27	41	73	110	...
2015	8	4	6	5	6	7	6	5	6	6	4	5	2	17	14	14	19	14	36	59	69	120

^a Total number of genotypes in each experiment is shown on the diagonal, and number of genotypes in common between pairs of experiments is shown in the off-diagonal cells.

variance accounted for by successively added factors. The estimated FA loadings were rotated to a principal components solution (Cullis et al. 2010), such that the first component axis accounted for the maximum amount of genetic covariance in the data and the second accounted for the next greatest amount, and so on for subsequent axes, and all the axes were orthogonal. A genetic correlation matrix between pairs of experiments was produced. The output from the FA analysis gave predictions of genotype performance in each experiment as regression empirical BLUPs (R-eBLUPs) (Cullis et al. 2010). For selected genotypes, the R-eBLUPs were plotted against loadings for principal axis 1 (PA1) of the individual experiments to produce a latent regression plot for which the slope is the PA1 score of that genotype (Smith et al. 2015). The overall resistance or susceptibility of genotypes was compared using a single value for each genotype as PA(1+2)-eBLUPs, where PAs are from the principal components solution. The PA(1+2)-eBLUP for each wheat genotype was calculated as the respective PA1 score multiplied by the average of the 22 rotated loadings for PA1 plus the respective PA2 score multiplied by the average of the 22 rotated loadings for PA2. These values were rescaled by addition of the estimate for the overall mean for wheat in units of $\ln(P. thornei \text{ nematodes/kg of soil} + 1)$ and then back-transformed by exponentiation where required.

These statistical analyses were performed using ASReml-R (Butler et al. 2009) in the R software environment (R Core Team 2016). Variance parameters were estimated using residual maximum likelihood estimation (Patterson and Thompson 1971).

Prediction of field P_f of *P. thornei* from *P. thornei* resistance. The population densities of *P. thornei* in the field after harvest of the two experiments at Macalister and the experiment at Formartin were subject to analysis of variance by depth interval after transformation by $\ln(x + 1)$. Significant differences were obtained among cultivars to 45 cm of depth at Macalister and 90 cm of depth at Formartin. Nonlinear regression analysis was conducted between the mean population densities of *P. thornei* in the soil profile (0 to 45 cm of depth at Macalister and 0 to 90 cm at Formartin) as response variables and PA(1+2)-eBLUPs of $\ln(P. thornei \text{ nematodes/kg of soil} + 1)$ from the combined glasshouse experiments as the explanatory variable using Genstat for Windows (VSN International 2014).

Prediction of NDVI and grain yield of wheat cultivars in field experiments from *P. thornei* resistance. The relationships between the grain yield of the cultivars in the three field experiments and the PA(1+2)-eBLUPs of $\ln(P. thornei \text{ nematodes} + 1)$ from combined glasshouse experiments were examined graphically and appropriate nonlinear regression equations fitted in Genstat for Windows (VSN International 2014). Similarly, the relationships between the area under the disease progress curve (AUDPC) of seven readings of NDVI taken to measure vegetation greenness at intervals from 64 to 126 days after sowing of the genotypes in the Formartin experiment (Robinson et al. 2019) were determined.

Rating of resistance of wheat genotypes. The single range of log-transformed PA(1+2)-eBLUPs for the 1,096 wheat genotypes from MET analysis of the combined experiments was divided into nine equal subranges. Genotypes within these nine categories were assigned alpha ratings as used for diseases in Australian wheat variety guides for growers (Lush 2018; Matthews et al. 2017) as follows: R, resistant to moderately resistant (R-MR), moderately resistant (MR), moderately resistant to moderately susceptible (MR-MS), moderately susceptible (MS), moderately susceptible to susceptible (MS-S), susceptible (S), susceptible to VS (S-VS), and VS.

RESULTS

Combined glasshouse experiments. A graphical display of the between-environments genetic correlation matrix between all pairs of 22 experiments from the MET analysis of *P. thornei*

population densities of wheat genotypes is shown in Figure 1. The data from the experiments were well correlated, having 75% of all correlation coefficients between pairs of experiments >0.70 and with a median value of 0.80. This shows strong agreement in relative nematode population densities for individual genotypes between experiments.

Predicted values from the FA model for experiment mean population density of *P. thornei*, genetic variance and error variance, as well as principal components loadings and percentage genetic variance accounted for by PA1 and PA2 in individual experiments, are shown in Table 3. An FA model of order 2 (designated FA-2) was retained as the final model, explaining 90% of the genetic variance over all experiments (79% by PA1 and 11% by PA2) (Table 3). The Akaike information criterion (value for FA-2 = 0 compared with FA-1 = 10 and FA-3 = 9) supported the decision to select the FA-2 model. Every experiment had at least 67% of genetic variance accounted for by this FA-2 model, and every experiment had $>55\%$ genetic variance accounted for by PA1 alone, except for the 2006 experiment, with 42% (Table 3). Loadings along PA1 were positive for all experiments, ranging from 0.169 to 0.709 (Table 3). Loadings along PA2 ranged from -0.358 to 0.389 , with experiments having larger negative loadings being 2010N, 2009C, 2010C, 2008, and 2002, whereas experiments having larger positive loadings were 2012N, 2005, 1997, 2014, 2015, 2006, and 2009N. There was no noticeable difference between these two groups of experiments in relation to the chronology of the experiments or variation of the method used (Table 1), or the mean final *P. thornei* population densities of the experiments (Table 3).

Latent regression plots of R-eBLUPs of population densities of *P. thornei* for PA1 against experiment loadings for the 22 experiments are shown for some selected reference genotypes in Figure 2. Genotypes with a positive slope or high PA1 score produced higher nematode densities in most experiments, while genotypes with a negative slope or low PA1 score produced lower nematode densities in most experiments. The deviation of R-eBLUPs from the line was an indication of additional variation for resistance of particular genotypes across the experiments measured in the dimension of PA2; that is, the PA2 score.

For genotypes that occurred in four or more experiments, scores for PA1 and PA2 and PA(1+2)-eBLUPs with probabilities of exceeding values of these for two reference genotypes are given in Supplementary Table S1, while these results for a subset of 60 genotypes mentioned in this article or present in the field experiments is given in Table 4.

Fig. 1. Between-experiments genetic correlation matrix from the factor analytic (order 2) model for resistance testing of *Pratylenchus thornei* in 22 glasshouse experiments from 1996 to 2015.

Relationship between field P_f of *P. thornei* and resistance. In the Macalister field experiments, there were significant relationships for final *P. thornei* population densities at soil profile depths of 0 to 15, 15 to 30, and 30 to 45 cm but not at depths below 45 cm whereas, in the Formartin experiment, there were significant relationships at all depths of 0 to 30, 30 to 60, and 60 to 90 cm (not shown). There were highly significant ($P < 0.001$) linear relationships between the field population densities of *P. thornei* in the deep soil profile (0 to 45 cm at Macalister and 0 to 90 cm at Formartin) after growth of wheat genotypes in the three field experiments and the glasshouse-derived PA(1+2)-eBLUPs of those genotypes (Fig. 3).

Relationship between crop greenness at high and low population densities of *P. thornei* and resistance. There were highly significant negative exponential relationships between AUDPC of NDVI measurements at both high and low *P. thornei* population densities in the field experiment at Formartin and glasshouse-derived PA(1+2)-eBLUPs of *P. thornei* population densities (Fig. 4).

Relationship between grain yield of wheat cultivars on *P. thornei*-infested sites and resistance. Grain yield of the wheat cultivars in the two field experiments at Macalister showed a significant negative sigmoidal relationship with glasshouse-derived measures of resistance of the same wheat cultivars expressed as PA(1+2)-eBLUPs of $\ln(\text{population density of } P. thornei \text{ nematodes/kg of soil})$ from combined glasshouse experiments (Fig. 5). There were highly significant negative exponential relationships between grain yields at both high and low *P. thornei* population densities in the field experiment at Formartin and glasshouse-derived PA(1+2)-eBLUPs of *P. thornei* population densities (Fig. 6A and B). Also, there was a highly significant exponential relationship between grain yield loss (calculated as the percentage difference in yields between low and high *P. thornei* population densities) in the field experiment at Formartin and glasshouse-derived PA(1+2)-eBLUPs of *P. thornei* population densities (Fig. 6C).

Resistance ratings of wheat genotypes. The PA(1+2)-eBLUPs of the 1,096 wheat genotypes (Table 4; Supplementary

Table S1) ranged from a minimum for CPI133872 (present in 13 experiments, with a back-transformed mean P_f of 7,171 *P. thornei* nematodes/kg of soil) to a maximum for cultivar Darwin (present in 4 experiments, with a back-transformed mean P_f of 104,192 *P. thornei* nematodes/kg of soil) (Table 4). The distribution of the 1,096 wheat genotypes in this study in relation to the nine categories of resistance ratings based on subranges of PA(1+2)-eBLUPs is given in Figure 7.

Most of the wheat genotypes (62%) tested in these experiments were in the three rating categories (S, S-VS, and VS) most susceptible to *P. thornei*. In contrast, only 2% of the wheat genotypes were in the most resistant three rating categories (R, MR-R, and MR). These most-resistant genotypes were mainly germplasm sources of resistance such as the synthetic hexaploid wheat CPI133872 (Thompson 2008; Zwart et al. 2004) or GS50a (a resistant selection from wheat cultivar Gatcher) (Thompson et al. 1999) and lines derived from back-crossing these into susceptible wheat cultivars; for example, QT8343 and QT9048 (Table 4). Two commercial cultivars, Impose CL Plus and Wyalkatchem, both adapted to the Mediterranean (western) grain region of Australia, were rated as MR. The category MR-MS included 10 named cultivars (Table 4), of which Gauntlet, Wallup, Sunmate, Suntime, Suntop, and Ventura are adapted to the subtropical (northern) grain region of eastern Australia, while Bolac, Kiora, Amarok, and Corack are adapted to the more temperate (southern) grain region of Australia. At the other end of the resistance spectrum were the VS wheat genotypes (Table 4), which included Petrie (a northern region cultivar), Brennan (a northern region forage wheat), and the southern region cultivars Darwin, Wedgetail, Annuello, and Forrest. The western region wheat cultivar Yandanooka, previously proposed as a VS reference cultivar (Sheedy et al. 2015), was categorized as S-VS in this study.

DISCUSSION

This is the first large-scale MET analysis of multiple glasshouse experiments to determine the resistance to *P. thornei* of an extensive range of wheat genotypes, in which the output has been used to

TABLE 3. Parameters from combined analysis using a factor analytic structure of 22 experiments (Exp) testing resistance to *Pratylenchus thornei* of wheat genotypes used for derivation of empirical best linear unbiased predictors of individual genotypes

Exp	Mean <i>P. thornei</i> nematodes/kg of soil ^a		Variance		Principal component loadings ^b		Genetic variance accounted for (%)		
	$\ln(x + 1)$	BTM	Genetic	Error	PA1	PA2	PA1	PA2	PA1+PA2
1996	10.663	42,753	0.046	0.169	0.196	-0.044	83	4	88
1997	10.965	57,804	0.105	0.270	0.277	0.169	73	27	100
1999	11.981	159,764	0.303	0.357	0.542	-0.097	97	3	100
2000	11.063	63,759	0.503	0.636	0.709	0.004	100	0	100
2001	12.092	178,504	0.336	0.379	0.579	-0.039	100	0	100
2002	11.945	154,095	0.180	0.266	0.394	-0.106	87	6	93
2003	10.597	39,995	0.435	1.438	0.602	0.034	83	0	84
2005	11.528	101,478	0.175	0.574	0.328	0.260	61	39	100
2006	11.564	105,263	0.068	0.815	0.169	0.141	42	29	71
2007	12.510	271,020	0.193	0.541	0.387	0.099	78	5	83
2008	11.226	75,026	0.127	0.368	0.331	-0.132	86	14	100
2009C	12.103	180,367	0.484	0.780	0.521	-0.232	56	11	67
2009N	12.313	222,542	0.271	0.647	0.507	0.117	95	5	100
2010C	12.327	225,763	0.407	0.777	0.597	-0.224	88	12	100
2010N	12.529	276,301	0.606	0.381	0.691	-0.358	79	21	100
2011C	11.153	69,746	0.335	0.618	0.514	-0.096	79	3	81
2011N	11.492	97,959	0.520	0.308	0.613	-0.056	72	1	73
2012C	10.069	23,590	0.441	1.019	0.571	0.108	74	3	76
2012N	9.315	11,102	0.606	1.031	0.674	0.389	75	25	100
2013	11.372	86,866	0.218	0.391	0.397	0.095	72	4	76
2014	10.952	57,044	0.247	0.350	0.413	0.155	69	10	79
2015	9.723	16,702	0.158	0.490	0.371	0.144	87	13	100
Overall	...	...	...	...	...	...	79	11	90

^a Abbreviations: $\ln$ = natural logarithm or $\log_e$, where e is Euler's number and BTM = back-transformed mean by exponentiation.

^b PA = principal axes after rotation to principal components solution.

predict relative final nematode populations and grain yield of wheat genotypes in independent field experiments. We ranked more than 1,000 wheat genotypes for resistance to *P. thornei* by combining results from 22 experiments conducted during the period 1996 to 2015. This was achieved using an FA approach with rotation of axes to a principal components solution. A strong effect of wheat genotype on the P_f of *P. thornei* in all experiments was exhibited in PA1, which can be considered a stable resistance axis accounting for 79% of the genetic variance. Furthermore, PA2, which accounted for an additional 11% of genetic variance, can be considered a measure of additional genetic variability for resistance across

different experiments. One possible cause of the greater variability for resistance of some wheat genotypes could be incomplete genetic fixation of the genotype, whereas other genotypes might have been fixed through single plant selection and a greater number of inbred generations. Another possible reason is unidentified environmental variation among the experiments resulting in some G×E effects.

The overall predicted value for the P_f of *P. thornei* based on PA(1+2)-eBLUPs from the FA-2 model can be considered the best single index for the resistance level of genotypes included in this investigation. A quantitative measure as used in this study rather

Fig. 2. Latent regression plots of predicted *Pratylenchus thornei* population density as regression empirical best linear unbiased predictors (R-eBLUPs) against estimated loadings for principal axis 1 (PA1) in 22 glasshouse experiments showing a selection of wheat genotypes with lower, intermediate, and higher nematode population densities and stabilities. Dark points indicate genotype present in the experiment associated with the loading, whereas light points indicate an estimate where genotype was absent from the experiment associated with the loading.

TABLE 4. Genotypic scores for principal axes 1 and 2 (PA1 and PA2) and predicted final population densities of *Pratylenchus thornei* for 60 selected wheat genotypes used in field experiments and present in 4 or more of 22 experiments comprising 1,096 wheat genotypes in a combined analysis^a

Rank ^b	Genotype	PA scores		<i>P. thornei</i> nematodes/kg of soil		Probability > reference		N ^c	Rating ^d
		PA1	PA2	PA(1+2)-eBLUP	BTM	R-MR	S-VS		
1	<i>CP1133872</i>	-3.783	0.917	8.88	7,171	0	0	13	R
4	QT8343	-2.839	-2.363	9.28	10,667	0.226	0	11	R-MR
5	QT9048	-2.694	-1.186	9.36	11,625	0.425	0	9	R-MR
6	<i>GS50a</i>	-2.666	-0.040	9.39	11,991	NA	0	22	R-MR
14	Impose CL Plus	-2.488	1.243	9.49	13,279	0.694	0	4	MR
17	QT9050	-2.246	-0.232	9.59	14,573	0.908	0	12	MR
20	<i>QT8447</i>	-2.086	0.577	9.67	15,898	0.969	0	12	MR
24	Wyalkatchem	-1.937	0.657	9.75	17,085	0.946	0	5	MR
28	Bolac	-1.790	0.356	9.81	18,214	0.967	0	4	MR-MS
30	Kiora	-1.728	-0.748	9.82	18,471	0.955	0	4	MR-MS
34	Gauntlet	-1.716	-0.355	9.84	18,675	0.989	0	5	MR-MS
40	Sunmate	-1.594	-0.700	9.89	19,672	0.974	0	4	MR-MS
41	Amarok	-1.597	-0.529	9.89	19,711	0.988	0	5	MR-MS
51	Corack	-1.523	1.016	9.95	20,868	0.996	0	4	MR-MS
62	Wallup	-1.368	0.329	10.01	22,225	0.999	0	4	MR-MS
65	Suntime	-1.305	-0.389	10.03	22,674	0.994	0	4	MR-MS
73	<i>Suntop</i>	-1.244	-0.979	10.05	23,132	0.999	0	4	MR-MS
75	Ventura	-1.249	0.038	10.06	23,411	1	0	9	MR-MS
79	Chara	-1.219	-0.059	10.07	23,717	1	0	11	MS
107	Glover	-1.026	0.048	10.17	26,029	1	0	5	MS
120	<i>Hartog</i>	-0.995	0.541	10.19	26,608	1	0	14	MS
145	Sunzell	-0.845	-0.091	10.25	28,282	1	0	5	MS
191	Leichhardt	-0.651	-0.267	10.34	30,914	1	0	11	MS
287	Sunvale	-0.412	-0.517	10.45	34,474	1	0	11	MS-S
310	Kidman	-0.385	0.000	10.47	35,206	1	0	5	MS-S
329	Giles	-0.338	0.172	10.49	36,061	1	0	11	MS-S
347	<i>Gregory</i>	-0.264	-0.532	10.52	36,974	1	0	10	MS-S
374	Spitfire	-0.169	-1.272	10.55	38,253	1	0.002	5	MS-S
378	Yenda	-0.189	-0.420	10.56	38,368	1	0.004	4	MS-S
384	Wylie	-0.213	0.712	10.56	38,560	1	0	9	MS-S
425	Bowerbird	-0.077	-0.443	10.61	40,416	1	0.001	6	MS-S
437	Baxter	-0.029	-1.548	10.61	40,700	1	0	12	MS-S
557	Sunco	0.142	-1.254	10.70	44,311	1	0.003	11	S
10	Lang	0.214	-0.874	10.74	46,119	1	0.005	11	S
654	Batavia	0.204	2.060	10.78	47,905	1	0.003	21	S
659	Hume	0.260	0.687	10.78	48,193	1	0.014	9	S
663	Sunguard	0.284	-0.020	10.78	48,242	1	0.029	5	S
674	Sunvex	0.278	0.520	10.79	48,484	1	0.054	5	S
688	Waagan	0.327	0.119	10.81	49,315	1	0.049	6	S
691	Banks	0.375	-1.295	10.81	49,414	1	0.016	11	S
715	Crusader	0.399	-0.151	10.84	50,817	1	0.058	6	S
816	<i>Cunningham</i>	0.547	-0.050	10.91	54,611	1	0.049	12	S
905	Janz	0.675	0.579	10.98	58,512	1	0.100	13	S-VS
935	Babbler	0.781	-0.530	11.01	60,535	1	0.186	4	S-VS
936	Stampede	0.772	0.139	11.02	60,839	1	0.219	6	S-VS
60	<i>Strzelecki</i>	0.815	0.261	11.04	62,254	1	0.185	10	S-VS
998	Kennedy	0.962	-0.872	11.09	65,577	1	0.263	13	S-VS
1,002	H91	0.956	-0.128	11.10	66,104	1	0.313	4	S-VS
1,004	Kukri	0.968	-0.423	11.10	66,236	1	0.317	4	S-VS
1,008	Gazelle	0.991	-0.712	11.11	66,635	1	0.315	5	S-VS
1,018	Gatcher	0.970	0.785	11.12	67,507	1	0.314	18	S-VS
1,054	Impala	1.162	-0.359	11.19	72,619	1	0.481	5	S-VS
1,059	Yandanooka	1.210	-1.222	11.20	73,423	1	NA	9	S-VS
1,070	Lincoln	1.243	1.158	11.25	77,187	1	0.581	4	S-VS
1,079	Forrest	1.380	0.176	11.30	81,145	1	0.684	5	VS
1,083	Annuello	1.445	-0.175	11.33	83,199	1	0.699	4	VS
1,084	Wedgetail	1.421	0.675	11.33	83,365	1	0.722	5	VS
1,091	Brennan	1.581	-0.050	11.40	88,875	1	0.803	5	VS
1,095	<i>Petrie</i>	1.719	-0.356	11.46	94,465	1	0.916	11	VS
1,096	Darwin	1.893	0.714	11.55	104,192	1	0.901	4	VS

^a Genotypes have been assigned resistance ratings based on subdivision of the PA1 and PA2 empirical best linear unbiased predictors (PA[1+2]-eBLUPs) range of natural logarithm (ln)(*P. thornei* nematodes per kilogram of soil) into nine arithmetically equal categories. BTM = back-transformed means by exponentiation. Probabilities of values greater than the reference genotypes GS50a (R-MR) and Yandanooka (S-VS) proposed by Sheedy et al. (2015) are given. NA = not applicable. Derived resistance ratings are R = resistant, R-MR = resistant to moderately resistant, MR = moderately resistant, MR-MS = moderately resistant to moderately susceptible, MS = moderately susceptible, MS-S = moderately susceptible to susceptible, S = susceptible, S-VS = susceptible to very susceptible, and VS = very susceptible. Genotypes chosen to be resistance references in these nine categories for future experiments are shown in italics.

^b Reference rank.

^c Number of experiments.

^d The alpha resistance rating based on PA(1+2)-eBLUP.

than a qualitative approach to characterizing the resistance to *P. thornei* is required in view of the knowledge that resistance to this nematode species is inherited quantitatively under the control of multiple genes having additive gene action (Thompson and Seymour 2011; Thompson et al. 2012b; Zwart et al. 2004).

The PA(1+2)-eBLUP values for genotypes were shown to be a valuable parameter in predicting relative field population densities of *P. thornei* developed under different wheat genotypes. These results validate the glasshouse methods for assessing resistance of wheat genotypes to *P. thornei* and provide confidence in the application of this information to field situations. Foremost among these applications are an ordinal alpha ratings for growers' sowing guides; however, they could also be valuable variables in crop growth models incorporating nematode population dynamics as influenced by wheat cultivar choice.

Fig. 3. Predictive ability of glasshouse-derived principal axes 1 and 2 empirical best linear unbiased predictors (PA[1+2]-eBLUPs) of natural logarithm (ln)(*Pratylenchus thornei* nematodes per kilogram of soil) for field population densities of *P. thornei* in the soil profile of two experiments at Macalister and one at Formartin for various wheat genotypes. Bar marker is least significant difference ($P = 0.05$). **A**, For Macalister experiment 1, soil profile to 45-cm depth, $FPD = 0.744GHBLUP + 1.330$, $R^2 = 0.73$, $P < 0.001$, $n = 11$. **B**, for Macalister experiment 2, soil profile to 45-cm depth, $FPD = 0.76GHBLUP + 1.330$, $R^2 = 0.65$, $P < 0.001$, $n = 19$. **C**, For Formartin, soil profile to 90-cm depth at low *P. thornei*: $FPD = 1.606GHBLUP - 9.20$, $R^2 = 0.92$, $P < 0.001$, $n = 16$, and at high *P. thornei*: $FPD = 1.335GHBLUP - 5.78$, $R^2 = 0.89$, $P < 0.001$, $n = 16$. FPD = field population density ln(*P. thornei* nematodes per kilogram of soil + 1) and $GHBLUP$ = glasshouse (PA1+2)-eBLUP of ln(*P. thornei* nematodes per kilogram of soil + 1).

Previously, genetic correlation ($r > 0.66$) was found for data on P_f of *P. thornei* between a single glasshouse experiment evaluating 47 genotypes of chickpea and six field experiments (five from the subtropical grain region and one from the warm temperate grain region of Victoria) sampled to either 15 or 30 cm of soil depth and evaluating a total of 85 chickpea genotypes in the one MET analysis (Rodda et al. 2016). Recently, high genetic correlation ($r > 0.9$) was found between environments for population densities of *P. thornei* in the top soil (0 to 10 or 0 to 15 cm) after harvest of 68 cereal genotypes in six field experiments in the temperate region of southern Australia, except where the fungal pathogen *Rhizoctonia solani* was damaging (Fanning et al. 2018). In the present study with wheat, we have preferred to analyze our glasshouse data in a MET analysis separately from any field data and then to use the output of the MET analysis to predict relative *P. thornei* population densities in the independent field experiments. This approach has validated our glasshouse methods for assessing *P. thornei* resistance of wheat genotypes and demonstrated their value for predicting relative final *P. thornei* population densities developed in the field when various wheat cultivars are grown. In addition, the glasshouse method provides surety of results each year compared with field testing, where some experiments can be lost through environmental

Fig. 4. Predictive ability of glasshouse-derived principal axes 1 and 2 empirical best linear unbiased predictors (PA[1+2]-eBLUPs) of natural logarithm (ln)(*Pratylenchus thornei* nematodes per kilogram of soil) for area under the disease progress curve (AUDPC) of seven measurements of crop greenness by normalized difference vegetation index (NDVI) at seven sensing times from 64 to 126 days after sowing of 28 wheat genotypes grown at two initial population densities of *P. thornei* at Formartin. Bar marker is least significant difference ($P = 0.05$). **A**, NDVI measurements at high initial population density (P_i) of *P. thornei*: $AUDPC = 46.63 - 0.00000016 \times 5.36^{(GHBLUP)}$, $R^2 = 0.62$, $P < 0.001$, $n = 28$. **B**, NDVI measurements at low P_i of *P. thornei*: $AUDPC = 43.7 - 0.00000000000286 \times 16.3^{(GHBLUP)}$, $R^2 = 0.54$, $P < 0.001$, $n = 28$. $AUDPC$ was measured in NDVI units and $GHBLUP$ = PA(1+2)-eBLUPs of ln(final population density of *P. thornei* nematodes per kilogram of soil) from combined glasshouse experiments.

extremes such as drought, flood, and hail or pests such as feral animals or fungal diseases. In contrast, only one glasshouse experiment—namely, that conducted in 2004—was deemed unsuitable for combined analysis because of aberrant results caused by manually processing the soil for nematode extraction when it was too wet.

In the deep clay soils of the subtropical grain region of Australia, *P. thornei* can occur in the soil profile to a 90-cm depth. Our results showed that the genotype PA(1+2)-eBLUPs from the combined glasshouse experiments were predictive of the genotype P_f to depths of 45 cm at Macalister and 90 cm at Formartin. This shows the influence of growing different wheat genotypes on *P. thornei* population densities throughout the soil profile, and that the combined analysis of the glasshouse experiments as described provides measures that can be used to rank genotypes on how they affect nematode population densities throughout the whole soil profile.

To produce resistance ratings of cultivars for wheat sowing guides, the subdivision of the range of PA(1+2)-eBLUPs into nine equal classes converted to nine α ratings as required is a useful simplification but, for other purposes, the numeric values of PA(1+2)-eBLUPs are preferable. Each year, there is a requirement to assess prospective new cultivars for resistance to *P. thornei* and our established database is an asset for this purpose. Adding new experimental data to the existing database for MET analysis will allow new wheat genotypes to be assessed reliably for resistance

Fig. 5. Predictive ability of glasshouse-derived principal axes 1 and 2 empirical best linear unbiased predictors (PA[1+2]-eBLUPs) of natural logarithm (ln)(*Pratylenchus thornei* nematodes per kilogram of soil) for grain yield of wheat cultivars in a *P. thornei*-infested field at Macalister for 23 late-maturing wheat genotypes in experiment 1 and 52 main-maturity wheat genotypes in experiment 2. Bar marker is least significant difference ($P = 0.05$). **A**, Grain yield in experiment 1: $GY = 0.931 + 1.519/[1 + \exp\{8.44 \times (GHBLUP - 10.632)\}]$, $R^2 = 0.47$, $P < 0.002$, $n = 23$. **B**, Grain yield in experiment 2: $GY = 1.546 + 0.9589/[1 + \exp\{11.31 \times (GHBLUP - 10.52)\}]$, $R^2 = 0.50$, $P < 0.001$, $n = 52$. GY = grain yield (t/ha) and $GHBLUP$ = PA(1+2)-eBLUPs of ln(final population density of *P. thornei* nematodes per kilogram of soil) from combined glasshouse experiments.

within the framework of covariance across experiments through concurrence of other genotypes, including the reference set. We are applying a similar approach to analysis of glasshouse resistance experiments for *P. neglectus* of wheat genotypes for growers' sowing guides (Lush 2018; Matthews et al. 2017), and the methods used here could be useful in other crops and with other nematode species.

The fact that the wheat genotypes in this study are representative of Australian cultivars and plant breeders' advanced breeding lines and that most were found to be susceptible to very susceptible to *P. thornei* indicates the need for concerted efforts to improve resistance levels. Excellent levels of resistance have been identified in several unadapted germplasm sources which are now being hybridized into adapted wheat backgrounds for plant

Fig. 6. Predictive ability of glasshouse-derived principal axes 1 and 2 empirical best linear unbiased predictors (PA[1+2]-eBLUPs) of natural logarithm (ln)(*Pratylenchus thornei* nematodes per kilogram of soil) for grain yield of wheat cultivars in a *P. thornei*-infested field at Formartin for 28 wheat genotypes grown with high and low initial population densities (P_i) of *P. thornei*. Bar marker is least significant difference ($P = 0.05$). **A**, Grain yield at high *P. thornei*: $GY = 4.113 - 0.000000087 \times 4.68^{(GHBLUP)}$, $R^2 = 0.58$, $P < 0.001$, $n = 28$. **B**, Grain yield at low *P. thornei*: $GY = 3.477 - 0.000000000101 \times 21.9^{(GHBLUP)}$, $R^2 = 0.45$, $P < 0.001$, $n = 28$. **C**, Grain yield loss: $GYL = 17.1 - 0.000006 \times 4.23^{(GHBLUP)}$, $R^2 = 0.56$, $P < 0.001$, $n = 28$. GY = grain yield (t/ha), GYL = grain yield loss (%), and $GHBLUP$ = PA(1+2)-eBLUPs of ln(final population density of *P. thornei* nematodes per kilogram of soil) in combined glasshouse experiments. GYL (%) = $100 \times [(GY \text{ at low } P_i - GY \text{ at high } P_i)/GY \text{ at low } P_i]$.

breeders' use to produce more resistant cultivars for growers (Sheedy et al. 2017).

From analysis of six experiments comparing methods for testing for resistance to *P. thornei*, in which 23 bread wheat genotypes were evaluated, Sheedy et al. (2015) selected 7 for use as provisional reference cultivars for future experiments. The more comprehensive analyses reported here, with 1,096 genotypes and 22 experiments, have permitted an improved selection of reference cultivars. One genotype has been selected to represent the resistance level in each of nine ordered categories ranging from R to VS based on equal subdivision of the range of values for PA(1+2)-eBLUPs in units of $\ln(P. thornei \text{ nematodes/kg of soil})$. These new reference genotypes for the nine resistance categories are R = CPI133872, R-MR = GS50a, MR = QT8447, MR-MS = Suntop, MS = Hartog, MS-S = Gregory, S = Cunningham, S-VS = Strzelecki, and VS = Petrie. These reference genotypes have been selected based on their overall levels of resistance within each category and on a low standard error of the PA(1+2)-eBLUP. Of those reference genotypes suggested by Sheedy et al. (2015), CPI133872 has been retained to represent the R category and GS50a has been retained to represent the R-MR category of resistance. Other genotypes have been nominated for categories in which no reference genotype was nominated by Sheedy et al. (2015), while others have been replaced by preferred reference genotypes.

Growers are primarily interested in producing grain, and the capacity of wheat cultivars to do this under field environmental conditions in soil heavily infested with *P. thornei* in the subtropical grain region of eastern Australia is provided as tolerance ratings in sowing guides (Lush 2018). Resistance ratings are also provided in sowing guides and they are often regarded as a measure of the impact that a cultivar will have on the population densities of *P. thornei* residual in the soil to attack a subsequent crop. However, from this study, it is apparent that the level of resistance or susceptibility of crop cultivars is a major determinant of their growth and grain yield on sites infested with *P. thornei* in this region. This effect of resistance on plant growth was illustrated diagrammatically by Roberts (2002), in which a resistant genotype produced not only fewer nematodes but also a larger plant than a tolerant genotype that allowed greater nematode reproduction. Clearly, because of the polycyclic nature of *P. thornei*, as the nematode numbers increase in the roots of susceptible wheat genotypes they cause more damage to the plant root systems, which results in poorer vegetative growth and grain yield than in wheat genotypes with greater levels of resistance. This better comprehension of the role that resistance plays in *P. thornei* population changes and grain yield of wheat cultivars in the subtropical grain region of

Australia emphasizes the value of genetic resistance for growers, the importance of the trait as a target for wheat breeding, and the ongoing need to accurately characterize the resistance to *P. thornei* of all wheat cultivars to be released by plant breeding companies for growers' use.

In summary, combining data on P_f of *P. thornei* for 1,096 wheat genotypes in 22 glasshouse experiments by a MET analysis was an effective way to compare the resistance of wheat genotypes. A two-factor model explained 90% of the genetic variance, with 79% of the genetic variance accounted for in PA1, regarded as a stable resistance axis, reflecting the high genetic correlation among experiments. PA2 explained an additional 11% of the genetic variance, indicating that the resistance levels of some genotypes could not be fully explained by PA1 alone. Genotype scores of PA(1+2)-eBLUPs in units of $\ln(P. thornei \text{ nematodes/kg of soil} + 1)$ from these glasshouse results were highly predictive of relative P_f of *P. thornei* to depth in the soil profile after growth of various wheat genotypes in three field experiments. There were also highly significant nonlinear relationships between glasshouse-derived resistance levels and genotype performance in the field assessed by greenness of vegetative biomass and grain yield. Subdivision of the range of PA(1+2)-eBLUPs into nine subranges is presented as an objective method for producing ordinal or alpha resistance ratings for growers' sowing guides. The majority of genotypes tested were in the top three susceptibility ratings, indicating the need for continued germplasm development to raise the level of resistance to *P. thornei* in wheat cultivars available to Australian growers.

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LITERATURE CITED

- Beckmann, G. G., and Thompson, C. H. 1960. Soils and Land Use in the Kurrawa Area, Darling Downs, Queensland. CSIRO Division of Soils, Soils Land Use Ser. No. 37. CSIRO, Melbourne, VIC, Australia.
- Beeck, C., Cowling, W. A., Smith, A. B., and Cullis, B. R. 2010. Analysis of yield and oil from a series of canola breeding trials. Part I. Fitting factor analytic mixed models with pedigree information. *Genome* 53:992-1001.
- Butler, D. G., Cullis, B. R., Gilmour, A. R., and Gogel, B. J. 2009. ASReml-R Reference Manual. Tech. Rep. 3. Queensland Department of Agriculture, Fisheries and Forestry, Brisbane, QLD, Australia.
- Cullis, B. R., Smith, A. B., Beeck, C. P., and Cowling, W. A. 2010. Analysis of yield and oil from a series of canola breeding trials. Part II. Exploring variety by environment interaction using factor analysis. *Genome* 53:1002-1016.
- De Waele, D., and Elsen, A. 2002. Migratory endoparasites: *Pratylenchus* and *Radopholus* species. Pages 175-206 in: *Plant Resistance to Parasitic Nematodes*. J. L. Starr, R. Cook, and J. Bridge, eds. CABI Publishing, Wallingford, U.K.
- Fanning, J., Linsell, K., McKay, A., Gogel, B., Munoz Santa, I., Davey, R., and Hollaway, G. 2018. Resistance to the root lesion nematodes *Pratylenchus thornei* and *P. neglectus* in cereals: Improved assessments in the field. *Appl. Soil Ecol.* 132:146-154.
- Farsi, M., Vanstone, V. A., Fisher, J. M., and Rathjen, A. J. 1995. Genetic variation in resistance to *Pratylenchus neglectus* in wheat and triticales. *Aust. J. Exp. Agric.* 35:597-602.
- Fortuner, R. 1977. *Pratylenchus thornei*. In: C.I.H. Description of Plant-Parasitic Nematodes, Set 7. Commonwealth Institute of Helminthology, Commonwealth Agricultural Bureaux, Farnham Royal, Slough, U.K.
- Gilmour, A. R., Cullis, B. R., and Verbyla, A. P. 1997. Accounting for natural and extraneous variation in the analysis of field experiments. *J. Agric. Biol. Environ. Stat.* 2:269-293.
- Harris, P., Biggs, A. J. W., Stone, B. J., Crane, L. N., and Douglas, N. J. 1999. Resource information book. in: *Central Darling Downs Land Management Manual* DNRQ990102. Queensland Department of Natural Resources, Brisbane, QLD, Australia.
- Isbell, R. F. 1996. *The Australian Soil Classification*, Rev. ed. CSIRO Publishing, Melbourne, VIC, Australia.

Fig. 7. Distribution of predicted population densities of *Pratylenchus thornei* based on equal subdivision of the range of principal axes 1 and 2 empirical best linear unbiased predictors (PA[1+2]-eBLUPs) in natural logarithm ($\ln$)(*P. thornei* nematodes per kilogram of soil + 1) units for 1,096 wheat genotypes from 22 experiments shown as nine corresponding α resistance ratings, where R = resistant, R-MR = resistant to moderately resistant, MR = moderately resistant, MR-MS = moderately resistant to moderately susceptible, MS = moderately susceptible, MS-S = moderately susceptible to susceptible, S = susceptible, S-VS = susceptible to very susceptible, and VS = very susceptible.

- Kelly, A., Smith, A., Eccleston, J., and Cullis, B. R. 2007. The accuracy of varietal selection using factor analytic models for multi-environment plant breeding trials. *Crop Sci.* 47:1063-1070.
- Lush, D. 2018. 2018 Wheat Varieties—Queensland. Grains Research and Development Corporation and the Queensland Department of Agriculture and Fisheries, Brisbane, QLD, Australia.
- Matthews, P., McCaffery, D., and Jenkins, L. 2017. Winter Crop Variety Sowing Guide 2017. NSW Department of Primary Industries, Sydney, NSW, Australia.
- Murray, G. M., and Brennan, J. P. 2009. Estimating disease losses to the Australian wheat industry. *Australas. Plant Pathol.* 38:558-570.
- Nombela, G., and Romero, M. D. 2001. Field response to *Pratylenchus thornei* of a wheat line with the *CreAet* gene for resistance to *Heterodera avenae*. *Eur. J. Plant Pathol.* 107:749-755.
- O'Reilly, M. M., and Thompson, J. P. 1993. Open pot culture proved more convenient than carrot callus culture for producing *Pratylenchus thornei* inoculum for glasshouse experiments. Pages 5-9 in *Proc. Pratylenchus Workshop*, Australas. Plant Pathol. Soc. Conf. Hobart, TAS, Australia.
- Owen, K. J., Clewett, T. G., and Thompson, J. P. 2012. What is the impact of winter grain crops on *Pratylenchus thornei* grown in rotation with tolerant and intolerant wheat? Page 31 in: *Proc. 7th Australas. Soilborne Dis. Symp.* W. J. Macleod, ed. Fremantle, WA, U.S.A.
- Owen, K. J., Clewett, T. G., and Thompson, J. P. 2014. Wheat biomass and yield increased when populations of the root-lesion nematode (*Pratylenchus thornei*) were reduced through sequential rotation of partially-resistant winter and summer crops. *Crop Pasture Sci.* 65:227-241.
- Patterson, H. D., and Thompson, R. 1971. Recovery of inter-block information when block sizes are unequal. *Biometrika* 58:545-554.
- PennState Extension. 2017. *Pythium*. Extension. <https://extension.psu.edu/pythium>
- Piepho, H.-P. 1998. Empirical best linear unbiased prediction in cultivar trials using factor-analytic variance-covariance structures. *Theor. Appl. Genet.* 97:195-201.
- R Core Team. 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Reen, R. A., Thompson, J. P., Clewett, T. G., Sheedy, J. G., and Bell, K. L. 2014. Yield response in chickpea cultivars and wheat following crop rotations affecting population densities of *Pratylenchus thornei* and arbuscular mycorrhizal fungi. *Crop Pasture Sci.* 65:428-441.
- Roberts, P. A. 2002. Concepts and consequences of resistance. Pages 23-41 in: *Plant Resistance to Parasitic Nematodes*. J. L. Starr, R. Cook, and J. Bridge, eds. CABI Publishing, Wallingford, U.K.
- Robinson, G. K. 1991. That BLUP is a good thing: The estimation of random effects. *Stat. Sci.* 6:15-32.
- Robinson, N. A., Sheedy, J. G., MacDonald, B. J., Owen, K. J., and Thompson, J. P. 2019. Tolerance of wheat cultivars to root-lesion nematode (*Pratylenchus thornei*) assessed by normalised difference vegetation index is predictive of grain yield. *Ann. Appl. Biol.* 174:388-401.
- Rodda, M. S., Hobson, K. B., Forknall, C. R., Daniel, R. P., Fanning, J. P., Pounsett, D. D., Simpfendorfer, S., Moore, K. J., Owen, K. J., Sheedy, J. G., Thompson, J. P., Hollaway, G. J., and Slater, A. T. 2016. Highly heritable resistance to root-lesion nematode (*Pratylenchus thornei*) in Australian chickpea germplasm observed using an optimised glasshouse method and multi-environment trial analysis. *Australas. Plant Pathol.* 45:309-319.
- Romero, D., and Nombela, G. 1999. Host response to *Pratylenchus thornei* of a wheat line carrying the *Cre2* gene for resistance to *Heterodera avenae*. *Nematology* 1:381-388.
- Sheedy, J. G., McKay, A. C., Lewis, J., Vanstone, V. A., Fletcher, S., Kelly, A., and Thompson, J. P. 2015. Cereal cultivars can be ranked consistently for resistance to root-lesion nematodes (*Pratylenchus thornei* & *P. neglectus*) using diverse procedures. *Australas. Plant Pathol.* 44:175-182.
- Sheedy, J. G., Robinson, N. A., Lin, J., Reen, R. A., Clewett, T. G., and Thompson, J. P. 2017. Pre-breeding to produce wheat cultivars that combine root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) resistance and tolerance. In: *Aust. Wheat Breeders Assembly*, Sydney, NSW, Australia.
- Sheedy, J. G., and Thompson, J. P. 2009. Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. *Australas. Plant Pathol.* 38:478-489.
- Smiley, R. W., and Nicol, J. M. 2009. Nematodes which challenge global wheat production. Pages 71-187 in: *Wheat Science and Trade*. B. F. Carver, ed. Wiley-Blackwell, Ames, IA, U.S.A.
- Smith, A. B., Cullis, B. R., and Thompson, R. 2001. Analysing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. *Biometrics* 57:1138-1147.
- Smith, A. B., Ganesalingam, A., Kuchel, H., and Cullis, B. R. 2015. Factor analytic mixed models for the provision of grower information from national crop variety testing programs. *Theor. Appl. Genet.* 128:55-72.
- Thompson, C. H., and Beckmann, G. G. 1959. Soils in the Toowoomba area, Darling Downs, Queensland. CSIRO Australia, Soil and Land Use Series, No. 28. CSIRO, Melbourne, VIC, Australia.
- Thompson, J. P. 1990a. Treatments to eliminate root-lesion nematode (*Pratylenchus thornei* Sher and Allen) from a vertisol. *Nematologica* 36: 123-127.
- Thompson, J. P. 1990b. Soil sterilization methods to show VA-mycorrhizae aid P and Zn nutrition of wheat in vertisols. *Soil Biol. Biochem.* 22: 229-240.
- Thompson, J. P. 2008. Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. *Aust. J. Agric. Res.* 59:432-446.
- Thompson, J. P., Brennan, P. S., Clewett, T. G., Sheedy, J. G., and Seymour, N. P. 1999. Progress in breeding wheat for tolerance and resistance to root-lesion nematode (*Pratylenchus thornei*). *Australas. Plant Pathol.* 28:45-52.
- Thompson, J. P., Clewett, T. G., and O'Reilly, M. M. 2015a. Optimising initial population density, growth time and nitrogen nutrition for assessing resistance of wheat cultivars to root-lesion nematode (*Pratylenchus thornei*). *Australas. Plant Pathol.* 44:133-147.
- Thompson, J. P., Clewett, T. G., and O'Reilly, M. M. 2015b. Temperature response of root-lesion nematode (*Pratylenchus thornei*) reproduction on wheat cultivars has implications for resistance screening and wheat production. *Ann. Appl. Biol.* 167:1-10.
- Thompson, J. P., and Haak, M. I. 1997. Resistance to root-lesion nematode (*Pratylenchus thornei*) in *Aegilops tauschii* Coss., the D-genome donor to wheat. *Aust. J. Agric. Res.* 48:553-559.
- Thompson, J. P., Mackenzie, J., and Sheedy, G. H. 2012a. Root-lesion nematode (*Pratylenchus thornei*) reduces nutrient response, biomass and yield of wheat in sorghum-fallow-wheat cropping systems in a subtropical environment. *Field Crops Res.* 137:126-140.
- Thompson, J. P., Owen, K. J., Stirling, G. R., and Bell, M. J. 2008. Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*): A review of recent progress in managing a significant pest of grain crops in northern Australia. *Australas. Plant Pathol.* 37:235-242.
- Thompson, J. P., Reen, R. A., Clewett, T. G., Sheedy, J. G., Kelly, A. M., Gogel, B. J., and Knights, E. J. 2011. Hybridisation of Australian chickpea cultivars with wild *Cicer* spp. increases resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*). *Australas. Plant Pathol.* 40:601-611.
- Thompson, J. P., and Seymour, N. P. 2011. Inheritance of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat landraces and cultivars from the West Asia and North Africa (WANA) region. *Crop Pasture Sci.* 62: 82-93.
- Thompson, J. P., Zwart, R. S., and Butler, D. 2012b. Inheritance of resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in five doubled-haploid populations of wheat. *Euphytica* 188:209-219.
- Trudgill, D. L. 1991. Resistance and tolerance of plant parasitic nematodes in plants. *Annu. Rev. Phytopathol.* 29:167-192.
- VSN International. 2014. GenStat for Windows, 17th ed. VSN International, Hemel Hempstead, U.K. <https://www.vsn.co.uk/software/genstat/>
- Wallace, H. R. 1987. A perception of tolerance. *Nematologica* 33:419-432.
- Webb, A. A., Grundy, M. J., Powell, B., and Littleboy, M. 1997. The Australian subtropical cereal belt: Soils, climate and agriculture. Pages 8-23 in: *Sustainable Crop Production in the Sub-tropics: An Australian Perspective*. A. L. Clarke and P. B. Wylie, eds. Queensland Department of Primary Industries, Toowoomba, QLD, Australia.
- Whish, J. P. M., Thompson, J. P., Clewett, T. G., Lawrence, J. L., and Wood, J. 2014. *Pratylenchus thornei* populations reduce water uptake in intolerant wheat cultivars. *Field Crops Res.* 161:1-10.
- Whish, J. P. M., Thompson, J. P., Clewett, T. G., Wood, J., and Rostad, H. E. 2017. Predicting the slow decline of root-lesion nematode (*Pratylenchus thornei*) during host-free fallows to improve farm management decisions. *Eur. J. Agron.* 91:44-53.
- Whitehead, A. G., and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. Appl. Biol.* 55:25-38.
- Williams, E. R. 1986. Row and column designs with contiguous replicates. *Aust. J. Stat.* 28:154-163.
- Zwart, R. S., Thompson, J. P., and Godwin, I. D. 2004. Genetic analysis of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat. *Plant Breed.* 123:209-212.