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 J. P. Thompson^{A,C}, J.G. Sheedy^A, N.A. Robinson^A ^AUniversity of Southern Queensland, Centre for Crop Health, Institute for Agriculture and the Environment, West Street, Toowoomba, Queensland 4350, Australia ^CCorresponding author, Email address john.thompson@usq.edu.au Telephone +61 7 46311148

11 ABSTRACT

12 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 13 14 determined from final nematode population densities in glasshouse experiments, but combining results across multiple experiments presents challenges. Here we use a factor 15 analytic method for multi-experiment analysis of final population densities of *P. thornei* for 16 17 1096 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 18 principal components solution. Principal axes 1 and 2 (PA1 and PA2) respectively accounted 19 20 for 79% and 11 % of the genetic variance over all experiments. Final population densities of 21 *P. thornei* as empirical best linear unbiased predictors (PA(1+2)-eBLUPs)) from the 22 combined glasshouse experiments were highly predictive (P < 0.001) of final nematode 23 population densities in the soil profile, of crop canopy greenness (NDVI) and of grain yield of wheat genotypes in *P. thornei* infested fields in the Australian subtropical grain region. 24 25 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 26 27 guides. Nine genotypes were nominated as references for future resistance experiments. 28 Most (62%) Australian wheat genotypes were in the most susceptible three categories (S, S– VS and VS). However, resistant germplasm characterized in this study could be used in plant 29 30 breeding to considerably improve the overall resistance of Australian wheat crops

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32 Abbreviations

A list of abbreviations is given in Table 1.

34 Additional keywords: root-lesion nematodes, wheat genotype resistance, MET analysis,

35 nematode population densities predictions, wheat yield prediction

36 INTRODUCTION

37 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 38 warm temperate zones such as the subtropical or northern grain region of eastern Australia 39 (Webb et al., 1997; Thompson et al., 2008). This region, which lies between Lat. 20°S in 40 Queensland and Lat. 32°S in New South Wales, is characterized by rain-fed agriculture 41 42 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 43 (Hordeum vulgare) and chickpea (Cicer arietinum), while major warm-season crops termed 44 45 'summer' crops are sorghum (Sorghum bicolor) and mungbean (Vigna radiata) with a number of other cereal, pulse and oilseed crop species grown to lesser extents. Deep cracking 46 clay soils (vertisols) are favoured for cropping in this region because of their high water-47 48 holding capacities to store fallow rainfall to supplement rainfall during the next crop phase (Webb et al., 1997). 49

50 *Pratylenchus thornei* is polyphagic being hosted by many cereal and pulse crop species including wheat and chickpea, which in other respects are valuable rotational crops for each 51 other. Some wheat genotypes can lose up to 65% yield (Thompson et al., 1999) and some 52 chickpea genotypes up to 25% yield (Reen et al., 2014) from high population densities of P. 53 thornei in the soil profile at sowing. It has been estimated that P. thornei costs 4.3% of the 54 total wheat production/year in the eastern Australian subtropical grain region with this loss 55 valued at AU\$38 M (Murray and Brennan, 2009). Other hosts of P. thornei among crop 56 57 species grown in this region include winter crops barley, triticale (*×Triticosecale*), durum (Triticum turgidum ssp. durum), and faba bean (Vicia faba), and summer crops mung bean, 58 59 black gram (Vigna mungo) and soybean (Glycine max) (Owen et al., 2012, 2014).

60 Management of *P. thornei* for wheat production depends on crop sequences that include moderately resistant crop species like sorghum (Thompson et al., 2008, 2012a; Owen et al., 61 2014), which is the major summer crop species in the subtropical grain region of Australia. 62 However, sorghum cannot be grown profitably across the entire region. Fixed rotations are 63 difficult to implement due to variable rainfall patterns in this region, and flexible crop 64 sequencing (termed opportunity cropping) is often practiced. *Pratylenchus thornei* survives 65 66 well in the clay soils of the region and, following growth of a susceptible wheat cultivar, ~ 3 years of fallow and/or resistant rotational crops are needed to reduce high nematode 67 68 population densities to below the threshold for damage (Owen et al., 2014; Whish et al., 69 2017) which is ~2,000 nematodes/kg soil for intolerant wheat cultivars (Thompson et al., 2008). Protection of the major at-risk crops through breeding resistant and tolerant genotypes 70 71 is essential for the long-term management of *P. thornei* in this region.

72 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 73 74 with little loss in the presence of high initial nematode population densities, and resistance is defined as the capacity of plant genotypes to limit nematode reproduction in the roots 75 (Trudgill, 1991; Roberts, 2002). Although tolerance and resistance can be measured 76 77 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 78 roots, but also as a consequence of the low population density can have maximum plant 79 80 growth at least equal to a tolerant genotype. In field experiments, P. thornei was shown to 81 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 82 average. Previously, Wallace (1987) suggested genetic factors that confer plants with 83 abilities to withstand stresses, like water and nutrient deficiencies, would function in a non-84

specific 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 *Pratylenchus neglectus* (Farsi, 1995; Thompson, 2008). Similarly,
other wheat genotypes resistant to cereal cyst nematode (*Heterodera avenae*) are susceptible
to *P. thornei* (Nombela and Romero, 1999, 2001; Thompson, 2008).

Resistance to nematodes is measured by the increase in nematode population densities 90 during plant growth. Resistance of crop genotypes to root-lesion nematodes can be assessed 91 in the field or glasshouse, with the more resistant genotypes resulting in lower final 92 population densities (P_f) of nematodes than the more susceptible genotypes. The advantage 93 94 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 95 conditions the initial population density of the site for the target nematode species has to be 96 97 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 98 plot in a field experiment to be determined (Fanning et al. 2018), thereby considerably 99 100 increasing the resources needed. Furthermore, better environmental control is possible under 101 glasshouse conditions resulting in a greater proportion of experiments successfully completed 102 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 103 profile to ~90 cm depth with various vertical distribution patterns (Owen et al., 2014; 104 105 Thompson et al., 1999), further complicating the determination of nematode population densities in the field. 106

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, 2017; Matthews et

110 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 111 112 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 113 glasshouse and field (Thompson et al., 2015a, 2015b). Over time, the glasshouse methods 114 have been modified to provide conditions conducive for both plant growth and nematode 115 116 reproduction so that differences in the final population densities among genotypes are controlled by genetic difference rather than environmental factors. 117

Resistance of a wheat genotype in the glasshouse method is assessed after 16 weeks of 118 119 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 120 range of resistance/susceptibility are included in each experiment for comparison with new 121 genotypes to be tested and to gauge nematode reproduction in an experiment. This method 122 allows ranking of genotypes on their resistance in any single experiment, but presents 123 124 difficulties in comparing results across multiple unbalanced experiments in which many of the genotypes under consideration differ from experiment to experiment. This lack of 125 126 balance arises from the on-going need to characterize the resistance of a changing suite of 127 advanced plant breeding lines prior to their potential release to growers. To combine results across experiments we previously used the average reproduction factor, RF = final population 128 density/initial population density. The range of average RF was then divided into nine 129 130 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 131 132 susceptible (VS) (Lush, 2017), known as an 'alpha' rating scale to distinguish it from previous methods of assigning numbers 1 to 9 to these ordinal categories. Despite 133 standardized experimental conditions, nematode reproduction rates can vary among 134

experiments. This results in higher average RF values and susceptibility ratings of genotypes
present in experiments with higher final population densities, but absent from experiments
with lower final population densities, 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 140 141 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. 142 bijugum) and interspecific hybrids (Thompson et al., 2011). This FA approach was based on 143 144 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 145 similarly to environments in MET analyses. Genotype by experiment $(G \times E)$ interaction 146 effects are modelled by a FA regression model in a mixed model framework. This is in order 147 to accommodate heterogeneity of genetic variance across experiments and heterogeneity of 148 covariance and hence correlation, between experiments, while adjusting for spatial trends. 149 These data sets from multiple glasshouse experiments on genotypes conducted over a period 150 of years are typically unbalanced with respect to the occurrence of genotypes among 151 152 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 153 (Cullis et al., 2010). The dimensionality of the data defined by the number of experiments 154 can be reduced by FA models to a number of underlying 'factors'. Best linear unbiased 155 predictors (BLUPs) (Robinson, 1991; Piepho, 1998) of nematode population density for the 156 genotypes are obtained from the variance parameter estimates. This FA approach has been 157 shown to provide improved prediction of genotype performance relative to traditional 158

variance component models and is also superior to conducting separate analysis of individualexperiments (Kelly et al., 2007).

In this paper we apply FA methods for a combined analysis of 22 replicated glasshouse 161 experiments conducted from 1996 to 2015 that were designed to characterize the resistance to 162 *P. thornei* of a large number of genetically fixed genotypes of wheat. We further show that 163 this approach to combining glasshouse resistance experiments provides a single strong 164 165 predictor of relative final population densities of P. thornei, crop growth and grain yield associated with specific wheat genotypes grown in P. thornei infested fields. This provides a 166 validated new method for ranking cereal genotypes on resistance to P. thornei for wheat 167 168 improvement purposes and for growers' sowing guides.

169 MATERIALS AND METHODS

Glasshouse experiments The data analyzed are from a series of experiments that we have 170 conducted annually to characterise resistance to P. thornei of advanced wheat lines for plant 171 172 breeding programs and wheat cultivars for growers sowing guides. Data from a total of 22 glasshouse experiments on resistance to P. thornei of 1096 unique wheat genotypes were 173 used for the combined analysis. Genotypes of other crops were present in some of these 174 experiments, namely durum, barley and triticale. Canary grass (Phalaris canariensis, cv. 175 Moroccan) as a resistant crop control and an unplanted soil control were also included in 176 some experiments. The number of genotypes, experimental designs and any differences in 177 experimental procedures for the experiments in each year are given in Table 2. 178

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 Beckman, 1959) containing 78% clay from Wellcamp (Lat. 27.55°S,
Long. 151.87°E; Elevation ~500 m, near Toowoomba, Australia). In experiments from 1996

to 2012, the soil was pasteurized at 70°C for 30 minutes to kill nematodes (Thompson, 1990a) 183 and fungal pathogens like Fusarium pseudograminearum (Thompson, 1990b) that might 184 damage the plants and thereby limit P. thornei reproduction. In later experiments (2013 to 185 2015) the temperature of pasteurization was increased to 85°C to ensure control of Pythium 186 spp. originating from oospores (PennState Extension, 2017). Each pot of soil was inoculated 187 at sowing with a suspension of *P. thornei* at a rate of 10,000/kg soil (oven dry equivalent) 188 extracted from open pot cultures on wheat (O'Reilly et al. 1993). The strain of P. thornei was 189 originally isolated from Formartin, Queensland, Australia (Lat. 27.46°S, Long. 151.43E; 190 191 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 192 conducted from 1999 to 2015, methods for pot culture with bottom watering, as described by 193 194 Sheedy and Thompson (2009), were used. A summary of conditions for each experiment is given in Table 2. Experiments were laid out on the glasshouse benches as three randomized 195 complete blocks which had row:column spatial arrangements (Williams 1986) except in the 196 first two experiments. 197

Water supply to the pots was ceased at ~15 weeks after sowing so that the soil dried down to 198 ~45% moisture content in preparation for sampling at 16 weeks after sowing. This moisture 199 content expedited processing of this soil type for nematode extraction. The soil and roots 200 from each pot were placed in a tray where they were cut and broken manually into pieces <1 201 cm, before thorough mixing. Soil moisture content was determined by drying a 150-g 202 subsample at 105°C in a forced draft oven for 48 hours. Nematodes were extracted from 150-203 g subsamples of soil in Whitehead trays (Whitehead and Hemming, 1965) for 48 hours at 204 22°C and concentrated using a 20-µm aperture sieve into ~15 mL water. Pratylenchus 205 thornei individuals were counted in a Peters 1-mL gridded slide (Chalex Corporation, 206

207 Portland, Oregon, USA) under a compound microscope and population densities expressed as
208 number/kg soil on an oven dry weight basis.

Field experiments Final population densities of *P. thornei* in the soil profile following the 209 210 growth of wheat genotypes in field experiments and canopy greenness and grain yield were used to validate the glasshouse assessments of resistance/susceptibility of wheat genotypes. 211 Two field experiments to assess advanced wheat breeding lines in comparison with existing 212 cultivars were conducted in 2001 on a Black Vertosol of the Bongeen soil type (Harris et al., 213 1999) containing 60% clay, near Macalister, Australia (Lat. 27.03°S, Long. 151.07°E; 214 215 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 216 laid out in the field in three blocks as a row:column design (Williams 1986), with plots being 217 218 8 m long by seven drill rows on 25 cm spacing. The plots were fertilized with 55 kg N/ha as 219 urea before sowing, and with 40 kg/ha Starter Z (Granulock Z, Incitec Pivot) supplying 4.4 kg N/ha, 8.7 kg P/ha, 1.6 kg S/ha, and 0.4 kg Zn/ha applied with the seed at sowing. Seeding 220 221 rate was adjusted based on grain weight and germination percentage of each genotype to sow 100 viable seeds/ m^2 . The cropping history of the land immediately prior to the experiments 222 was wheat cv. Hybrid Mercury, double cropped to black gram cv. Regur (Vigna mungo), then 223 clean fallowed until the wheat experiments were sown 14 months later. During the course of 224 225 the wheat growing season, symptoms of damage from root-lesion nematodes were noted on 226 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, 227 and 19 in Experiment 2) were soil sampled to assess final population densities of root-lesion 228 229 nematodes. Deep soil samples were taken with a vehicle-mounted hydraulic soil corer using push tubes of 45 mm diameter. Four positions were sampled per plot from the middle rows at 230 approximately even intervals along the harvested length of 6 m of each plot. The four soil 231

cores were subdivided and composited into one bulk in each of the following layers 0–15,
15–30, 30–45, 45–60, 60–90, 90–120 and 120–150 cm depth and placed in polythene bags.
The soil was broken into pieces manually, mixed thoroughly, and 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. *Pratylenchus thornei* was
identified (Fortuner, 1977) and counted as described in Section 2.2.

238 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. 239 240 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% 241 clay, near Formartin. The land was managed in a 4-year rotation of sorghum, long fallow, 242 243 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 244 susceptible wheat cultivar Kennedy were grown in a 3-replicate strip design as the first wheat 245 crop in the 4-year rotation to generate low and high P. thornei population densities 246 respectively. All test wheat cultivars were sown into both low (2,570 P. thornei /kg soil at 0-247 90 cm from back-transformation of ln(x+1) mean following QT8343), and high (9,090 P. 248 *thornei* /kg soil at 0–90 cm from back-transformation of ln(x+1) mean following cv. 249 Kennedy) population densities in the following year (Robinson et al. 2019). Other field 250 251 procedures were similar to those described for Macalister except the rate of urea applied before sowing supplied 100 kg N/ha to each crop. 252 After grain harvest in November, the soil was sampled by taking two cores/plot with a 253 254 hydraulically driven push tube to 90 cm depth at one third intervals along the plot, and

subdivided and composited in intervals of 0–30, 30–60 and 60–90 cm. Nematode population

densities and soil water contents were determined as described above for the Macalisterexperiments in Section 2.3.1.

Statistical analysis of glasshouse experiments A consideration for the estimation of 258 parameters in a multi-experiment model is the concurrence of genotypes, that is, the number 259 of genotypes in common between pairs of experiments (Table 3). Most pairs of glasshouse 260 experiments had suitable numbers of concurrent genotypes, except for the experiment 2009N, 261 262 which had only two genotypes in common with eight other experiments (Table 3). The data on final *P. thornei* population densities were transformed by natural logarithms to ensure 263 264 homogeneity of variance over the range of fitted values. The multi-experiment analysis modelled ln(*P. thornei*/kg soil+1) in a linear mixed model framework, following the 265 approach of Smith et al. (2001). A fixed term was included for experiment effects, and a 266 267 random term was included for replicate effects for all experiments. A FA variance structure was fitted to the genotype x experiment effects, allowing for a different genetic variance for 268 each experiment, and heterogeneous covariance (and hence correlation) between each pair of 269 270 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 271 272 (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 273 274 modelling process, where significant. A 'crop type' factor was included in the analysis as a 275 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 276 90% of the genetic variance over all experiments was explained. The effective number of 277 278 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 variance accounted for by successively 279 added factors. The estimated FA loadings were rotated to a principal components solution 280

281 (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 282 on for subsequent axes, and all the axes were orthogonal. A genetic correlation matrix 283 284 between pairs of experiments was produced. The output from the FA analysis gave predictions of genotype performance in each experiment as regression empirical best linear 285 unbiased predictors (R-eBLUPs) (Cullis et al., 2010). For selected genotypes, the R-eBLUPs 286 287 were plotted against loadings for PA1 of the individual experiments to produce a latent regression plot for which the slope is the PA1score of that genotype (Smith et al. 2015). The 288 289 overall resistance or susceptibility of genotypes was compared using a single value for each genotype as PA(1+2)-eBLUPs where PA denotes principal axes from the principal 290 components solution. The PA(1+2)-eBLUP for each wheat genotype was calculated as the 291 292 respective PA1 score multiplied by the average of the 22 rotated loadings for PA1 plus the 293 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 294 295 $\ln(P. thornei/kg soil+1)$ and then back-transformed by exponentiation where required. These statistical analyses were performed using ASReml-R (Butler et al., 2009) in the R 296 software environment (R Core Team, 2016). Variance parameters were estimated using 297 residual maximum likelihood (REML) estimation (Patterson and Thompson, 1971). 298 Prediction of field final population densities of P. thornei from P. thornei resistance The 299 population densities of *P. thornei* in the field after harvest of the two experiments at 300 301 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 302 cultivars to 45 cm depth at Macalister and to 90 cm depth at Formartin. Non-linear 303 304 regression analysis was conducted between the mean population densities of *P. thornei* in the

soil profile (0–45 cm depth at Macalister and 0–90 cm at Formartin) as response variables

and PA(1+2)-eBLUPs of ln(*P. thornei*/kg soil+1) from the combined glasshouse experiments
as the explanatory variable using Genstat[®] for WindowsTM (VSN International, 2012).
Prediction of NDVI and grain yield of wheat cultivars in field experiments from *P*.

308 Prediction of NDVI and grain yield of wheat cultivars in field experiments from P 309 thornei resistance

310 The relationships between the grain yield of the cultivars in the three field experiments and the PA(1+2)-eBLUPs of ln(P. thornei+1) from combined glasshouse experiments were 311 examined graphically and appropriate non-linear regression equations fitted in Genstat[®] for 312 313 WindowsTM (VSN International, 2012). Similarly the relationships between the area under the disease progress curve (AUDPC) of seven readings of Normalized Difference Vegetation 314 Index (NDVI) taken to measure vegetation greenness at intervals from 64 to 126 days after 315 sowing of the genotypes in the Formartin experiment (Robinson et al., 2019) were 316 determined. 317

Rating of resistance of wheat genotypes The single range of log transformed PA(1+2)-

eBLUPs for the 1096 wheat genotypes from MET analysis of the combined experiments was

320 divided into nine equal sub-ranges. Genotypes within these nine categories were assigned

alpha ratings as used for diseases in Australian wheat variety guides for growers (Lush, 2017;

- 322 Matthews et al., 2017) as follows: resistant (R), resistant to moderately resistant (R–MR),
- 323 moderately resistant (MR), moderately resistant to moderately susceptible (MR–MS),
- moderately susceptible (MS), moderately susceptible to susceptible (MS–S), susceptible (S),
- susceptible to very susceptible (S–VS), and very susceptible (VS).

326 **RESULTS**

327 Combined glasshouse experiments A graphical display of the between environments

328 genetic correlation matrix between all pairs of 22 experiments from the MET analysis of *P*.

329 *thornei* population densities of wheat genotypes is shown in Fig. 1. The data from the

experiments were well correlated having 75% of all correlation coefficients between pairs of
experiments greater than 0.70 and with a median value of 0.80. This shows strong agreement
in relative nematode population densities for individual genotypes between experiments.

333 Table 4 shows predicted values from the FA model for experiment mean population density of P. thornei, genetic variance and error variance, as well as principal components 334 loadings and percentage genetic variance accounted for by PA1 and PA2 in individual 335 experiments. An FA model of order 2 (designated FA-2) was retained as the final model, 336 explaining 90% of the genetic variance over all experiments (79% by PA1 and 11% by PA2) 337 338 (Table 4). 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 339 67% of genetic variance accounted for by this FA-2 model, and every experiment had greater 340 341 than 55% genetic variance accounted for by PA1 alone, except for the 2006 experiment with 42% (Table 4). Loadings along PA1 were positive for all experiments, ranging from 0.169 to 342 0.709 (Table 4). Loadings along PA2 ranged from -0.358 to 0.389, with experiments having 343 larger negative loadings being 2010N, 2009C, 2010C, 2008 and 2002, while experiments 344 having larger positive loadings were 2012N, 2005, 1997, 2014, 2015, 2006 and 2009N. 345 There was no noticeable difference between these two groups of experiments in relation to 346 the chronology of the experiments or variation of the method used (Table 2), or the mean 347 final *P. thornei* population densities of the experiments (Table 4). 348

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 Fig. 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 Accessory Table 1, while these results for a subset of 50 genotypes mentioned in this paper and/or present in the field experiments is given in Table 5.

Relationship between field final population densities of *P. thornei* and resistance In the

361 Macalister field experiments, there were significant relationships for final *P. thornei*

population densities at soil profile depths of 0–15 cm, 15–30 cm and 30–45 cm, but not at

depths below 45 cm whereas in the Formartin experiment there were significant relationships

at all depths of 0–30 cm, 30–60 cm and 60–90 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 (0-45 cm at Macalister and 0-90 cm at Formartin) after growth of

367 wheat genotypes in the three field experiments and the glasshouse derived PA(1+2)-eBLUPs

368 of those genotypes (Fig. 3).

369 Relationship between crop greenness at high and low population densities of

370 *Pratylenchus thornei* and resistance There were highly significant negative exponential
371 relationships between AUDPC of NDVI measurements at both high and low *P. thornei*372 population densities in the field experiment at Formartin and glasshouse derived PA(1+2)-

373 eBLUPs of *P. thornei* population densities. (Fig. 4).

374 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

- 376 showed a significant negative sigmoidal relationships with glasshouse derived measures of
- 377 resistance of the same wheat cultivars expressed as PA(1+2)-eBLUPs of ln(population
- density of *P. thornei*/kg 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 1096 wheat genotypes
(Accessory Table 1 and Table 5) ranged from a minimum for CPI133872 (present in 13
experiments with a back-transformed mean final population density of 7,171 *P. thornei*/kg
soil) to a maximum for cv. Darwin (present in four experiments with a back-transformed
mean final population density of 104,192 *P. thornei*/kg soil) (Table 5). The distribution of
the 1096 wheat genotypes in this study in relation to the nine categories of resistance ratings
based on sub-ranges of PA(1+2)-eBLUPs is given in Fig. 7.

Most of the wheat genotypes (62%) tested in these experiments were in the most 393 susceptible three rating categories (S, S-VS, and VS) to P. thornei. In contrast, only 2% of 394 395 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 396 synthetic hexaploid wheat CPI133872 (Thompson, 2008; Zwart et al., 2004) or GS50a (a 397 398 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 399 (Table 5). Two commercial cultivars, Impose CL Plus and Wyalkatchem, both adapted to the 400 401 Mediterranean (western) grain region of Australia, were rated as MR. The category MR-MS included ten named cultivars (Table 5) of which Gauntlet, Wallup, Sunmate, Suntime, Suntop 402 and Ventura are adapted to the subtropical (northern) grain region of eastern Australia, while 403

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 very susceptible (VS)
wheat genotypes (Table 5) which included Petrie (a northern region cultivar) and Brennan (a
northern region forage wheat), and the southern region cultivars Darwin, Wedgetail,
Annuello and Forrest. The western region wheat cv. Yandanooka, previously proposed as a
VS reference cultivar (Sheedy et al., 2015), was categorized as S–VS in this study.

410 **DISCUSSION**

This is the first large scale MET analysis of multiple glasshouse experiments to determine 411 the resistance to *P. thornei* of an extensive range of wheat genotypes in which the output has 412 been used to predict relative final nematode populations and grain yield of wheat genotypes 413 in independent field experiments. We ranked more than one thousand wheat genotypes for 414 resistance to P. thornei by combining results from 22 experiments conducted during the 415 period 1996 to 2015. This was achieved using a FA approach with rotation of axes to a 416 417 principal components solution. A strong effect of wheat genotype on the final population density of P. thornei in all experiments was exhibited in PA1, which can be considered a 418 stable resistance axis accounting for 79% of the genetic variance. Further, PA2 which 419 accounted for an additional 11% of genetic variance, can be considered a measure of 420 additional genetic variability for resistance across different experiments. One possible cause 421 of the greater variability for resistance of some wheat genotypes could be incomplete genetic 422 fixation of the genotype, whereas other genotypes might have been fixed through single plant 423 selection and a greater number of inbred generations. Another possible reason is unidentified 424 environmental variation among the experiments resulting in some GxE effects. 425 426 The overall predicted value for the final population densities of *P. thornei* based on PA(1+2)-eBLUPs from the FA-2 model can be considered the best single index for the 427 resistance level of genotypes included in this investigation. A quantitative measure as used in 428

429	this study rather than a qualitative approach to characterising the resistance to P. thornei is
430	required in view of the knowledge that resistance to this nematode species is inherited
431	quantitatively under the control of multiple genes having additive gene action (Zwart et al.,
432	2004: Thompson and Seymour, 2011: Thompson et al., 2012b).

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 as ordinal alpha ratings for growers' sowing guides, but they could also be valuable variables in crop growth models incorporating nematode population dynamics as influenced by wheat cultivar choice.

Previously, genetic correlation (r > 0.66) was found for data on final population densities 440 of P. thornei between a single glasshouse experiment evaluating 47 genotypes of chickpea 441 442 and six field experiments (five from the subtropical grain region and one from the warm 443 temperate grain region of Victoria) sampled to either 15 or 30 cm soil depth evaluating a total of 85 chickpea genotypes in the one MET analysis (Rodda et al. 2016). Recently, high 444 genetic correlation (r>0.9) was found between environments for population densities of *P*. 445 thornei in the top soil (0-10 cm or 0-15 cm) after harvest of 68 cereal genotypes in six field 446 experiments in the temperate region of southern Australia, except where the fungal pathogen 447 *Rhizoctonia solani* was damaging (Fanning et al. 2018). In the present study with wheat, we 448 have preferred to analyse our glasshouse data in a MET analysis separately from any field 449 450 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 451 methods for assessing P. thornei resistance of wheat genotypes and demonstrated their value 452 453 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 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.

460 In the deep clay soils of the subtropical grain region of Australia, P. thornei can occur in the soil profile to 90 cm depth. Our results showed that the genotype PA(1+2)-eBLUPs from 461 the combined glasshouse experiments were predictive of the genotype final population 462 463 densities to depths of 45 cm at Macalister and to 90 cm at Formartin. This shows the influence of growing different wheat genotypes on *P. thornei* population densities throughout 464 the soil profile, and that the combined analysis of the glasshouse experiments as described 465 provides measures that can be used to rank genotypes on how they affect nematode 466 population densities throughout the whole soil profile. 467

468 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 alpha ratings as required 469 is a useful simplification, but for other purposes the numeric values of PA(1+2)-eBLUPS are 470 471 preferable. Each year there is a requirement to assess prospective new cultivars for resistance to P. thornei and our established data base is an asset for this purpose. Adding new 472 473 experimental data to the existing data base for MET analysis will allow new wheat genotypes to be assessed reliably for resistance within the framework of covariance across experiments 474 475 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 476 genotypes for growers sowing guides (Lush, 2017; Matthews et al. 2017), and the methods 477 478 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 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. 485 thornei in which 23 bread wheat genotypes were evaluated, Sheedy et al. (2015) selected 486 seven for use as provisional reference cultivars for future experiments. The more 487 488 comprehensive analyses reported here with 1096 genotypes and 22 experiments have permitted an improved selection of reference cultivars. One genotype has been selected to 489 represent the resistance level in each of nine ordered categories ranging from resistant to 490 491 very susceptible based on equal subdivision of the range of values for PA(1+2)-eBLUPs in units of ln(*P. thornei*/kg soil). These new reference genotypes for the nine resistance 492 493 categories are: (R) CPI133872, (R–MR) GS50a, (MR) QT8447, (MR–MS) Suntop, (MS) 494 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 495 category and on a low standard error of the PA(1+2)-eBLUP. Of hose reference genotypes 496 suggested by Sheedy et al. (2015), CPI133872 has been retained to represent the R category 497 and GS50a has been retained to represent the R-MR category of resistance. Other genotypes 498 have been nominated for categories in which no reference genotype was nominated by 499 500 Sheedy et al. (2015), while others have been replaced by preferred reference genotypes. 501 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 502 subtropical grain region of eastern Australia is provided as tolerance ratings in sowing guides 503

504 (e.g. Lush 2017). 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. 505 thornei residual in the soil to attack a subsequent crop. However, from this study it is 506 507 apparent that the level of resistance/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 508 resistance on plant growth was illustrated diagrammatically by Roberts (2002) in which a 509 510 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 511 512 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 513 vegetative growth and grain yield than in wheat genotypes with greater levels of resistance. 514 515 This better comprehension of the role that resistance plays in *P. thornei* population changes 516 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 517 breeding, and the ongoing need to accurately characterize the resistance to P. thornei of all 518 wheat cultivars to be released by plant breeding companies for growers' use. 519 520 In summary, combining data on final population densities of P. thornei for 1096 wheat genotypes in 22 glasshouse experiments by a MET analysis was an effective way to compare 521 the resistance of wheat genotypes. A two factor model explained 90% of the genetic 522 523 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 524 additional 11% of the genetic variance indicating the resistance levels of some genotypes 525 526 could not be fully explained by PA1 alone. Genotype scores of PA(1+2)-eBLUPs in units of $\ln(P. thornei/kg \text{ soil } +1)$ from these glasshouse results were highly predictive of relative final 527

528 population densities of *P. thornei* to depth in the soil profile after growth of various wheat

529 genotypes in three field experiments. There were also highly significant non-linear relationships between glasshouse-derived resistance levels and genotype performance in the 530 field assessed by greenness of vegetative biomass and grain yield. Subdivision of the range 531 of PA(1+2)-eBLUPs into nine sub-ranges is presented as an objective method for producing 532 ordinal/alpha resistance ratings for growers' sowing guides. The majority of genotypes tested 533 were in the top three susceptibility ratings, indicating the need for continued germplasm 534 535 development to raise the level of resistance to *P. thornei* in wheat cultivars available to Australian growers. 536

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Abbreviation	Meaning
AUDPC	area under the disease progress curve
BTM	back-transformed mean
FA	factor analysis
FA-k	factor analytic model of order k
G×E	genotype \times experiment
ln	natural logarithm or \log_e where e is Euler's number
NDVI	Normalized difference vegetation index
R-eBLUP	regression empirical best linear unbiased predictor
РА	principal axes from varimax rotation of FA axes
PAk	principal axis k
PA(1+2)-eBLUP	empirical best linear unbiased predictor based on PA1 and PA2
Pi	initial population density of nematodes
P _f	final population density of nematodes
RF	reproduction factor
MET	multi-environment trial

720 resistance to *Pratylenchus thornei*

		Experimental				Under-		Inoculum
.	Number	design		Watering	Nutrient	bench	- ·	mode
Experiment	of	No. of	No. of	system ^a	Application ^b	control of	Inoculum	
(year conducted)	genotypes	Pows	Columns			soll temperature ^c	(nematodes/kg	
1006	111	PRd	PR	tom	achutian	temperature	2500	in coil
1990	00			top	solution	no	2500	in soil
1997	90			top	solution	по	2500	111 SO11
1999	125	KC ¹	RC	bottom	solution	yes	10000	suspension
2000	126	RC	RC	bottom	solution	yes	10000	suspension
2001	118	RC	RC	bottom	solution	yes	10000	suspension
2002	129	10	39	bottom	solution	yes	10000	suspension
2003	154	11	42	bottom	Osmocote ^c	yes	10000	suspension
2005	143	18	24	bottom	Osmocote	yes	10000	suspension
2006	139	18	24	bottom	Osmocote	yes	10000	suspension
2007	228	29	24	bottom	Osmocote	yes	10000	suspension
2008	27	9	9	bottom	Osmocote	yes	10000	suspension
$2009C^{f}$	45	9	15	bottom	Osmocote	yes	10000	suspension
2009N ^g	117	13	27	bottom	Osmocote	yes	10000	suspension
2010C	156	36	13	bottom	Osmocote	yes	10000	suspension
2010N	156	36	13	bottom	Osmocote	yes	10000	suspension
2011C	110	11	30	bottom	Osmocote	yes	10000	suspension
2011N	156	39	12	bottom	Osmocote	yes	10000	suspension
2012C	110	33	10	bottom	Osmocote	yes	10000	suspension
2012N	143	39	11	bottom	Osmocote	yes	10000	suspension
2013	144	36	12	bottom	Osmocote	yes	10000	suspension
2014	110	33	10	bottom	Osmocote	yes	10000	suspension
2015	120	36	10	bottom	Osmocote	yes	10000	suspension

^aDetails 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 by Sheedy and Thompson

723 (2009) respectively. For top watering the soil was brought to 56% moisture content as required, while

for bottom watering the soil was held at a constant 2 cm water tension. ^bNutrient application:

solution = nutrients added from solutions to provide (mg/kg soil) 200 NO₃–N, 25 P, 88 K, 36 S, 285

726 Ca and 5 Zn; and Osmocote = 1 g of Osmocote @ native gardens plus micronutrients (17–1.6–8.7

727 NPK) slow-release fertilizer pellets (Scotts Australia Pty Ltd., Baulkham Hills, Australia). ^cSoil

temperature controlled at 22°C.

All experiments had three replicates laid out as randomized complete blocks. ${}^{d}RB$ = randomized

block design only; eRC = additional row column design, but exact positions not available for the

731 combined analysis of experiments. ${}^{f}C$ = preponderance of released cultivars in the experiment; ${}^{g}N$ =

732 preponderance of breeders' advanced lines in the experiment

Table 3. Concurrence of genotypes between experiments testing for resistance to

735 *Pratylenchus thornei* in 22 glasshouse experiments conducted from 1996 to 2015. 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.

Exp.																						
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
Exp.	1996	1997	1999	2000	2001	2002	2003	2005	2006	2007	2008	2009C	2009N	2010C	2010N	2011C	2011N	2012C	2012N	2013	2014	2015

Experiment	Mean P. thorn	<i>ei/</i> kg soil	Genetic variance	Error variance	Principal component loadings		% Genetic variance accounted for (VAF)			
	ln(x+1)	BTM ^a			PA1 ^b	PA2	PA1	PA2	PA1+PA2	
1996	10.663	42753	0.046	0.169	0.196	-0.044	83	4	88	
1997	10.965	57804	0.105	0.270	0.277	0.169	73	27	100	
1999	11.981	159764	0.303	0.357	0.542	-0.097	97	3	100	
2000	11.063	63759	0.503	0.636	0.709	0.004	100	0	100	
2001	12.092	178504	0.336	0.379	0.579	-0.039	100	0	100	
2002	11.945	154095	0.180	0.266	0.394	-0.106	87	6	93	
2003	10.597	39995	0.435	1.438	0.602	0.034	83	0	84	
2005	11.528	101478	0.175	0.574	0.328	0.260	61	39	100	
2006	11.564	105263	0.068	0.815	0.169	0.141	42	29	71	
2007	12.510	271020	0.193	0.541	0.387	0.099	78	5	83	
2008	11.226	75026	0.127	0.368	0.331	-0.132	86	14	100	
2009C	12.103	180367	0.484	0.780	0.521	-0.232	56	11	67	
2009N	12.313	222542	0.271	0.647	0.507	0.117	95	5	100	
2010C	12.327	225763	0.407	0.777	0.597	-0.224	88	12	100	
2010N	12.529	276301	0.606	0.381	0.691	-0.358	79	21	100	
2011C	11.153	69746	0.335	0.618	0.514	-0.096	79	3	81	
2011N	11.492	97959	0.520	0.308	0.613	-0.056	72	1	73	
2012C	10.069	23590	0.441	1.019	0.571	0.108	74	3	76	
2012N	9.315	11102	0.606	1.031	0.674	0.389	75	25	100	
2013	11.372	86866	0.218	0.391	0.397	0.095	72	4	76	
2014	10.952	57044	0.247	0.350	0.413	0.155	69	10	79	
2015	9.723	16702	0.158	0.490	0.371	0.144	87	13	100	
						Overall	79	11	90	

741	Table 4.	Parameters from combined analysis using a factor analytic structure of 22)
/ · ±		i arameters moni comonica anarysis asing a ractor anarytic stractare or 22	-

experiments testing resistance to *Pratylenchus thornei* of wheat genotypes used for derivation

of empirical best linear unbiased predictors of individual genotypes given in Table 5.

 $^{a}BTM = back$ -transformed mean by exponentiation; $^{b}PA = Principal axes after rotation to principal components solution$

751 Table 5. Genotypic scores for PA1 and PA2 and predicted final population densities of Pratylenchus thornei for 60 selected wheat genotypes used in field experiments and present 752 in four or more of 22 experiments comprising 1096 wheat genotypes in a combined analysis. 753 Genotypes have been assigned resistance ratings based on subdivision of the PA(1+2)-754 eBLUP range of $\ln(P. \text{ thornei/kg soil})$ into nine arithmetically equal categories. BTM = 755 back-transformed means by exponentiation. Probabilities of values greater than the reference 756 757 genotypes GS50a and Yandanooka proposed by Sheedy et al. (2015) are given.. Derived resistance ratings are R: resistant, R-MR: resistant to moderately resistant, MR: moderately 758 resistant, MR-MS moderately resistant to moderately susceptible, MS: moderately 759 760 susceptible, MS-S: moderately susceptible to susceptible, S: susceptible, S-VS susceptible to very susceptible, VS very susceptible. Genotypes chosen to be resistance references in these 761 nine categories for future experiments are highlighted in the Table.

Resist-		Prin	cipal		Probability > reference cvs.					
ance		axes s	scores	P. thornei/k	P. thornei/kg soil		50a Yandanooka		rating based	
Rank	Genotype	Ρ Δ1	ΡΔ2	PA(1+2)-	BTM	R_MR	S_VS	evnts	on PA(1+2)- eBLUP	
<u>1</u>	CPI133872	-3 783	0.917	<u>8 88</u>	7171		<u> </u>	13	R	
4	OT8343	-2.839	-2.363	9.28	10667	0.226	0	11	R-MR	
5	OT9048	-2.694	-1.186	9.36	11625	0.425	0	9	R-MR	
	GS50a	<mark>-2.666</mark>	<mark>-0.040</mark>	<mark>9.39</mark>	<mark>11991</mark>	NA	0	22	<mark>R-MR</mark>	
	Impose CL									
14	Plus	-2.488	1.243	9.49	13279	0.694	0	4	MR	
17	QT9050	-2.246	-0.232	9.59	14573	0.908	0	12	MR	
<mark>20</mark>	<mark>QT8447</mark>	<mark>-2.086</mark>	<mark>0.577</mark>	<mark>9.67</mark>	<mark>15898</mark>	<mark>0.969</mark>	<mark>0</mark>	<mark>12</mark>	<mark>MR</mark>	
24	Wyalkatchem	-1.937	0.657	9.75	17085	0.946	0	5	MR	
28	Bolac	-1.790	0.356	9.81	18214	0.967	0	4	MR-MS	
30	Kiora	-1.728	-0.748	9.82	18471	0.955	0	4	MR-MS	
34	Gauntlet	-1.716	-0.355	9.84	18675	0.989	0	5	MR-MS	
40	Sunmate	-1.594	-0.700	9.89	19672	0.974	0	4	MR-MS	
41	Amarok	-1.597	-0.529	9.89	19711	0.988	0	5	MR-MS	
51	Corack	-1.523	1.016	9.95	20868	0.996	0	4	MR-MS	
62	Wallup	-1.368	0.329	10.01	22225	0.999	0	4	MR-MS	
65	Suntime	-1.305	-0.389	10.03	22674	0.994	0	4	MR-MS	
<mark>73</mark>	Suntop	<mark>-1.244</mark>	<mark>-0.979</mark>	10.05	<mark>23132</mark>	<mark>0.999</mark>	0	<mark>4</mark>	MR-MS	
75	Ventura	-1.249	0.038	10.06	23411	1	0	9	MR-MS	
79	Chara	-1.219	-0.059	10.07	23717	1	0	11	MS	
107	Glover	-1.026	0.048	10.17	26029	1	0	5	MS	
<mark>120</mark>	Hartog	<mark>-0.995</mark>	<mark>0.541</mark>	10.19	<mark>26608</mark>	<mark>1</mark>	<mark>0</mark>	<mark>14</mark>	MS	
145	Sunzell	-0.845	-0.091	10.25	28282	1	0	5	MS	
191	Leichhardt	-0.651	-0.267	10.34	30914	1	0	11	MS	
287	Sunvale	-0.412	-0.517	10.45	34474	1	0	11	MS-S	
310	Kidman	-0.385	0.000	10.47	35206	1	0	5	MS-S	
329	Giles	-0.338	0.172	10.49	36061	1	0	11	MS-S	
<mark>347</mark>	Gregory	-0.264	-0.532	10.52	<mark>36974</mark>	1	0	<mark>10</mark>	MS-S	
374	Spitfire	-0.169	-1.272	10.55	38253	1	0.002	5	MS-S	
378	Yenda	-0.189	-0.420	10.56	38368	1	0.004	4	MS-S	
384	Wylie	-0.213	0.712	10.56	38560	1	0	9	MS-S	
	2	_			-					

425	Bowerbird	-0.077	-0.443	10.61	40416	1	0.001	6	MS-S
437	Baxter	-0.029	-1.548	10.61	40700	1	0	12	MS-S
557	Sunco	0.142	-1.254	10.70	44311	1	0.003	11	S
10	Lang	0.214	-0.874	10.74	46119	1	0.005	11	S
654	Batavia	0.204	2.060	10.78	47905	1	0.003	21	S
659	Hume	0.260	0.687	10.78	48193	1	0.014	9	S
663	Sunguard	0.284	-0.020	10.78	48242	1	0.029	5	S
674	Sunvex	0.278	0.520	10.79	48484	1	0.054	5	S
688	Waagan	0.327	0.119	10.81	49315	1	0.049	6	S
691	Banks	0.375	-1.295	10.81	49414	1	0.016	11	S
715	Crusader	0.399	-0.151	10.84	50817	1	0.058	6	S
<mark>816</mark>	Cunningham	<mark>0.547</mark>	<mark>-0.050</mark>	<mark>10.91</mark>	<mark>54611</mark>	1	<mark>0.049</mark>	<mark>12</mark>	<mark>S</mark>
905	Janz	0.675	0.579	10.98	58512	1	0.100	13	S-VS
935	Babbler	0.781	-0.530	11.01	60535	1	0.186	4	S-VS
936	Stampede	0.772	0.139	11.02	60839	1	0.219	6	S-VS
<mark>60</mark>	Strzelecki	<mark>0.815</mark>	<mark>0.261</mark>	<mark>11.04</mark>	<mark>62254</mark>	1	<mark>0.185</mark>	<mark>10</mark>	<mark>S-VS</mark>
998	Kennedy	0.962	-0.872	11.09	65577	1	0.263	13	S-VS
1002	H91	0.956	-0.128	11.10	66104	1	0.313	4	S-VS
1004	Kukri	0.968	-0.423	11.10	66236	1	0.317	4	S-VS
1008	Gazelle	0.991	-0.712	11.11	66635	1	0.315	5	S-VS
1018	Gatcher	0.970	0.785	11.12	67507	1	0.314	18	S-VS
1054	Impala	1.162	-0.359	11.19	72619	1	0.481	5	S-VS
1059	Yandanooka	1.210	-1.222	11.20	73423	1	NA	9	S-VS
1070	Lincoln	1.243	1.158	11.25	77187	1	0.581	4	S-VS
1079	Forrest	1.380	0.176	11.30	81145	1	0.684	5	VS
1083	Annuello	1.445	-0.175	11.33	83199	1	0.699	4	VS
1084	Wedgetail	1.421	0.675	11.33	83365	1	0.722	5	VS
1091	Brennan	1.581	-0.050	11.40	88875	1	0.803	5	VS
<mark>095</mark>	Petrie	<mark>1.719</mark>	<mark>-0.356</mark>	<mark>11.46</mark>	<mark>94465</mark>	1	<mark>0.916</mark>	<mark>11</mark>	<mark>VS</mark>
1096	Darwin	1.893	0.714	11.55	104192	1	0.901	4	VS

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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.

771

772 Fig. 2. Latent regression plots of predicted *Pratylenchus thornei* population density as R-

- eBLUPs against estimated loadings for principal axis one (PA1) in 22 glasshouse
- experiments showing a selection of wheat genotypes with lower, intermediate and higher
- nematode population densities and stabilities. Dark blue points indicate genotype present in
- the experiment associated with the loading, whereas light red points indicate an estimatewhere genotype was absent from the experiment associated with the loading.
- 778

Fig. 3. Predictive ability of glasshouse-derived PA(1+2)-eBLUPs of ln(*P. thornei*/kg 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 l.s.d. (P=0.05).
- (a) For Macalister Experiment 1, soil profile to 45 cm depth
- 783 $FPD = 0.744GHBLUP + 1.330, R^2 = 0.73, P < 0.001, n = 11$
- (b) For Macalister Experiment 2, soil profile to 45 cm depth
- 785 $FPD = 0.76GHBLUP + 1.330, R^2 = 0.65, P < 0.001, n = 19$
- 786 (c) For Formartin, soil profile to 90 cm depth
- 787 At low *P. thornei*: $FPD = 1.606GHBLUP 9.20, R^2 = 0.92, P < 0.001, n = 16$
- 788 At high *P. thornei*: FPD = 1.335GHBLUP 5.78, $R^2 = 0.89$, P < 0.001, n = 16
- where FPD = Field population density $\ln(P. thornei/kg soil+1)$ and GHBLUP = Glasshouse
- 790 (PA1+2)-eBLUP of $\ln(P. thornei/kg \text{ soil }+1)$
- 791
- **Fig. 4.** Predictive ability of glasshouse-derived PA(1+2)-eBLUPs of ln(*P. thornei*/kg 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_{12}
- 796 *Pratylenchus thornei* at Formartin. Bar marker is l.s.d. (*P*=0.05).
- 797 (a) NDVI measurements at high *P. thornei*
- 798 $AUDPC = 46.63 0.00000016 * 5.36^{(GHBLUP)}, R^2 = 0.62, P < 0.001, n = 28$
- (b) NDVI measurements at low *P. thornei*:
- 800 $AUDPC = 43.7 0.0000000000286 * 16.3^{(GHBLUP)}, R^2 = 0.54, P < 0.001, n = 28$
- 801 where 'AUDPC' area under the disease progress curve in NDVI units and 'GHBLUP' is
- PA(1+2)-eBLUPs of ln(final population density of *P. thornei*/kg soil) from combined
- 803 glasshouse experiments
- **Fig.5.** Predictive ability of glasshouse-derived PA(1+2)-eBLUPs of ln(*P. thornei*/kg soil) for
- grain yield of wheat cultivars in a *Pratylenchus thornei*-infested field at Macalister for 23 late
- 806 maturing wheat genotypes in Experiment 1 and 52 main maturity wheat genotypes in 807 Experiment 2. Bar marker is 1.s.d. (P=0.05).
- 808 (a) Grain yield in Experiment 1:
- 809 $GY = 0.931 + 1.519 / (1 + \exp(8.44 * (GHBLUP 10.632)), R^2 = 0.47, P = 0.002, n = 23$
- 810 (b) Grain yield in Experiment 2:

- 811 $GY = 1.546 + 0.9589 / (1 + \exp(11.31*(GHBLUP 10.52)))R^2 = 0.50, P < 0.001, n = 52$ 812 where 'GY' is grain yield (t/ha) and 'GHBLUP' is PA(1+2)-eBLUPs of ln(final population
- density of *P. thornei*/kg soil) from combined glasshouse experiments.
- 814
- **Fig. 6.** Predictive ability of glasshouse-derived PA(1+2)-eBLUPs of ln(*P. thornei*/kg soil)
- 816 for grain yield of wheat cultivars in a *Pratylenchus thornei*-infested field at Formartin for 28
- 817 wheat genotypes grown with high and low initial population densities of *P. thornei*. Bar 818 marker is l.s.d. (P=0.05).
- 819 (a) Grain yield at high *P. thornei*:
- 820 $GY = 4.113 0.00000087 * 4.68^{(GHBLUP)}, R^2 = 0.58, P < 0.001, n = 28$
- (b) Grain yield at low *P. thornei*:
- 822 $GY = 3.477 0.000000000101 * 21.9^{(GHBLUP)}, R^2 = 0.45, P < 0.001, n = 28$
- 823 (c) Grain yield loss
- 824 $GYL = -17.1 + 0.000006 * 4.23^{(GHBLUP)}, R^2 = 0.56, P < 0.001, n = 28$
- 825 Where '*GY*' is grain yield (t/ha), '*GYL*' is grain yield loss (%) and '*GHBLUP*' is PA(1+2)-
- eBLUPs of ln(final population density of *P. thornei*/kg soil) in combined glasshouse
- experiments. Grain yield loss (%) =100*(grain yield at low P_t grain yield at high P_t)/grain yield at low P_t
- 829
- **Fig. 7.** Distribution of predicted population densities of *Pratylenchus thornei* based on equal
- subdivision of the range of PA(1+2)-eBLUPs in ln(P. thornei/kg soil+1) units for 1096 wheat
- genotypes from 22 experiments shown as nine corresponding alpha resistance ratings: R
- resistant, R–MR resistant to moderately resistant, MR moderately resistant, MR–MS
- 834 moderately resistant to moderately susceptible, MS moderately susceptible, MS-S
- 835 moderately susceptible to susceptible, S susceptible, S–VS susceptible to very susceptible,
- 836 VS very susceptible.
- 837 838





842 model for resistance testing of *Pratylenchus thornei* in 22 glasshouse experiments from 1996

843 to 2015.



848

Fig. 2. Latent regression plots of predicted Pratylenchus thornei population density as R-849 eBLUPs against estimated loadings for principal axis one (PA1) in 22 glasshouse 850 experiments showing a selection of wheat genotypes with lower, intermediate and higher 851 nematode population densities and stabilities. Dark points indicate genotype present in the 852 experiment associated with the loading, whereas light points indicate an estimate where 853

genotype was absent from the experiment associated with the loading. 854







Fig. 3. Predictive ability of glasshouse-derived PA(1+2)-eBLUPs of ln(*P. thornei*/kg soil)

858 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 l.s.d. (P=0.05).

860 (a) For Macalister Experiment 1, soil profile to 45 cm depth

861 $FPD = 0.744GHBLUP + 1.330, R^2 = 0.73, P < 0.001, n = 11$

(b) For Macalister Experiment 2, soil profile to 45 cm depth

863 $FPD = 0.76GHBLUP + 1.330, R^2 = 0.65, P < 0.001, n = 19$

864 (c) For Formartin, soil profile to 90 cm depth

865 At high *P. thornei*: *FPD* = 1.335GHBLUP - 5.78, $R^2 = 0.89$, P < 0.001, n = 16

- 866 At low *P. thornei*: $FPD = 1.606GHBLUP 9.20, R^2 = 0.92, P < 0.001, n = 16$
- where FPD = Field population density $\ln(P. thornei/kg soil+1)$ and GHBLUP = Glasshouse
- 868 (PA1+2)-eBLUP of ln(*P. thornei*/kg soil +1)
- 869



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871

872	Fig. 4. Predictive ability of glasshouse-derived PA(1+2)-eBLUPs of ln(<i>P. thornei</i> /kg soil)
873	for area under the disease progress curve (AUDPC) of seven measurements of crop greenness
874	by Normalised 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
- 876 *Pratylenchus thornei* at Formartin. Bar marker is l.s.d. (*P*=0.05).
- 877 (c) NDVI measurements at high *P. thornei*

878
$$AUDPC = 46.63 - 0.00000016 * 5.36^{(GHBLUP)}, R^2 = 0.62, P < 0.001, n = 28$$

- (d) NDVI measurements at low *P. thornei*:
- 880 $AUDPC = 43.7 0.0000000000286 * 16.3^{(GHBLUP)}, R^2 = 0.54, P < 0.001, n = 28$
- 881 where 'AUDPC' area under the disease progress curve in NDVI units and 'GHBLUP' is
- 882 PA(1+2)-eBLUPs of ln(final population density of *P. thornei*/kg soil) from combined
- 883 glasshouse experiments



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888

Fig. 5. Predictive ability of glasshouse-derived PA(1+2)-eBLUPs of ln(*P. thornei*/kg soil)
for grain yield of wheat cultivars in a *Pratylenchus 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 l.s.d. (P=0.05).
- 893 (a) Grain yield in Experiment 1:

894 $GY = 0.931 + 1.519 / (1 + \exp(8.44 * (GHBLUP - 10.632)), R^2 = 0.47, P = 0.002, n = 23$

- (b) Grain yield in Experiment 2:
- 896 $GY = 1.546 + 0.9589 / (1 + \exp(11.31*(GHBLUP 10.52)))R^2 = 0.50, P < 0.001, n = 52$
- 897 where 'GY' is grain yield (t/ha) and 'GHBLUP' is PA(1+2)-eBLUPs of ln(final population
- 898 density of *P. thornei*/kg soil) from combined glasshouse experiments.
- 899





Fig. 6. Predictive ability of glasshouse-derived PA(1+2)-eBLUPs of ln(*P. thornei*/kg soil)
for grain yield of wheat cultivars in a *Pratylenchus thornei*-infested field at Formartin for 28
wheat genotypes grown with high and low initial population densities of *P. thornei*.

904 (a) Grain yield at high *P. thornei*: Bar marker is l.s.d. (*P*=0.05).

905
$$GY = 4.113 - 0.00000087 * 4.68^{(GHBLUP)}, R^2 = 0.58, P < 0.001, n = 28$$

906 (b) Grain yield at low *P. thornei*:

907 $GY = 3.477 - 0.000000000101 \times 21.9^{(GHBLUP)}, R^2 = 0.45, P < 0.001, n = 28$

908 (c) Grain yield loss

909
$$GYL = -17.1 + 0.000006 * 4.23^{(GHBLUP)}, R^2 = 0.56, P < 0.001, n = 28$$

- 910 Where 'GY' is grain yield (t/ha), 'GYL' is grain yield loss (%) and 'GHBLUP' is PA(1+2)-
- 911 eBLUPs of ln(final population density of *P. thornei*/kg soil) in combined glasshouse
- 912 experiments. Grain yield loss (%) =100*(grain yield at low P_t grain yield at high P_t)/grain
- 913 yield at low P_t
- 914



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919	Fig. 7. Distribution of predicted population densities of <i>Pratylenchus thornei</i> based on equal
920	subdivision of the range of PA(1+2)-eBLUPs in ln(P. thornei/kg soil+1) units for 1096 wheat
921	genotypes from 22 experiments shown as nine corresponding alpha resistance ratings: R
922	resistant, R-MR resistant to moderately resistant, MR moderately resistant, MR-MS
923	moderately resistant to moderately susceptible, MS moderately susceptible, MS-S

- moderately susceptible to susceptible, S susceptible, S–VS susceptible to very susceptible,
- 925 VS very susceptible.

926

927 Accessory Table 1. Genotypic scores for PA1 and PA2 and predicted final population 928 densities of *Pratylenchus thornei* for 171 wheat genotypes present in four or more of 22 929 930 experiments out of 1096 wheat genotypes in a combined analysis compared with two reference genotypes. Genotypes have been assigned resistance ratings based on subdivision 931 of the PA(1+2)-eBLUP range into nine arithmetically equal categories. BTM = back-932 transformed means by exponentiation. Reference genotypes GS50a and Yandanooka were 933 proposed by Sheedy et al. (2015). Derived resistance ratings are R: resistant, R-MR: 934 resistant to moderately resistant, MR: moderately resistant, MR-MS moderately resistant to 935 936 moderately susceptible, MS: moderately susceptible, MS-S: moderately susceptible to susceptible, S: susceptible, S-VS susceptible to very susceptible, VS very susceptible. 937 Genotypes chosen to be resistance references in these nine categories for future experiments 938 939 are highlighted in the Table.

Resist-	Principal		Pro refe	Alpha resistance		
ance	axes scores	P. thornei/kg soil	GS50a	Yandanooka	No.	rating based

Rank	Genotype	ΡΔ1	ΡΔ2	PA(1+2)- eBLUP	BTM	R-MR	S_VS	expts	on PA(1+2)- eBLUP
<u>1</u>	CPI133872	-3 783	0.917	<u>8 88</u>	7171	0	<u> </u>	<u>13</u>	R
4	OT8343	-2.839	-2.363	9.28	10667	0.226	0	11	R-MR
5	QT9048	-2.694	-1.186	9.36	11625	0.425	0	9	R-MR
6	GS50a	-2.666	-0.040	9.39	119 <u>-</u> 0	NA	0 0	22	R-MR
10	OT9310	-2.528	0.441	9.46	12886	0.663	0	5	R-MR
	Impose CL								
14	Plus	-2.488	1.243	9.49	13279	0.694	0	4	MR
17	QT9050	-2.246	-0.232	9.59	14573	0.908	0	12	MR
18	QT9616	-2.143	1.116	9.66	15599	0.929	0	5	MR
<mark>20</mark>	<mark>QT8447</mark>	<mark>-2.086</mark>	<mark>0.577</mark>	<mark>9.67</mark>	<mark>15898</mark>	<mark>0.969</mark>	<mark>0</mark>	<mark>12</mark>	MR
24	Wyalkatchem	-1.937	0.657	9.75	17085	0.946	0	5	MR
28	Bolac	-1.790	0.356	9.81	18214	0.967	0	4	MR-MS
30	Kiora	-1.728	-0.748	9.82	18471	0.955	0	4	MR-MS
34	Gauntlet	-1.716	-0.355	9.84	18675	0.989	0	5	MR-MS
35	LPB06-1209	-1.669	-1.075	9.85	18901	0.987	0	4	MR-MS
40	Sunmate	-1.594	-0.700	9.89	19672	0.974	0	4	MR-MS
41	Amarok	-1.597	-0.529	9.89	19711	0.988	0	5	MR-MS
48	WW21570	-1.521	0.002	9.93	20577	0.996	0	5	MR-MS
51	Corack	-1.523	1.016	9.95	20868	0.996	0	4	MR-MS
57	QT8620	-1.443	0.211	9.97	21417	0.999	0	6	MR-MS
58	QT12903	-1.401	-0.429	9.98	21654	0.983	0	4	MR-MS
62	Wallup	-1.368	0.329	10.01	22225	0.999	0	4	MR-MS
65	Suntime	-1.305	-0.389	10.03	22674	0.994	0	4	MR-MS
<mark>73</mark>	Suntop National States of	-1.244	-0.979	10.05	23132	0.999	<u>0</u>	<mark>4</mark> 0	MR-MS
/5	Ventura	-1.249	0.038	10.06	23411	1	0	9	MR-MS
/8 70	EGA Burke	-1.259	1.019	10.07	23646	0.999	0	8	MS
/9	Chara	-1.219	-0.059	10.07	23/17	1	0		MS
81	Drysdale	-1.181	0.125	10.10	24221	0.999	0	0	MS MS
83 105		-1.183	0.562	10.10	24342	0.999	0	4	MS MS
105	QAL2000	-1.039	0.219	10.16	25925	1	0	0	MS MS
107 120		-1.020	0.048	10.17	20029	1	0	3 14	MS
120 126	Parsia 20	-0.995 1.004	0.341 1.562	10.19 10.20	20008 26975	1 0.000	U O	14 5	MS
120		-1.004	0.251	10.20	20873	0.999	0	3	MS
144	LFB10-0018	-0.914	-0.251	10.22	27303	0.999	0	4	MS
144	Sunzell	-0.830	0.200	10.25	28282	1	0	0 5	MS
145	Livingston	-0.845	-0.091	10.23	20202	1	0	5 1	MS
16/	Wills	-0.752	-0.0+2 0.122	10.28	29145	1	0	+	MS
104	Sunstate	-0.752	1 320	10.30	29045	1	0	9	MS
183	Merinda	-0.775	-0.324	10.30	30332	1	0	12	MS
184	OT12134	-0.009	-1 237	10.32	30423	0 000	0.002	12 4	MS
186	OT12302	-0 669	-0 501	10.32	30545	0.999	0.002	т 4	MS
191	Leichhardt	-0.651	-0.267	10.34	30914	1	0.002	т 11	MS
198	Pelsart	-0.665	0.207	10.34	31131	1	0	11	MS
170	Impress CL	0.005	0.000	10.55	51151	1	0	11	1110
197	Plus	-0.637	-0.257	10.35	31131	1	0	4	MS
204	AUS4930	-0.650	0.907	10.36	31476	1	0	4	MS
228	Sunbrook	-0.543	-0.810	10.38	32272	1	0	6	MS-S

225	Jade	-0.561	-0.228	10.38	32272	1	0	5	MS-S
252	Diamondbird	-0.513	0.614	10.42	33422	1	0	5	MS-S
256	Mackellar	-0.480	-0.201	10.42	33556	1	0	4	MS-S
259	Condo	-0.477	-0.013	10.43	33690	1	0.002	4	MS-S
286	Pugsley	-0.452	0.604	10.45	34405	1	0.001	4	MS-S
287	Sunvale	-0.412	-0.517	10.45	34474	1	0	11	MS-S
300	Beaufort	-0.394	-0.207	10.46	34925	1	0.001	4	MS-S
301	Peake	-0.410	0.299	10.46	34925	1	0.001	4	MS-S
310	Kidman	-0.385	0.000	10.47	35206	1	0	5	MS-S
325	Eaglehawk	-0.329	-0.479	10.49	35881	1	0.003	5	MS-S
329	Giles	-0.338	0.172	10.49	36061	1	0	11	MS-S
332	QT13333	-0.348	0.959	10.50	36315	1	0	4	MS-S
<mark>347</mark>	Gregory	<mark>-0.264</mark>	<mark>-0.532</mark>	10.52	<mark>36974</mark>	1	<mark>0</mark>	<mark>10</mark>	MS-S
353	Sunbri	-0.267	0.061	10.53	37234	1	0.001	7	MS-S
371	Correll	-0.231	0.375	10.55	38062	1	0.004	4	MS-S
374	Spitfire	-0.169	-1.272	10.55	38253	1	0.002	5	MS-S
378	Yenda	-0.189	-0.420	10.56	38368	1	0.004	4	MS-S
384	Wylie	-0.213	0.712	10.56	38560	1	0	9	MS-S
393	Derrimut	-0.175	-0.242	10.56	38715	1	0.005	4	MS-S
416	Bounty	-0.133	0.291	10.59	39814	1	0.008	5	MS-S
425	Bowerbird	-0.077	-0.443	10.61	40416	1	0.001	6	MS-S
433	Ruby	-0.080	-0.172	10.61	40537	1	0.011	5	MS-S
435	Scout	-0.071	-0.266	10.61	40659	1	0.003	5	MS-S
437	Baxter	-0.029	-1.548	10.61	40700	1	0	12	MS-S
439	Young	-0.078	0.037	10.61	40700	1	0.007	4	MS-S
449	WW12885	-0.056	0.027	10.62	41109	1	0.012	4	MS-S
469	SUN577A	0.015	-1.485	10.64	41605	1	0.003	4	MS-S
491	Steel	0.006	0.086	10.65	42361	1	0.009	5	MS-S
489	Marombi	-0.001	0.323	10.65	42361	1	0.011	4	MS-S
495	Worrakatta	-0.004	0.469	10.66	42403	1	0.008	4	MS-S
503	Kord CL Plus	0.026	-0.133	10.66	42658	1	0.015	4	MS-S
505	Cook	0.046	-0.657	10.66	42701	1	0.002	10	MS-S
513	QT13164	0.020	0.405	10.67	42872	1	0.023	4	S
516	Dart	0.042	-0.154	10.67	42958	1	0.006	4	S
532	Whistler	0.052	0.143	10.68	43346	1	0.014	4	S
542	Bowie	0.068	0.387	10.69	43826	1	0.011	6	S
549	Axe	0.113	-0.702	10.69	44045	1	0.019	4	S
557	Sunco	0.142	-1.254	10.70	44311	1	0.003	11	S
	Hatchet CL								
555	Plus	0.106	-0.127	10.70	44311	1	0.031	4	S
556	Sentinel	0.084	0.640	10.70	44311	1	0.020	4	S
559	Krichauff	0.114	-0.275	10.70	44355	1	0.009	6	S
564	Merlin	0.151	-1.147	10.71	44577	1	0.006	5	S
587	King Rock Elmore CL	0.160	-0.112	10.72	45432	1	0.019	4	S
593	Plus	0.156	0.319	10.73	45660	1	0.024	4	S
608	Braewood	0.197	-0.338	10.74	46073	1	0.020	5	S
610	Lang	0.214	-0.874	10.74	46119	1	0.005	11	S
614	Ovalo	0.204	-0.287	10.74	46257	1	0.045	4	S
622	QT7208	0.208	-0.018	10.75	46536	1	0.012	6	S

626	Catalina	0.204	0.340	10.75	46676	1	0.006	9	S
648	Emu Rock	0.259	0.091	10.77	47762	1	0.029	4	S
653	Virest	0.305	-1.284	10.78	47809	1	0.039	5	S
651	Guardian	0.285	-0.698	10.78	47809	1	0.041	4	S
654	Batavia	0.204	2.060	10.78	47905	1	0.003	21	S
659	Hume	0.260	0.687	10.78	48193	1	0.014	9	S
658	Barham	0.286	-0.161	10.78	48193	1	0.044	4	S
663	Sunguard	0.284	-0.020	10.78	48242	1	0.029	5	S
674	Sunvex	0.278	0.520	10.79	48484	1	0.054	5	S
679	Pardalote	0.253	1.549	10.79	48629	1	0.044	4	S
681	Hunter	0.282	0.700	10.79	48727	1	0.068	4	S
688	Waagan	0.327	0.119	10.81	49315	1	0.049	6	S
689	Wentworth	0.359	-0.857	10.81	49364	1	0.028	8	S
691	Banks	0.375	-1.295	10.81	49414	1	0.016	11	S
698	Jaeger	0.344	0.320	10.82	49910	1	0.110	4	S
705	Lancer	0.391	-0.655	10.83	50261	1	0.057	4	S
709	Sunlin	0.343	0.998	10.83	50361	1	0.032	8	S
711	Estoc	0.376	0.124	10.83	50462	1	0.080	4	S
714	Trojan	0.382	0.292	10.84	50766	1	0.062	4	S
715	Crusader	0.399	-0.151	10.84	50817	1	0.058	6	S
717	Hornet Justica CL	0.393	0.111	10.84	50868	1	0.057	6	S
720	Plus	0.385	0.453	10.84	50969	1	0.079	4	S
743	Tenfour	0.433	0.111	10.86	51843	1	0.102	4	S
772	Ellison	0.490	-0.210	10.88	52996	1	0.056	8	S
779	Potam	0.490	0.241	10.89	53369	1	0.048	7	S
787	Wylah	0.550	-1.246	10.89	53690	1	0.093	6	S
794	H45	0.527	-0.212	10.90	53959	1	0.082	5	S
806	Shield	0.505	0.845	10.90	54230	1	0.103	4	S
807	Carinya	0.522	0.363	10.90	54284	1	0.117	5	S
809	Mitch	0.536	0.001	10.90	54338	1	0.107	4	S
811	Preston	0.561	-0.745	10.90	54393	1	0.112	4	S
<mark>816</mark>	Cunningham	<mark>0.547</mark>	<mark>-0.050</mark>	10.91	<mark>54611</mark>	1	<mark>0.049</mark>	<mark>12</mark>	<mark>S</mark>
826	Harper	0.560	-0.251	10.91	54775	1	0.111	4	S
832	Clearfield Jnz	0.560	0.110	10.92	55049	1	0.122	4	S
850	Orion	0.578	-0.055	10.92	55380	1	0.095	5	S
853	Rosella	0.590	-0.281	10.93	55547	1	0.113	5	S
855	SQP Revenue	0.548	1.134	10.93	55602	1	0.098	5	S
862	Manning	0.631	-1.137	10.93	55881	1	0.098	4	S
873	Naparoo	0.623	0.003	10.94	56612	1	0.148	4	S
881	Espada	0.633	0.105	10.95	57010	1	0.153	4	S
899	QT12667	0.668	0.261	10.97	58046	1	0.214	4	S-VS
905	Janz	0.675	0.579	10.98	58512	1	0.100	13	S-VS
907	Cobalt	0.686	0.337	10.98	58629	1	0.167	5	S-VS
935	Babbler	0.781	-0.530	11.01	60535	1	0.186	4	S-VS
936	Stampede	0.772	0.139	11.02	60839	1	0.219	6	S-VS
949	Tennant	0.816	-0.522	11.03	61573	1	0.223	5	S-VS
955	Envoy	0.818	-0.075	11.04	62006	1	0.224	4	S-VS
960	Strzelecki	<mark>0.815</mark>	<mark>0.261</mark>	<mark>11.04</mark>	<mark>62254</mark>	1	<mark>0.185</mark>	<mark>10</mark>	<mark>S-VS</mark>

968	Sapphire	0.814	0.790	11.05	62692	1	0.261	6	S-VS
969	QALBis	0.841	-0.057	11.05	62754	1	0.246	6	S-VS
988	Mansfield	0.894	-0.368	11.07	64022	1	0.261	5	S-VS
998	Kennedy	0.962	-0.872	11.09	65577	1	0.263	13	S-VS
999	Rudd	0.932	0.437	11.10	65906	1	0.321	5	S-VS
1002	H91	0.956	-0.128	11.10	66104	1	0.313	4	S-VS
1004	Kukri	0.968	-0.423	11.10	66236	1	0.317	4	S-VS
1008	Gazelle	0.991	-0.712	11.11	66635	1	0.315	5	S-VS
1018	Gatcher	0.970	0.785	11.12	67507	1	0.314	18	S-VS
1021	Phantom	1.032	-0.461	11.13	68185	1	0.365	4	S-VS
1023	H46	1.035	-0.539	11.13	68254	1	0.364	6	S-VS
1026	Petrel	1.033	-0.170	11.14	68527	1	0.378	5	S-VS
1034	Sunsoft98	1.057	-0.002	11.15	69493	1	0.394	7	S-VS
1043	Excalibur	1.106	0.062	11.17	71181	1	0.440	7	S-VS
1045	QT12663	1.118	-0.311	11.17	71181	1	0.460	4	S-VS
1047	Dakota	1.115	0.087	11.18	71538	1	0.456	6	S-VS
1050	Gladius	1.124	0.174	11.18	71897	1	0.467	4	S-VS
1053	Currawong	1.150	-0.070	11.19	72547	1	0.4801	4	S-VS
1054	LR Impala	1.162	-0.359	11.19	72619	1	0.481	5	S-VS
1059	Yandanooka	1.210	-1.222	11.20	73423	1	NA	9	S-VS
1065	QAL1064	1.208	0.477	11.23	75131	1	0.536	4	S-VS
1070	Lincoln	1.243	1.158	11.25	77187	1	0.581	4	S-VS
1074	Opata85	1.336	0.052	11.28	79300	1	0.652	7	VS
1079	Forrest	1.380	0.176	11.30	81145	1	0.684	5	VS
1083	Annuello	1.445	-0.175	11.33	83199	1	0.699	4	VS
1084	Wedgetail	1.421	0.675	11.33	83365	1	0.722	5	VS
1091	Brennan	1.581	-0.050	11.40	88875	1	0.803	5	VS
1095	Petrie Petrie	<mark>1.719</mark>	<mark>-0.356</mark>	<mark>11.46</mark>	<mark>94465</mark>	<mark>1</mark>	<mark>0.916</mark>	<mark>11</mark>	<mark>VS</mark>
1096	Darwin	1.893	0.714	11.55	104192	1	0.901	4	VS