USE OF NORMALISED DIFFERENCE VEGETATION INDEX (NDVI) TO ASSESS TOLERANCE OF WHEAT CULTIVARS TO ROOT-LESION NEMATODES (*PRATYLENCHUS THORNEI*)

A thesis submitted by

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For the award of

Master of Science (Advanced Research)

2019

Abstract

Increasing food production is crucial to adequately nourish the global population. Wheat is a staple cereal as a major food source in many countries worldwide, and growing it is attractive and lucrative to growers, traders, and to whole countries' economies. Growers in Australia have become heavily reliant on wheat as their primary winter season crop and economic income. Continuous cropping of wheat cultivars that are susceptible to the root-lesion nematode, *Pratylenchus thornei* has caused increased population densities in the soil leading to significant yield loss of intolerant crops. Yield losses of up to 70% have been demonstrated. Therefore, growers are reliant on wheat cultivars that are tolerant to *P. thornei* to minimise yield losses. Wheat breeders need to develop regionally adapted cultivars with improved tolerance to *P. thornei*. The use of new technologies, such as normalised difference vegetation index (NDVI), could assist with selecting cultivars with improved tolerance. This forms the focus of the research questions posed in Chapter one, and the review of literature is addressed in Chapter two of this thesis.

The aim of this study was to investigate NDVI, measured by GreenSeeker™, as a tool to improve the selection of *P. thornei* tolerance of wheat genotypes in research and wheat breeding programs. To do this, three two-stage field experiments tested 36 wheat cultivars. In the first stage, two wheat cultivars, one moderately resistant and one susceptible to *P. thornei* were grown as plots in replicated experimental designs to establish low and high nematode population densities respectively. In the second stage, these plots were sown with 36 wheat cultivars across low and high nematode population densities. NDVI measurements were taken regularly during the growing season and grain was harvested at crop maturity from each plot. The NDVI values for intolerant wheat cultivars were inversely related to *P. thornei* population densities. The NDVI values for tolerant cultivars were independent of *P. thornei* population densities. Cultivars could be classified into groups by their response to *P. thornei* as determined by the predicted NDVI values. Higher *P. thornei* compared to lower population densities improved the correlation between the NDVI predicted tolerance and grain yield for the wheat cultivars. These correlations were observed when comparing the area under disease progress curve (AUDPC) with respect to NDVI and by single critical point sensing (CPS). An advantage of AUDPC with respect to NDVI compared to CPS was that even on population densities, as low as 1245 *P. thornei*/kg soil, AUDPC-NDVI is predictive of tolerance ($R^2 = 0.35$, $P>0.001$). It was found that CPS can be used to predict the tolerance of wheat cultivars at approximately 1000°Cd after sowing on land with initial

population densities greater than 2500 *P. thornei*/kg soil. These results are presented in Chapter three as a accepted article in the journal, Annals of Applied Biology.

This study demonstrated that NDVI can be used to predict tolerance of wheat cultivars to *P. thornei*. More research is required to determine the suitability of NDVI on small plots, such as three row plots that are used in breeding programs, and this is described in the concluding chapter (Chapter four). Briefly, there is additional scope to determine whether NDVI can be used to predict the tolerance of other important crops, such as chickpea and barley that are known to suffer yield losses due to *P. thornei*. Furthermore, wheat breeders have options to use aerial technologies in their phenotyping programs with the availability of unmanned aerial vehicles (UAV) with the capacity to have NDVI, multispectral or thermal cameras. Ultimately, the development of a high throughput tool using UAV that accurately predicts the tolerance of a cultivar to *P. thornei* will enable more rapid development by researchers and plant breeders of germplasm and adapted cultivars with superior tolerance to *P. thornei*.

Certification of Thesis

This thesis is the work of Neil Alan Robinson, except where otherwise acknowledged, with the majority of the authorship of the paper presented as a thesis by Publication undertaken by the student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Supervisor: Professor John Thompson

Associate Supervisor: Dr Kirsty Owen

Student and supervisors signatures of endorsement are held at the University.

Statement of Contribution

This thesis is presented as a thesis by publication. Chapter three is an accepted journal article published in Annals of Applied Biology. The details of this publication with the agreed contribution for the candidate and co-authors is given below:

Robinson, NA, Sheedy, JG, MacDonald, BJ, Owen, KJ & Thompson, JP, 2019, 'Tolerance of wheat cultivars to root-lesion nematode (*Pratylenchus thornei*) assessed by Normalised Difference Vegetation Index (NDVI) is predictive of grain yield', *Annals of Applied Biology*, vol. 174, pp. 388–401 (accepted for publication – 21 February 2019). Impact factor: 2.046.

The contributions by authors is as follows:

- Robinson, NA (55%) – concept, design, experimentation, analysis, drafting and revising of journal article for submission
- Sheedy, JG (10%) – advice, concept, design and editing of journal article
- MacDonald, BJ (10%) – analysis and editing of journal article
- Owen, KJ (10%) – editing and revising of journal article
- Thompson, JP (15%) – concept, analysis, editing and revising of journal article for submission

Acknowledgements

I wish to express my sincere gratitude and appreciation to everyone who supported me during the time I undertook a Master's degree. I am blessed in so many ways to have the opportunity to study for a higher degree, and to have the support of my employer, the University of Southern Queensland, and for the encouragement of those around me. On a personal note, this has been a great period and chapter of my life that I will look back on it fondly for so many reasons.

Firstly, to my supervisors, colleagues and the University of Southern Queensland. I hope that you feel a sense of achievement, and that you may join with me in celebrating the submission of this Master's thesis. To my principal supervisor, Professor John Thompson, thank you for your leadership, your guidance, your encouragement and your time that you have generously given. To my co-supervisor, Dr Kirsty Owen, thank you for your time, your knowledge and your editing prowess, and for being someone who devotes the time to listen. Thank you and I look forward to working alongside you both.

To my colleague Jason Sheedy, thank you for all your help, discussions and support along the way. To Tim Clewett, Roslyn Reen, Michelle Thompson, Jing Ling, Hannah Rostad and Mei Wang, thank you for all your help and contributions. Thank you to all of the members of the Action Learning Group who reviewed and provided feedback in preparation of the manuscript, the confirmation of candidature document, and listening to practice presentations.

Thank you to the University of Southern Queensland, in particular the Centre for Crop Health, for being my employer and supporting my part-time study. This has been a rewarding experience, and I look forward continuing as a member of the Centre with the quest of using science to advance the stability and profitability of the agricultural sector.

I would also like to acknowledge the support I received from the Queensland Department of Agriculture and Fisheries, in particular James Henderson and Graham Pauli with their valuable help with field experiments, and Bethany Macdonald, Michael Mumford and Alison Kelly for their statistical support.

A special thank you to Alec Gwynne and family, for their long term support of our *P. thornei* research. Thank you for your generosity in supplying farm land for our experiments, and your commitment and willingness to support our research endeavours. Thank you to the

Grains Research and Development Corporation (GRDC), for funding DAQ00171 and USQ00019, in particular Dr Omid Ansari and John Sheppard for instigating this higher degree study within these projects. Thank you to the Australian Commonwealth Government under the Research Training Program (RTP) Fees Offset scheme for funding the tuition fees associated with this study.

And finally, to my family and friends. Thank you all for being understanding and accommodating of me over the period of my enrolment, and spurring me on by making time for me to study when needed. To my parents and parent's in-law, thank you for your love, encouragement and support. To my daughter, Sophie. A big thank you to our 'little one' for challenging me on the value of having a balanced family, work and study lifestyle. To my wife, Philippa, thank you for your love, encouragement, prayers and your willingness to sacrifice time to make this degree possible. And thank you to my Lord Jesus, for your innumerable blessings, wisdom and strength.

List of Figures

This list of figures with page numbers below are representative of the figures used in Chapters two and four of this thesis. A secondary list of figures is included on pages 65 and 66 of this thesis that are representative of the figures used in the accepted journal article.

List of Table

This list of table with page numbers below are representative of the Table used in Chapter two of this thesis. This list does not include the Tables in the accepted journal article (Chapter three).

Abbreviations and Acronyms

APSIM	Agriculture production systems simulator
AUD	Australian dollar
AUDPC	Area under disease progress curve
BCA	Biological control agent
BLUP	Best linear unbiased prediction
BTM	Back transformed mean
CPS	Critical point sensing
DAS	Days after sowing
GYL	Grain yield low
GYH	Grain yield high
HTPP	High throughput phenotyping platform
NDVI	Normalised difference vegetation index
PCA	Principal component analysis
PC1	Principal component 1
PC2	Principal component 2
PC3	Principal component 3
PAW	Plant available water
QDAF	Queensland Department of Agriculture and Fisheries
QTL	Quantitative trait locus
RLN	Root-lesion nematode
R_{NIR}	Reflectance near infrared
R_{RED}	Reflectance visible red radiation
UAV	Unmanned aerial vehicle
USQ	University of Southern Queensland
VAF	Variance accounted for
VI	Vegetation index
YTI	Yield tolerance index

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1. Chapter One – Introduction

1.1. Project rationale

Cereal production in the subtropical grain region of eastern Australia is impacted by both abiotic and biotic factors. Crop yields depend on the interaction of sowing date, rainfall, soil fertility, plant populations, and pests and diseases (Lee et al. 2010). This thesis aims to explore one of these biotic factors, the root-lesion nematode *Pratylenchus thornei*, and to investigate whether a novel technology, normalised difference vegetation index (NDVI), will help with characterising wheat cultivars for tolerance to *P. thornei*.

Pratylenchus thornei is a major pest worldwide (Smiley et al. 2014; Thompson et al. 2010) and is responsible for decreasing wheat yields (Thompson et al. 2012; Van Gundy et al. 1974). For example, in the United States of America, *P. thornei* has caused yield loss in the Pacific Northwest region (Smiley et al. 2014). Meanwhile, in the subtropical grain region of eastern Australia, yield losses of up to 65% have been reported for intolerant wheat cultivars (Thompson et al. 1999) and with *P. thornei* found in 67% of fields (Thompson et al. 2010) the cost to this region was estimated at AUD $38 million/year (Murray & Brennan 2009) in lost wheat production.

In order to limit production losses by *P. thornei*, wheat breeders in the subtropical grain region of eastern Australia have incorporated genes for tolerance into commercially adapted pedigrees by selecting for high yielding cultivars at a field site with high *P. thornei* population densities (Thompson et al. 1999). As part of this process, breeding lines and wheat cultivars have been assessed for *P. thornei* tolerance using visual scores of symptoms in the plant tops during the vegetative stages of plant growth and by final grain yield (Thompson et al. 1999). There are potential problems with either of these assessment methods, as (i) visual scores are subjective and there can be variation in scores between operators who conduct visual assessments (Bock et al. 2010) and (ii) grain yield results collected at the end of season (in November and December), are at risk because of the prevalence of damaging summer storms and hail events. Additionally, grain harvest may not be feasible due to labour and financial constraints where experiments comprise many thousands of experimental breeding lines and cultivars that need to be screened for tolerance. Alternative methods to visual scores and grain harvest to predict the tolerance of wheat cultivars to *P. thornei* are needed.

This Master's thesis is an investigation into the suitability of the NDVI to predict the tolerance of wheat cultivars to *P. thornei*. Predicting the tolerance of wheat cultivars to *P. thornei* will ensure that wheat breeders develop cultivars that can produce superior yields when grown in infested fields. The stress response of intolerant wheat cultivars, include symptoms like, chlorosis of the lower leaves and reduced plant biomass. Advantageously, Greenseeker™ (NTech Industries Inc., Ukiah, CA, USA) measures NDVI, and is an instrument that can accurately identify stressed plants (Walsh et al. 2013). Measuring the greenness of plants by NDVI has been routinely used in some breeding programs (Araus et al. 2008) to give an objective assessment of plant growth and to increase the phenotyping capacity for plant breeders (Kumar et al. 2016).

1.2. Research questions

The overall aim of the research presented in this thesis was to determine whether NDVI can be used as a phenotypic tool to predict the tolerance of wheat cultivars to *P. thornei*. If NDVI were able to predict *P. thornei* tolerance, then Greenseeker™ could be used as a selection tool in wheat breeding programs on suitable sites. Six research questions (listed below) were posed to investigate the suitability of NDVI to predict the tolerance of wheat cultivars to *P. thornei*:

1. Can NDVI discriminate the vegetative growth of wheat cultivars grown on land with a high population density of *P. thornei*?
2. Are the NDVI values obtained predictive of grain yield?
3. Since NDVI is not trait specific, is it necessary to have a low population density of *P. thornei* as a control treatment or is high population densities sufficient, and what is the threshold for the high population density?
4. Is it necessary to measure NDVI regularly during the growing season to establish an area under the disease progress curve (AUDPC) or is critical point sensing sufficient and if so, what is that critical point?
5. Can numerical methods including multivariate analysis by cluster analysis and principal components analyses, calculation of AUDPC, calculation of nematode tolerance indices based on NDVI and grain yield, multiple regression analysis, and critical point analysis be utilised to answer questions 1–4?
6. Are the results consistent across experiments conducted in different years?

1.3. Publication arising

Robinson, NA, Sheedy, JG, MacDonald, BJ, Owen, K & Thompson, JP, 2019, 'Tolerance of wheat cultivars to root-lesion nematode (*Pratylenchus thornei*) assessed by Normalised Difference Vegetation Index (NDVI) is predictive of grain yield', *Annals of Applied Biology*, vol. 174, pp. 388–401 (accepted for publication – 21 February 2019)

1.4. Conference presentations arising

Robinson, N, Sheedy, JG, Mumford, M, Kelly, A & Thompson, JP, 2016, 'Using normalized difference vegetation index (NDVI) to select wheat genotypes for tolerance to the root-lesion nematode *Pratylenchus thornei'*, Proceedings of *9th Australasian Soilborne Disease Symposium*, New Zealand, p. 67

Robinson, N, Sheedy, J, Thompson, J, Mumford, M & Kelly, A, 2017, 'Use of Normalised Difference Vegetation Index (NDVI) to assess tolerance of cereal cultivars to root-lesion nematode (*Pratylenchus thornei*)', *Queensland Department of Agriculture and Fisheries Seminar Series*, Toowoomba. **(Oral presentation)**

Robinson, N, Owen, K & Thompson, J, 2017, 'Use of normalised difference vegetation index (NDVI) to assess tolerance of cereal cultivars to root-lesion nematode (*Pratylenchus thornei*)', In: R. Zwart & K. Owen (eds.), *Science Protecting Plant Health 2017 Workshop 'Management of plant-parasitic nematodes through crop rotation, plant breeding and other means*', Toowoomba, p. 6 **(Oral presentation)**

2. Chapter Two – Literature Review

2.1. Introduction

Securing adequate food for an ever-increasing world population is a concern of many societies and a focal point of numerous governments (Potgieter et al. 2013). The dependency on agriculture can be assessed in two ways, firstly to adequately nourish six billion people worldwide, and secondly, to effectively manage half of the global land area (Richards et al. 2007). Climate variability, increasing populations, a reduction of arable land availability, and changing weather patterns have provided challenges to plant breeders to increase yield to ensure food security (Furbank & Tester 2011). Conservation agriculture or sustainable cropping aims to minimise environmental degradation and maximise agricultural production (Govaerts et al. 2007; Stirling 2014). As a result, Australia has seen an annual yield increase of 1 to 3% in rain-fed environments (Trethowan et al. 2002).

The United States Agency for International Developments, Famine Early Warning Network, monitors food security and emphasises the need to use novel technologies to achieve sustainable supply (Brown & de Beurs 2008). Australia's food security is currently coordinated by liaisons between local agronomists and the Australian Bureau of Statistics which then reports to the Australian Government for policy decisions (Potgieter et al. 2013). Government officials are using remote technologies and satellite imaging as aids for decision and policy making. These same technologies are readily available to researchers and plant breeders at an experimental level, and are able to deliver results with accuracy and repeatability.

The following literature review will address the use of normalised difference vegetation index (NDVI) from a research perspective, to estimate the effects of the root-lesion nematode, *Pratylenchus thornei* on the yield of wheat cultivars, and the potential application of NDVI in wheat breeding programs to phenotype wheat cultivars and germplasm for tolerance to *P. thornei.*

2.2. Introduction: Nematodes (Phylum: Nematoda)

Nematodes are the most abundant multicellular organisms on earth and are found in almost all environments worldwide (Castillo & Vovlas 2007). Terrestrial nematodes are classified by the morphology of their mouthparts into trophic groups that determine their unique roles in the soil food web (Stirling 2014). Of these trophic groups, the plant-parasitic nematodes, which have stylets or needle-like mouth-parts, are extensively studied because of their

detrimental impact on worldwide crop production. Reducing their population densities to levels that are below those that cause economic damage to production is critical in agricultural systems (Schmidt et al. 2017). The root-lesion nematodes (*Pratylenchus* spp. and *Radophilus* spp.) are ranked among the most damaging three groups of plant parasitic nematodes alongside cyst nematodes (*Heterodera* spp. and *Globodera* spp.) and root-knot nematodes (*Meloidogyne* spp.) (Jones et al. 2013). In Australia, the root-lesion nematodes (*Pratylenchus* spp.) are ranked as the most economically important nematode genus due to their impact on production of rain-fed cereal crops (Jones & Fosu-Nyarko 2014), caused by their wide host range (Castillo et al. 2008) and their distribution throughout major broadacre cropping regions in Australia (Nicol 1996; Robinson et al. 2014; Thompson et al. 2010; Vanstone 2007).

2.2.1. Root-lesion nematodes: *Pratylenchus* spp.

There are 68 *Pratylenchus* species described worldwide (Castillo & Volvas 2007). Root-lesion nematodes (RLN) are migratory endo-parasites (Fortuner 1977; Roberts 1982; Taylor & Evans 1998) where females can lay eggs inside and outside of the root (Agrios 1997; Jones et al. 2013). The lifecycle of *Pratylenchus* spp. begins at the egg stage, followed by four distinct juvenile stages (J1, J2, J3 and J4) that are defined by moulting, degree of development of gonads and overall size (Castillo & Vovlas 2007). The adult stage, is reproductively active and can lay eggs (Agrios 1997). *Pratylenchus* spp., except for the egg and J1 stages, are able to penetrate, infect and damage the root cortex of susceptible plants (Jones et al. 2013). The lifecycle of RLN is shown in Figure 2.1.

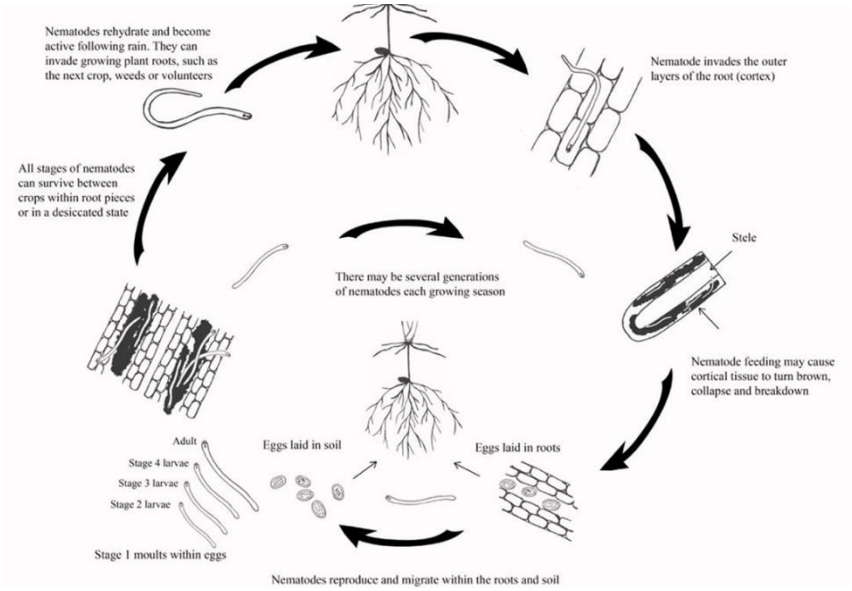

Figure 2.1 The lifecycle of the root-lesion nematode (*Pratylenchus* spp.) (Source: Agrios 1997).

2.2.2. Root-lesion nematode: *Pratylenchus thornei*

Male *P. thornei* are rare (Castillo & Volvas 2007), and adult females (Figure 2.2) reproduce by mitotic parthenogenesis (De Waele & Elsen 2002). The adult female *P. thornei* are 0.45–0.77 mm in length, with the vulva positioned at 73–80% of the total body length, and the length of the stylet is 17–19 µm (Sher & Allen 1953). With a lifecycle between 25–35 days when temperatures are between 20–25°C (Castillo et al. 1995), *P. thornei* population densities can rapidly increase when a susceptible plant is grown (Thompson et al. 2015). When no host is present and there is very limited water, *P. thornei* can survive in the soil for extended periods by decreasing the metabolic rate (Hoeskstra et al. 2014) which is known as anhydrobiosis (Castillo & Volvas 2007). The J4 life stage can more readily survive soil desiccation compared with the other life stages (Thompson et al. 2016).

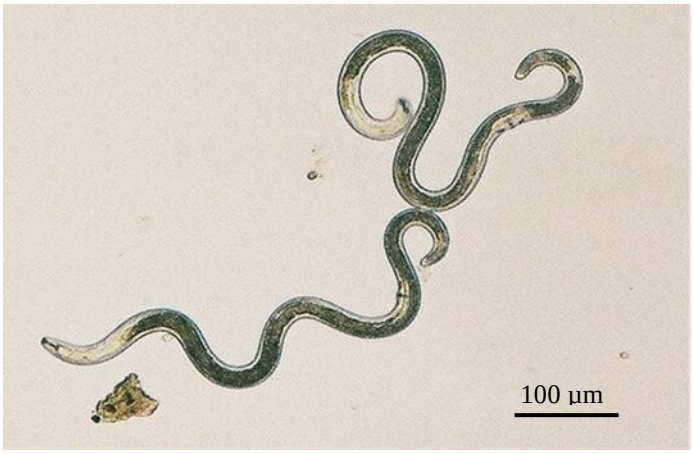

Figure 2.2 Two adult female *Pratylenchus thornei*. The length of the adult *P. thornei* is approximately 600 µm (Source: USQ Crop Nematology)

Necrotic lesions develop when *P. thornei* infest the roots of host crops (Baxter & Blake 1968; De Waele & Elsen 2002; Nicol & Ortiz-Monasterio 2004), destroying cell walls by migration through epidermal and cortical cells (Castillo et al. 1998), and thus slowing plant growth (Jones et al. 2013). Intolerant wheat cultivars consequently have reduced soil water extraction velocities due to root damage (Whish et al. 2014) and therefore take up less available water and nutrients from the soil (May et al. 2016), which causes chlorosis of lower leaves, stunted plant development and reduced yields (De Waele & Elsen 2002). These symptoms are shown in Figure 2.3, when an intolerant wheat cultivar is grown on high population densities (LHS) compared to low population densities (RHS) of *P. thornei*.

Figure 2.3 The effects of *Pratylenchus thornei* on plant tops of an intolerant wheat cultivar at Formartin, Darling Downs on high (LHS) and low (RHS) population densities. The reduced biomass and leaf chlorosis of intolerant wheat cultivars are consequential symptoms of *P. thornei* damage of the roots (Source: Jason Sheedy, USQ).

Yield loss or reduced biomass due to *Pratylenchus* spp. are common in wheat-producing regions worldwide, including the United States of America (May et al. 2016; Smiley & Nicol 2009), Australia (Owen et al. 2014; Thompson et al. 2012), Mexico (Van Gundy 1974) and Israel (Orion et al. 1979). In the Pacific northwest of America, 60% of fields sampled had populations densities of *Pratylenchus* spp. exceeding the economic damage threshold (Yan et al. 2008) of 2000 *P. neglectus* per kg soil (Smiley et al. 2005b). The economic loss in production was valued at US$8–20 per hectare (Smiley et al. 2014). In Sonora Mexico, yield losses in wheat caused by *P. thornei* were reported 50 years ago (Van Gundy 1974). In Australia, the value of potential losses to wheat production from *P. thornei* was valued at AUD$104 million for all Australia grain growing regions (Murray & Brennan 2009). In south eastern Australia both *P. thornei* and *P. neglectus* are commonly found in the same fields (Hollaway et al. 2000) and nematode densities are dependent on cropping intensities (Hollaway et al. 2008). *Pratylenchus neglectus* is more commonly found than *P. quasiteroides* and *P. thornei* in Western Australia fields (Anon 2018). In the subtropical grain region of eastern Australia, *P. thornei* is the most prevalent (Thompson et al. 2010) and damaging of the RLN species to broadacre wheat crops, reducing yields by 17% annually (Murray & Brennan 2009). In the southern and western cropping regions of Australia, the impact of *P. thornei* is estimated to be 1.8 and 3.0% annual yield loss respectively (Murray & Brennan 2009).

The subtropical grain region of eastern Australia has a range of different soil types, with self-mulching Vertosols (Isbell 1996) favoured for grain production. These soils have a high clay content and can potentially store large amounts of water (Hochman et al. 2001) from the summer dominant rainfall patterns during October to March. During this period, 60% of the annual rainfall (Boschma et al. 2017) is captured by the soil and can be used by winter cereal and legume crops that are grown in profitable rotations (Cox et al. 2010). Soils with a greater clay content, have a generally deeper soil depth distribution of *Pratylenchus* spp. compared with sandier textured soils (Taylor & Evans 1998).

In the subtropical grain region of eastern Australia, wheat is the predominant winter broadacre crop (Unkovich et al. 2009; Anon 2016) and is generally grown in rain fed environments. In production terms, wheat is the largest grain crop (1.4 million tonnes), and second only to sorghum (*Sorghum bicolor*) (1.5 million tonnes) for all crops in 2015 in Queensland (Anon 2016). In 2018, in the subtropical grain region of Queensland and New

South Wales, 3.7 million ha of wheat was grown and yielded on average 1.4 t/ha (ABARE 2018).

Intense production (>50 years) of crops that are susceptible to *P. thornei* in the subtropical region of eastern Australia has favoured *P. thornei* (Thompson et al. 2010). A survey of 795 wheat fields within the region found *P. thornei* was present in 67% of these fields (Thompson et al. 2010). In 31% of samples, population densities exceeded the estimated threshold for damage to intolerant wheat cultivars (>2000 *P. thornei*/kg soil) (Thompson et al. 2010). In later surveys of this same region from 2010 to 2014, approximately 50% of fields had *P. thornei* above this damage threshold (Robinson et al. 2014).

The following section will focus on the tolerance and resistance of cultivars to *P. thornei*, and will also review the potential impact of biological and chemical controls to reduce yield loss.

2.3. Tolerance and resistance

Incorporating resistance and tolerance genes into plant cultivars to protect against plant pathogens can help to secure food production worldwide (Politowski & Browning 1978). In plant nematology, the terms tolerance and resistance refer to two different traits. Tolerance is the capacity of a cultivar or host to maintain yield (Ney et al. 2013) and therefore, a crop that is tolerant to nematodes yields well when grown in fields infested with nematodes (Cook & Evans 1987; Roberts 1982). Resistance is the capacity of a cultivar or host to prevent pathogen reproduction (Ney et al. 2013; Trudgill 1991). Both tolerance and resistance are genetically independent (Smiley & Nicol 2009), therefore if a wheat cultivar is tolerant to *P. thornei*, it does not imply that the cultivar is resistant.

2.3.1. Tolerance to *Pratylenchus thornei*

Tolerance is an important trait for production of high yielding cultivars in *P. thornei*-infested fields. Genetic tolerance to a disease can only be quantified when damage occurs to a plant, because the level of tolerance may be over-estimated due to a lowered nematode pressure (Wallace 1987) if the plant is resistant (Simms & Triplett 1994). Furthermore, genetic tolerance only ensures that yield loss is minimised, because the susceptibility of the cultivar determines the change in pathogen population density (Bingham et al. 2009). A resistant plant can appear tolerant, however a susceptible plant could have varying levels of tolerance (Wallace 1987). A wheat cultivar that is tolerant to nematodes can maintain yield potential when grown in fields that are infested with moderate to high nematode population densities

(Smiley et al. 2005a, 2014; Thompson et al. 2012). For example, the biomass and yield of the intolerant wheat cultivar Strzelecki was reduced by 77% and 62% respectively when the initial population density was approximately 8000 *P. thornei*/kg (Owen et al. 2014). In another study, Strzelecki lost 53% of yield when grown on high compared to low population densities of *P. thornei* (Whish et al. 2014). Similarly, in South Australia, the yield of the wheat cultivar Warigal decreased by 27% (Nicol et al. 1999) and in Sonora, Mexico wheat growth was reduced when grown in fields with high *P. thornei* populations (Van Gundy et al. 1974).

Wheat breeders have recognised that tolerance to *P. thornei* is required in cultivars and good progress has been made to select and release cultivars to minimise yield losses (Thompson et al. 1999; 2008). The first wheat cultivar specifically bred for *P. thornei* tolerance in the subtropical grain region of eastern Australia was the cultivar Pelsart (Brennan et al. 1994). Selection for tolerance among other plant breeding lines resulted in other wheat cultivars, including Sunvale (released 1993), and Baxter (released 1999) that had superior tolerance to *P. thornei* compared with other cultivars (Thompson et al. 1999). By 2007, the wheat cultivar EGA Wylie was the only cultivar that had a higher level of tolerance than Sunvale (Lush et al. 2007; Thompson et al. 2008). However, by 2018, ~ 60% of the 44 wheat cultivars recommended for the subtropical grain region of eastern Australia had tolerance superior to Sunvale (which is currently rated as moderately tolerant–moderately intolerant), and were rated as moderately tolerant, with low risk of yield loss (Lush 2018).

Another example, is the classification of cultivars into four tolerance groups depending on the percentage of yield loss caused by high *P. thornei* populations, compared to yields of cultivars grown in aldicarb-treated soils (Smiley et al. 2014). In their study, three out of the four cultivars tested in the Pacific Northwest of the United States suffered yield losses greater than 11% (Smiley et al. 2014). The importance of phenotyping for tolerance is shown in the example in Figure 2.4, whereby the highest yielding cultivar in this experiment, yielded approximately seven times that of the lowest yielding cultivar when evaluated in a single yield trial in 2013 at a field site with high population densities of *P. thornei*.

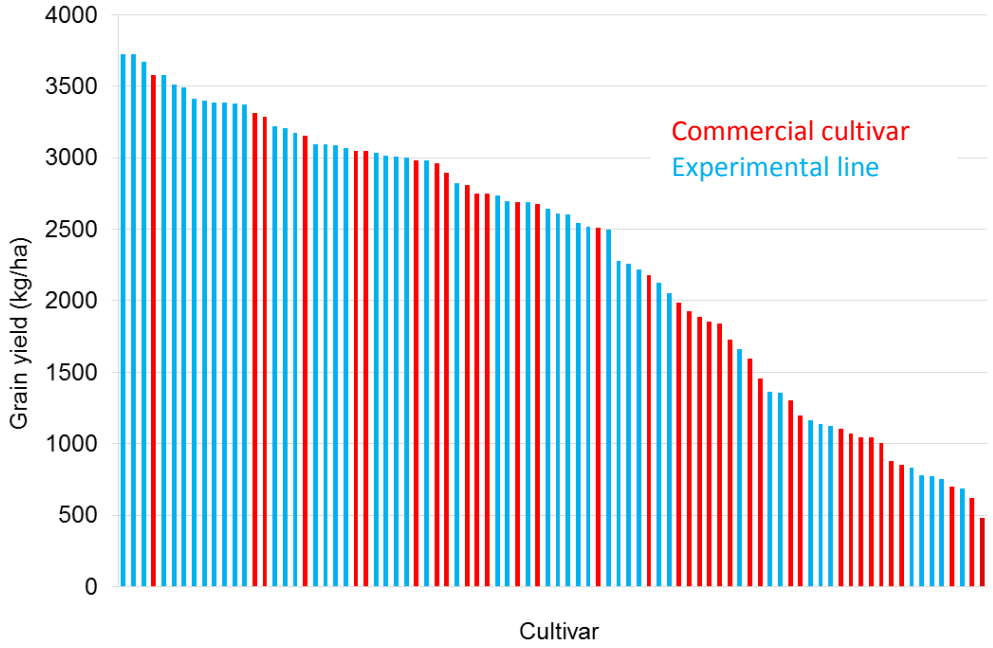

Figure 2.4 An example of the range of tolerance levels determined by yield (kg/ha) for the commercial wheat cultivars (red columns) and experimental lines (blue columns) tested in a National Variety Trial in 2013 at the dedicated *Pratylenchus thornei* field site on the Darling Downs, Queensland. The initial *P. thornei* population densities were 6663 nematodes/kg soil (0–0.9m). (Source: USQ Crop Nematology).

The mechanism of tolerance to plant diseases remains unclear (Bingham et al. 2009), but is probably induced as a consequence of the nematodes parasitising the plant (Wallace 1987). Quantifying tolerance is problematic (Ney et al. 2013), because the mechanism of tolerance is influenced by environmental factors, like plant available water and nutrients, other pathogens and temperature (Wallace 1983). For example, one of the responses of an intolerant wheat cultivar to *P. thornei* is chlorosis of the lower leaves of the plant (Thompson et al. 1995) which reduces leaf area index (Whish et al. 2014), causing reduced plant stands and stunting (Doyle et al. 1987; Thompson et al. 2008; Van Gundy et al. 1974). This can be attributed to impaired root function (Thompson et al. 2012; Trudgill 1991; Whish et al. 2014). These symptoms are non-specific to *P. thornei* intolerance in wheat (Nicol et al. 1999) and can be easily confused with water and nutritional deficiencies. Similarly, in other crops like potatoes, water stress developed in plants where roots were damaged by potato cyst nematodes (Evans et al. 1975). When wheat is water stressed at critical periods of growth development, in particular from 20–30 days pre-anthesis to 10 days post-anthesis, the potential grain number is reduced (Sadras & McDonald 2012). In terms of tolerance, the

reduced early season growth of intolerant cultivars where the crop is unable to fully extract water and nutrients from the soil (Wallace 1987; Whish et al. 2014), contributes to water stress during key growth development stages, and reduced grain yield is the consequence.

Studies have suggested mechanisms whereby the plant can partition resources and change organ architecture to increase the photosynthetic capacity of the upper leaves, thereby compensating against yield loss (Bingham et al. 2009; Ney et al. 2013). Chlorosis of the lower leaves is perhaps a response of the plant to allocate resources to the newer, upper leaves, to ensure seed set survival, but in doing so the parent growth is reduced (Hatfield 1997). Unfortunately, the yield potential of a cultivar is set early in the season (Whish et al. 2014). From a wheat-RLN management perspective to maintain yield potential, sowing spring wheat into cool soils, ~15°C, is beneficial (Van Gundy et al. 1974), as the reproduction rates of *P. thornei* are reduced in cool soils (Thompson 2015; Thompson et al. 2015) resulting in good early root growth (Whish et al. 2014) prior to the soil warming to temperatures between 20–25°C that are ideal for optimum nematode reproduction rates (Thompson et al. 2015).

Minimising yield loss can be viewed as the protection of the three components that contribute to yield, namely, (i) the number of ears per unit area, (ii) the number of grains per ear and (iii) average grain weight (Bingham et al. 2009). This suggests the tolerance mechanism can be potentially categorised at various levels, as described in Figure 2.5 (Ney et al. 2013) that regulate the production of tillers and heads, plant height and yield in wheat. Van Gundy and colleagues (1974) noted that the symptoms of *P. thornei* attack appeared within 20 days of an intolerant wheat cultivar being sown. Plants may recover to some extent due to adventitious root production, but subsequently the number of heads (ears) and head (ear) size are reduced (Van Gundy et al. 1974). However, below-ground, there are opportunities for plants that are capable or tolerant, to replace damaged roots due to nematode attack with new, effective roots (Seinhorst 1965). For example, the moderately tolerant Australian wheat cultivars, Oxley and Cook, were found to have significantly more seminal roots, and subsequently more tillers, than the less tolerant cultivar Gatcher at 47 days after sowing (Thompson et al. 2012). Similarly in another study, plant height, number of heads per plant and number of tillers are reduced for the intolerant wheat cultivar, Warrigal, compared with tolerant cultivars GS50a and AUS4930 (Nicol 1996). This is perhaps a survival mechanism of intolerant plants, whereby plant death is less common (Sheedy 2014; Van Gundy 1974) than reduced seed set (Hatfield 1997) to ensure progeny.

Interestingly, chlorosis of lower leaves does not occur for tolerant cultivars (Sheedy *per. comms* 2014, see Table 1 in section 2.4.1). This response suggests that the replacement of damaged roots with new roots by a tolerant cultivar is efficient in acquiring sufficient soil water and nutrients to maintain good growth.

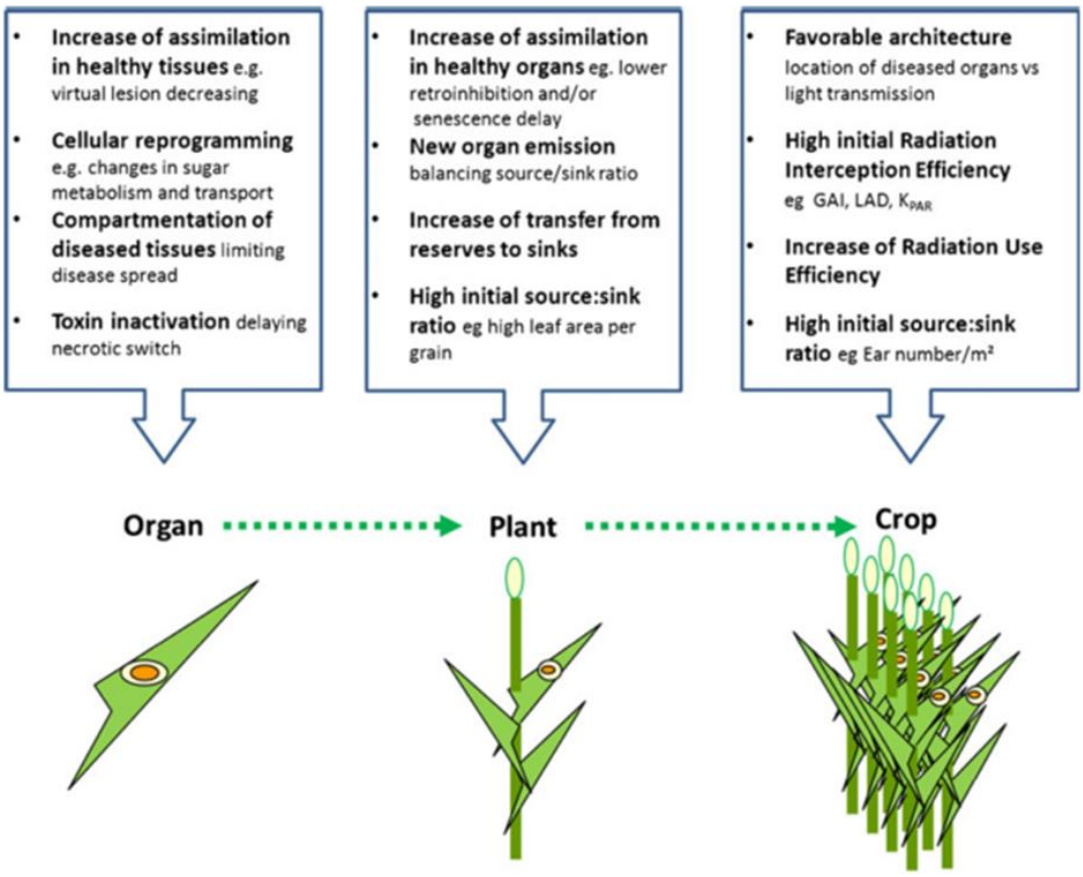

Figure 2.5 The tolerance mechanism to stress employed by plants at the organ, plant and crop levels (Source: Ney et al. 2013).

A review of plant tolerance mechanisms to other abiotic constraints, revealed that although salt tolerance in plants has been extensively studied (Deinlein et al. 2014; Gupta & Huang 2014; Volkov & Biebly 2017) the mechanism of this severe limitation to production (Munns & Gilliham 2015) is still largely unknown (Deinlein et al. 2014; Gupta & Huang 2014). Despite this, researchers recognise it is imperative that breeding for tolerance continues by incorporating tolerance genes (Wallace 1987) to alleviate against yield losses (Volkov & Beilby 2017). Similarly, improving tolerance levels in wheat to *P. thornei* to limit yield losses is essential (Thompson et al. 1999; Thompson et al. 2008; Whish et al. 2017).

Potentially, molecular markers could assist with identification of tolerance genes. Molecular studies have identified QTLs in wheat that are responsible for resistance to *P. thornei* (Linsell et al. 2014; Toktay et al. 2006; Zwart et al. 2005; Zwart et al. 2010), but little is known of the QTLs linked with tolerance to *P. thornei* in wheat. Promisingly, QTLs have been identified in *Medicago littloralis* that are linked with tolerance to *P. neglectus* (Oldach et al. 2014). Discovering new tolerance genes and QTLs will increase the level of protection that tolerance brings to crops.

Tolerance is a mechanism to protect against yield loss, but should be complemented with disease resistance (Ney et al. 2013) to stop *P. thornei* populations increasing (Whish et al. 2017). Seinhorst (1965) suggested there are tolerance limits that a plant can withstand up to certain nematode densities without losing yield, and populations need to be managed so as not to cause yield loss. Disease resistance is generally found to be less durable than tolerance as the pathogen consistently applies selection pressure to the host to break the resistance (Newton 2016). However, combining tolerance and resistance in a wheat cultivar can increase yield. A 17% yield benefit was observed when experimental wheat lines had combined resistance and tolerance, compared with tolerance alone to *P. thornei* (Thompson et al. 2001). Breeders should aim to develop wheat cultivars with resistance as well as tolerance to *P. thornei* (Trudgill 1991).

2.3.2. Resistance to *Pratylenchus thornei*

Genetic resistance has been applied at the crop and cultivar level to control *P. thornei* populations (Cook 2004) where populations are reduced without applying chemicals (Trugill 1991). Crops likes sorghum, millet (*Panicum miliaceum*), canaryseed (*Phalaris canaeriensis*) and sunflowers (*Helianthus annuus*) are resistant or poor hosts of *P. thornei* (O'Brien 1983; Owen at al. 2014; Thompson et al. 2008), whereas in wheat cultivars the best level of resistance is partial resistance (Jones et al. 2013; Thompson et al. 2008), with cultivars recommended for the subtropical grain region of eastern Australia ranging from very-susceptible to moderately-resistant (Lush 2016; Sheedy et al. 2015). Breeding for resistance to *P. thornei* is the most effective management strategy (May et al. 2016). There have been well documented breeding efforts that have incorporated genetic resistance into cultivars that now have complete resistance to parasitic nematodes. Notably, the incorporation of the Cre1, Cre5 and Cre8 resistance genes on chromosomes 2B, 2A and 6B respectively into wheat cultivars and rotations with resistant crops have essentially

eliminated the threat of cereal cyst nematode (CCN) (*Heterodera avenae*) to wheat production in Australia (Eastwood et al. 1994; Eastwood 2018).

The mechanism of resistance in wheat to *P. thornei* is not completely understood. In one study, it is thought the nematode is able to penetrate wheat roots of the cultivar Sokoll (a resistant derivative of a synthetic hexaploid wheat line) and upon entry of the root a compound within the root greatly reduces the mobility of the nematode and thereby inhibiting the development of the nematode past the juvenile stage (Linsell et al. 2014). Genetic resistance is a defence system of the plant that prevents reproduction that benefits the present crop, while reduction of parasitic nematodes populations continues to benefit subsequent intolerant crops (Smiley & Nicol 2009).

At the genetic level, the major genes that confer resistance to *P. thornei* have been identified in germplasm of synthetic-hexaploid wheat (Zwart et al. 2004), domestic bread (Thompson and Haak 1997) and landrace wheat lines (Sheedy & Thompson 2009; Sheedy et al. 2012; Thompson & Seymour 2011). Introgressing these genes from different sources into commercial bread wheat cultivars is a feasible way to include multiple genes for additive resistance on each of the three wheat genomes (Sheedy et al. 2012). Reliance on a single gene for resistance can devastate major crops (McIntosh et al. 2018) and the resistance is less durable than polygenic resistance (McDonald & Linde 2002). For example, the breakdown of the single stripe rust (*Puccinia striiformis* f. sp. *tritici)* resistance gene (*Yr24/26*) (McIntosh et al. 2018) now threatens to decrease Chinese wheat production (Han et al. 2015). However, breeding for resistance to *P. thornei* is effective because; (i) the asexual reproduction of *P. thornei* lowers the risk of resistance breakdown (McDonald & Linde 2002; Fortuner 1977), and ii) multiple sources of genetic resistance are available to breeders to develop cultivars with superior levels of polygenic resistance by combining genes with additive effect (Linsell 2013; Thompson et al. 2008; Zwart et al. 2004). The subsequent advantages of cultivars resistant to *P. thornei* is that (i) growers have access to seed that is resistant, negating the need for nematicides or chemical alternatives to control populations, and (ii) resistant crops can be grown more frequently without increasing nematode populations (Castillo & Vovlas 2007).

Presently, screening for resistance to *P. thornei* can be undertaken in glasshouses (Thompson et al. 2008) under controlled conditions that regulate soil moisture, temperature and initial nematode densities (Toktay et al. 2012). The experiments are robust and

repeatable results are independent of the geographic effects or screening procedures (Sheedy et al. 2015). Resistance to *P. thornei* does not necessarily confer resistance to *P. neglectus* (Smiley & Nicol 2009), a species which is also found throughout Australian grain-producing regions (Hollaway et al. 2000; Thompson et al. 2010). Wheat cultivars that have tolerance and resistance to both *P. thornei* and *P. neglectus* are favoured to decrease population densities thereby yielding well and limiting risk to subsequent crops (Smiley et al. 2014).

2.3.3. Biological and chemical control of *Pratylenchus* species

This section of the literature review will focus on biological and chemical control as methods to minimise damage caused by *P. thornei.*

2.3.3.1. Using biological control agents (BCA) to reduce *Pratylenchus* species

Biological control is a management strategy that can be employed in an ecosystem to alleviate the pressure applied to crop production by a pest (Stirling 2014). Biological control agents (BCA) are organisms such as bacteria, nematodes, insects, that are used to control plant diseases (Pal & McSpadden Gardener 2006) and reduce population densities of *Pratylenchus* spp. (Crampton 2017). The modes of action for BCA are varied. Assays by Samac and Kinkel (2001) found that *P. penetrans* populations were reduced in alfalfa (*Medicago sativa*) with inoculation at planting of *Streptomyces* strains in growth chamber experiments. Combining genetic resistance in alfalfa with *Streptomyces* strains further reduced *P. penetrans* populations (Samac & Kinkel 2001). In another study, populations of *P. braychyurus* were successfully decreased by 25–50%, when soybean (*Glycine max*) was sown into soils that contained high concentrations of *Pasteuria thornei* (Confort & Massayuki 2018). *Pasteuria* is a bacterial parasite that attaches to the plant parasitic nematodes, infects, grows and multiplies within the nematode and prevents the host from reproducing (Stirling 2014; Stirling et al. 2017). *Pasteuria thornei* parasitises *Pratylenchus* spp. (Starr & Sayre 1988), and more specifically has been found on *P. thornei* in the subtropical region of eastern Australia (Stirling 2014). However, their limited distribution in fields and relatively low parasitism of *P. thornei* in this region (Li et al. 2012), and their intolerance of abrasion caused by tillage (Stirling et al. 2017) reduces the potential impact of *Pasteuria thornei* as a BCA (Seymour et al. 2016). Conservative farming practices that use minimal tillage to conserve soil water should have a positive effect on the survival of *Pasteuria* (Stirling et al. 2017). Populations of *Pratylenchus* spp. can be reduced by

Pasteuria thornei, however, their impact on the broadacre production scale is inconclusive and potentially limited.

2.3.3.2. Using nematode suppressive soils to reduce *Pratylenchus thornei* population densities

Soils that are suppressive to plant parasitic nematodes rely on ecosystems (agroecosystems) and biotic factors that are antagonistic to the nematodes (Stirling 2014). The intensification of resistant crops in field rotations and practising stubble retention or conservation agriculture may enhance the suppressiveness of soil to *P. thornei* (Li et al. 2017). However, suppressiveness will be limited in fields where crop rotations include susceptible crops (Westphal 2011). Intensification of resistant crops in short sequences reduces populations (Trudgill 1991) and was found to be more effective at lowering *P. thornei* population densities than amending the soil with additional organic matter (Li et al., 2017). Similarly, although increasing soil organic matter is beneficial, this may not necessarily decrease *P. thornei* populations effectively (Thompson et al. 2008). The method is reliant on resistant crops being grown intensively to increase organic matter of the soil and breeding wheat cultivars with resistance to *P. thornei* would be beneficial in this system.

2.3.3.3. Using chemical control to reduce *Pratylenchus thornei* population densities

Understanding the magnitude of yield loss caused by *P. thornei* can be attributed to using nematicides in research (Doyle et al. 1987; Reen et al. 2014; Smiley et al. 2014; Van Gundy et al. 1974; Thompson et al. 2012). A decline in the production of wheat crops grown in paddocks with histories of previous wheat crops was noticed by growers in Queensland (Thompson et al. 2012) and in northern New South Wales (Doyle et al. 1987). Numerous, field experiments investigated the effect of nematicides and fertiliser treatments on wheat grown in *P. thornei*-infested soils (Clewett et al. 1993; Doyle et al. 1987; Smiley et al. 2005a, 2015; Thompson et al. 2012). It was found that nematicides, in particular the oxime carbamate, aldicarb, benefited wheat growth and yield by protecting the roots of wheat plants from *P. thornei*, although *P. thornei* densities below 0.15 m did not change (Doyle et al. 1987). Supplementing aldicarb with the addition of nitrogen fertiliser caused the greatest response by increasing biomass and yield (Thompson et al. 2012). The legacy to continued research and development of wheat cultivars with tolerance to *P. thornei* can be attributed to the success of the early research using nematicides.

Presently aldicarb is not available (Cone et al. 2016), and nematicides are not currently used (Smiley et al. 2014) because they are not economical (Kimpinski et al. 2005; Van Gundy et al. 1974), are toxic to non-target organisms and pose a risk to the environment and personal health (Oldach et al. 2014). Meanwhile, resistant rotational crops are effective at managing *P. thornei* populations through the whole soil profile (Reen et al. 2014). Not only is crop rotation safer than chemical control, but the resistant crop can also be harvested, generating an economic return for the grower.

On the broadacre agricultural scale, the use of genetic tolerance and resistance are most feasible options to manage *P. thornei* (Oldach et al., 2014; Toktay et al. 2012). The limited success of suppressive soils (Westphal 2011) and biological control (Stirling 2014), and the unfeasibility of chemical control, sees the broadacre cropping system being reliant on plant breeding technologies to improve the tolerance and resistance of cultivars, and to adopt resistant crop rotations that manage *P. thornei* to below damaging population levels (Smiley & Nicol 2009).

2.4. Determining tolerance to *Pratylenchus thornei*

Tolerance needs to be tested in field environments and not glasshouse pot experiments (Cook & Evans 1987). An example of this is the study by Rebetzke et al. (2013), where the spike density of the wheat cultivar Janz, significantly differed between potted plants and field crops in response to different rates of nitrogen, indicating that researchers need to be cautious extrapolating results from pot experiments. In the subtropical region of eastern Australia, wheat cultivars are tested for tolerance on rain-fed land within a commercially operated farming enterprise on the Darling Downs, Queensland. The field site is managed so that other soilborne diseases are not limiting factors and has assisted wheat breeders to improve the level of tolerance of wheat cultivars for the region (Thompson et al. 1999). The 20-ha site is divided into four cropping strips managed in a 4-year rotation such that each year, one cropping strip has evenly distributed damaging populations of *P. thornei* for assessing tolerance of wheat cultivars and other crop (Figure 2.6). The following section details the experimental methods used to estimate the tolerance of wheat cultivars to *P. thornei*, using visual assessments and grain yield as estimators of tolerance.

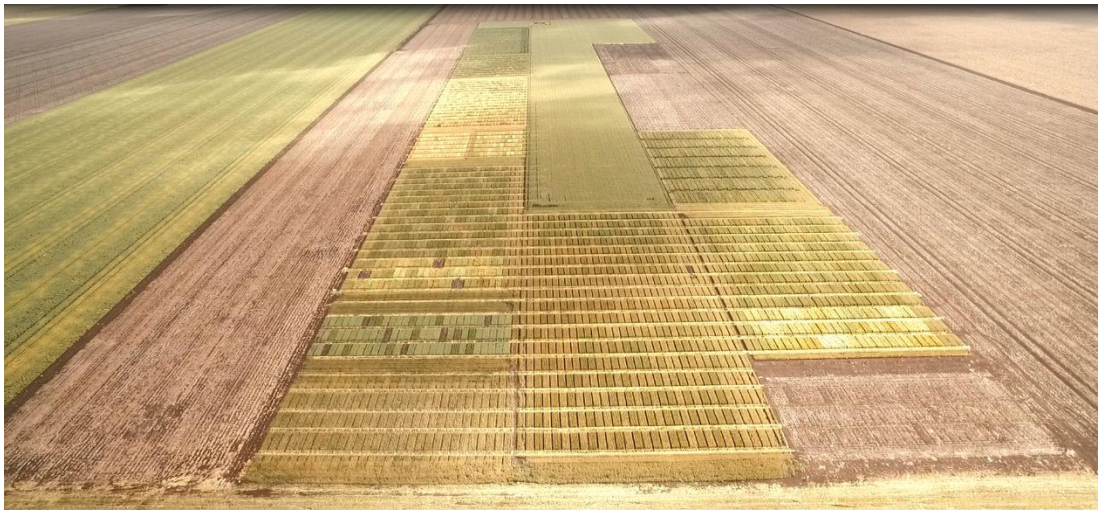

Figure 2.6 The 20-ha field site dedicated to assessing wheat cultivars for tolerance to *Pratylenchus thornei* on the Darling Downs, Queensland (Source: Adam Quade, Queensland Department of Agriculture and Fisheries).

2.4.1. Visual assessment

The human eye is complex. The eye presents potential sources of error in visual rating systems because the level of light and colour are perceived differently among individuals (Bock et al. 2010). Intrinsic ability, value preference, plant size, colour-blindness, are some common sources of error in visually assessing disease severity (Bock et al. 2010). Anyone can be trained quickly to report disease severity (Bockus et al. 2007), however, they are prone to individual subjectiveness (Christopher et al. 2014). A further complication of visual rating assessments is the interaction of these factors, and prolonged days in inclement and hostile environments when assessor fatigue may impact the quality of the work.

Approximately 2000 early-generation wheat breeding lines are screened against known check cultivars at the mid-tillering stage of plant development for *P. thornei* tolerance in field experiments in the subtropical region of eastern Australia (Thompson et al. 1999). These short 3-row plots are unreplicated, and the visual assessment is the only information recorded, as the plots are not harvested for yield. Two trained observers on two days (approximately a fortnight apart) assess for tolerance when symptoms are most evident (Thompson et al. 1999). A trained observer has experience in recognising the above-ground symptoms of *P. thornei* damage in the wheat plots. The symptoms of intolerance are chlorosis of the lower leaves, reduced tillering and poor canopy closure of the inter row space. The visual scoring system used is a one to nine scale (one = very intolerant; nine = tolerant) as described in Table 2.1 (Lush 2018; Sheedy 2014).

Table 2.1 The visual classification of wheat cultivars to *Pratylenchus thornei* on a one to nine scoring system when assessing the symptoms of intolerance in field experiments (Source: Modified from Lush 2018 and Sheedy *pers comms.* 2014).

Score	Classification[a]	Symptoms at mid to late stem elongation[b]
1	Very intolerant	Whole plant chlorotic, stunted and possibly purple. Limited leaf development. May produce a single head.
2	Intolerant–very intolerant	Very severe lower leaf chlorosis and necrosis. Reduced tillering. Plants visibly stressed. Leaf biomass dramatically reduced.
3	Intolerant	Severe lower leaf chlorosis and necrosis. Reduced tillering. Plants visibly stressed. Leaf biomass reduced.
4	Moderately intolerant–intolerant	Obvious lower leaf chlorosis and necrosis. Reduced tillering. Leaf biomass reduced.
5	Moderately intolerant	Moderate lower leaf chlorosis. Leaf biomass does not fill inter-row gap.
6	Moderately tolerant–moderately intolerant	Some lower leaf chlorosis. Leaf biomass virtually fills inter-row gap.
7	Moderately tolerant	Minor lower leaf chlorosis. Leaf biomass virtually fills inter-row gap.
8	Tolerant–moderately tolerant	Very few visible symptoms. Leaf biomass fully covers inter-row gap.
9	Tolerant	No visible symptoms. Leaf biomass fully covers inter-row gap.

Modified from sources: [a] tolerance classifications (Lush 2018), [b] description of plant symptoms for each alpha scale (Sheedy *pers. comms*, 2014).

2.4.2. Measuring grain yield

The greatest concern for growers is losing grain yield and overall profitability of their cropping land. One of the first signs of an increasing nematode problem is localised areas of poor growth in fields, and the reduction in grain yield (Van Gundy 1974). Growing tolerant wheat cultivars will minimise yield loss and for that reason, growers require accurate information of tolerance levels of commercially available wheat cultivars that can be determined by grain yield (Figure 2.7). This section describes the methods, namely using (i) nematicidal treatments (Smiley et al. 2014) to produce low and high populations, (ii) treatments that involve different crop resistance levels, to manipulate *P. thornei* population densities and (iii) assessing for tolerance on damaging populations only, as ways to measure the yield response of wheat cultivars to *P. thornei* (Thompson et al. 2008). Methods (i) and (ii) estimate the intolerance of a cultivar by the level of yield loss between the treatments (Smiley et al. 2014).

Figure 2.7 An experimental-plot header harvesting grain to determine cultivar tolerance to *Pratylenchus thornei* by grain yield (Source: Adam Quade, Queensland Department of Agriculture and Fisheries).

2.4.2.1. Using chemical control to manipulate *Pratylenchus thornei* population densities

Chemical control of *P. thornei* is the predecessor to the methods described below for assessing wheat cultivars for tolerance to *P. thornei*. Chemicals with nematicidal modes of action, aldicarb ($C_7H_{14}N_2O_2S$) and fenamiphos ($C_{13}H_{22}NO_3PS$) and the fumigants, chloropicrin (CCl_3NO_2) and dazomet ($C_5H_{10}N_2S_2$) decreased *P. thornei* populations, with a positive yield response to the wheat cultivars tested (Thompson et al. 2012). Aldicarb at

application rates greater than 2.5kg/ha reduced nematode populations by 70–90% in southern Australian environments (Taylor et al. 1999), and reduced populations in the northwest of the United States of America (Smiley et al. 2005a). Aldicarb was found to be the most effective nematicide in the subtropical region of eastern Australia and has been fundamental in the recognition of *P. thornei* as a major problem in the region (Clewett et al. 1993; Thompson et al. 2012). More information is available in section 2.3.3.3, but with aldicarb being no longer available (Cone 2016), other alternatives are needed to study the tolerance trait.

2.4.2.2. Pre-cropping with susceptible and resistant crops to develop low and high *Pratylenchus thornei* population densities

Growers can manage *P. thornei* populations in their fields by rotating with resistant crops. Partially-resistant winter and summer crops such as oats (*Avena sativa*), linseed (*Linum usitatissimum*), sorghum, sunflower and cotton (*Gossypium* spp.) (Hollaway et al. 2002; Owen et al. 2010; Thompson et al. 2012; Owen et al. 2014) and bare fallows (Nicol et al. 1999; Whish et al. 2017) are not only advantageous for growers to manage *P. thornei*, but also can be an important tool to determine the tolerance of wheat cultivars (Taylor et al. 1999; Vanstone et al. 1998). Establishing low and high nematode populations in the same field reduces the variances associated with different soil types, plant available water contents, paddock histories, other diseases, and sporadic rainfall, compared with using different fields. Spatial variation in fields can mask the experimental effects and contribute to experimental error (Federer & Corsa 2008), and potentially undermine the dominant effect that in this case is related to the impact of *P. thornei.*

Crops or cultivars with different levels of resistance can be used to create *P. thornei* population differentials in the same field (Owen et al. 2014; Reen et al. 2014; Taylor et al. 1999; Thompson et al. 2012; Vanstone et al. 1998) allowing for a range of nematode populations for research use. This approach requires a minimum of two years for each experiment, the first (Year 1) where different nematode populations are established by growing resistant and susceptible cultivars/ crops, followed in the next season (Year 2) by the comparative trial of cultivars. However, two partially resistant crops or cultivars, or fallows are required to lower nematode populations below damaging thresholds (Owen et al. 2014; Whish et al. 2017). An alternate approach was used in a chickpea yield loss study, where a *P. thornei* moderately resistant canaryseed (*Phalaris canariensis*) and susceptible

wheat were grown to maintain low population densities and to increase population densities respectively throughout the soil profile (Reen et al. 2014). It was from these population densities, that a yield tolerance index can be calculated by the yield response of each chickpea cultivar on low and high population densities (Reen et al. 2014). A nil *P. thornei* control is very difficult to achieve. Studies by Peck et al. (1983) showed that even after eight years of fallow, populations were low, but not nil. A tolerance index (%) can be derived for each cultivar tested by dividing the yield obtained on the high population by the yield obtained on the low *P. thornei* population multiplied by 100.

2.4.2.3. Screening for tolerance on high *Pratylenchus thornei* population densities only

The tolerance of wheat cultivars is generally consistent across years in the subtropical grain region of eastern Australia (Thompson et al. 1999). When assessing wheat cultivars for tolerance, cultivars can be sown in soil with high, economically damaging *P. thornei* population densities with check cultivars covering a range of known tolerance levels, and grain yield can be used as a measure of tolerance (Thompson et al. 1999; 2008). Thompson et al. (2008) describes how a tolerance index is highly correlated with grain yield when two wheat breeding experiments were grown in a field infested with high populations of *P. thornei*. The yield tolerance index (YTI) used was derived by dividing the yield of a test cultivar by that of the most tolerant cultivar, in this case Sunvale (Thompson et al. 2008). Although final grain yield is currently the best indicator of *P. thornei* tolerance, conducting yield experiments requires sufficient space to grow representative plots and resources to harvest the plots. However, hail or flooding may damage experiments preventing harvest. Being able to assess for tolerance during the season, either visually or by NDVI, is firstly an insurance system for capturing data, but secondly a more applicable method to screen many thousands of breeding lines objectively through high throughput phenotyping platforms (HTPP).

2.5. Development of remote sensing technologies

Between 1955 and 1991, USA and the USSR competed for dominance and technological development for space exploration (Erickson 2018). During this time, the first Russian satellite, Sputnik, was launched into orbit on 4 October 1957 (Wood 2018) and American-led NASA astronauts first walked on the moon on 20 July 1969 (Erickson 2018). Meanwhile, in a bid to better understand the earth's atmospheric conditions and weather events, a plethora of satellites orbited the earth with advanced cameras and climatic instruments

(Maskova et al. 2008). Orbiting satellites are now able to provide remote sensing information that can be used in agriculture models that can forecast wheat production (Schut et al. 2009), measure altered light reflectance induced by diseased canopy changes (Lee et al. 2010) and monitor variability of soil constraints and their associated impacts on yield (Dang et al. 2011).

2.5.1. Normalised difference vegetation index (NDVI)

New technologies are paving the way to monitor the interactions of biotic and abiotic stresses in crops and their impacts on yields (Mahlein et al. 2012). Non-invasive spectral measurements have a role in crop research, plant phenotyping and plant breeding programs (Jansen et al. 2014; Mahlein et al. 2012). Remote sensing technology is able to detect the spread of plant disease (Mahlein et al. 2012) by vegetation indices (VI) that use two or more bands of light that are spectrally transformed (Maskova et al. 2008). One of these VI can predict the relative greenness of plants, based on a normalised index measuring the near infrared and red reflectance bands, namely normalised difference vegetation index (NDVI) (Rouse et al. 1974; Birch 2016).

NDVI is a slope-based VI range that considers the state and abundance of green cover and biomass by the contrast between the reflectance of visible red (R_{Red}) and near infrared light (R_{NIR}) radiation (Silleos et al. 2006; Verhulst & Govaerts 2010). NDVI first came to prominence in studies of the vegetation of the Great Plains in America (Rouse et al. 1973). The equation of NDVI is (Verhulst & Govaerts 2010):

$$NDVI = (R_{NIR} - R_{Red}) / (R_{NIR} + R_{Red})$$

High reflectance of the NIR band is due to the internal structure of leaves, while low reflectance of the red band is due to chlorophyll absorption of energy (Silleos et al. 2006; Zhitao et al. 2014). Thus the greener the biomass the higher the NDVI value. This index can quantitatively measure the health and growth of canopies, and growth responses due to water and nutrient stresses (Silleos et al. 2006). At approximately 800 nm, in the near infrared wavelength range, stressed plants have lower light reflectance than healthy plants (Figure 2.8a). A NDVI value can be derived from the amount of visible and near infrared light being reflected, as shown in the example given in Figure 2.8b, where the measured NDVI value is 0.72 and 0.14 for the healthy (LHS) and unhealthy plant (RHS) respectively (Simmon 2018).

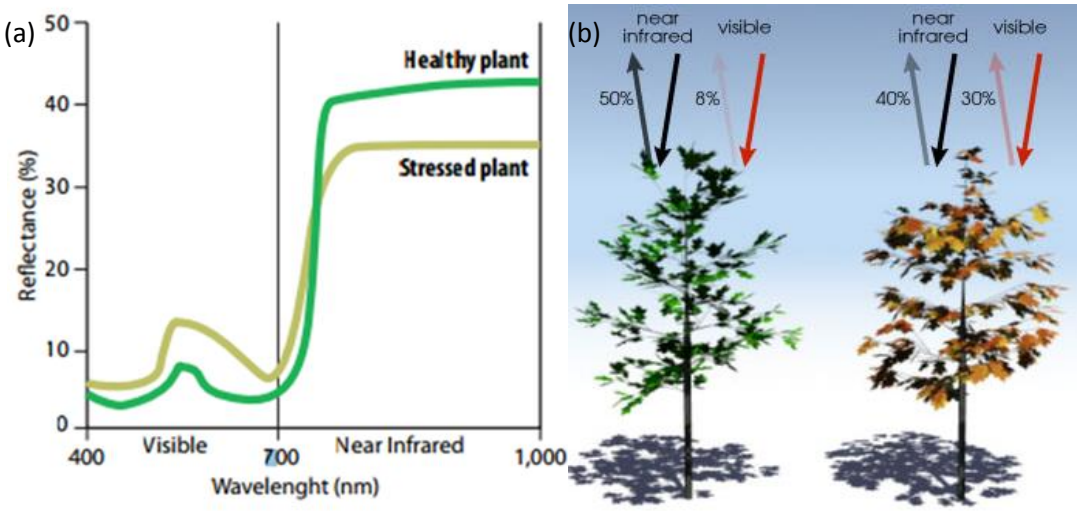

Figure 2.8 Healthy plants, have higher reflectance at near-infrared wavelengths and (a) these wavelengths are not visible to the eye (Source: Verhulst & Govaerts 2010) and (b) normalised difference vegetation index (NDVI) can be calculated for healthy and unhealthy plants by the reflectance of near infrared and visible light (Source: Simmon 2018).

NDVI has been used in commercial and research settings (Brown & de Beurs 2008; Christopher et al. 2014; Mkhabela et al. 2011) to monitor crop growth. NDVI scores the greenness (Jansen et al. 2014) of a plant or crop on a single index (Araus et al. 2008) between minus one and plus one, with minus one indicating non vegetated surfaces (water surfaces), zero indicating no vegetation (bare ground), and plus one indicating the maximum value for greenness (vegetated areas) (Silleos et al. 2006). When leaf area of plants rapidly increases post-emergence, the reflectance measured by NDVI is proportional to canopy coverage (Wang et al. 2016). As well as being sensitive to degree of cover, NDVI is sensitive to plant colour as influenced by a range of abiotic and biotic stresses.

2.5.1. Normalised difference vegetation index (NDVI) in research applications

NDVI is used in research programs via a handheld device, e.g., Greenseeker™ that is efficient, non-destructive to plants and is not prone to interference that can occur with satellite imaging (Crusiol et al. 2017). Strong, statistically significant relationships have been found between NDVI and crop physical variables (Shi et al. 2016) and it has been used for disease screening and yield prediction. For example, NDVI was significantly correlated with visual disease scores when assessing *Cercospora* leaf-spot disease in sugar beet breeding (*Beta vulgaris*) programs (Jansen et al. 2014). Spot blotch (caused by *Cochliobolus sativus*) in wheat was accurately phentoyped by NDVI (Kumar et al. 2016). Grain yield in

winter wheat was predicted by NDVI where different rates of nitrogen were applied (Walsh et al. 2013). NDVI has been successfully used to measure canopy characteristics (Christopher et al. 2014; Verhulst & Govaerts 2010), and to predict vineyard biomass in limited water and nitrogen conditions (Stamatiadis et al. 2010). There is no known publication in which NDVI has been used to determine the tolerance of wheat cultivars to *P. thornei*.

2.5.2. Confounding factors that may limit the interpretation of data generated by normalised difference vegetation index (NDVI)

The application of NDVI can be limited because the measured value can be influenced by various factors and plant traits (Govaerts et al. 2007). For example, in drought resistance studies using NDVI, the experiments had to be free from other biotic and abiotic stresses (Tuberosa 2012) to avoid confounding influences. Therefore, research using NVDI needs to be limited to one particular influence, whether abiotic or biotic. This section will explore factors that might confound the interpretation of data acquired by NDVI.

2.5.2.1. The effect of time of sensing on normalised difference vegetation index (NDVI) and integrating sequential measurements

The timing of measurements can affect the usefulness of NDVI (Labus et al. 2002). In this section, timing can refer to the different crop stages or the time of day itself. NDVI values obtained by Greenseeker™ are not affected by cloud cover or light intensity because the instrument is fitted with an inbuilt light source (NTech Industries Inc, Ukiah, CA, USA), although other environmental conditions, such as water and nutrient stress can influence NDVI values. When assessing for plant nitrogen uptake by NDVI, different growth stages of different cultivars of winter wheat confounded the results (Li et al. 2008). In a field study of soybean, 9 am was found to be the best time of day for NDVI readings to accurately screen for drought responses (Crusiol et al. 2017). In another study, the highest NDVI values obtained for soybean were at 8 am, after which the values progressively decreased until 2 pm, when they increased again until 8 am the following day (Zhitao et al. 2014). This is due to the increasing amounts of solar radiation that occur throughout the daylight hours that alter the structure of the soybean canopy, and therefore influence the NDVI values, rather than the function of the device (Chávez et al. 2014). In conclusion, the potential confounding factors of time of day and difference in crop stage on the accuracy of NDVI, suggest that

NDVI values are best captured for all plots in a single experiment over a relatively short period of time (1–2 hours), and at a similar period of the day.

Sequential NDVI values can be used to monitor changes in vegetation (Ricotta et al, 1999). Although accumulated NDVI measurements taken periodically throughout the growing season were the best indicator of grain yield in crops like barley (*Hordeum vulgare*), field pea (*Pisum sativa*), canola (*Brassica napus*) and spring wheat, a single NDVI measurement at one to two months before harvest also could accurately estimate the yield of those crops (Mkhabela et al. 2011). The area under the disease progress curve (AUPDC) calculated by NDVI is correlated with the manual disease scores obtained from a study of *Verticillium dahlia* resistance in strawberry, *Fragaria x ananassa* (Cockerton et al. 2018). The AUDPC is a measure of disease development (Nayak et al. 2018), and is a quantitative single number of the interaction between host, pathogen, environment and time (Mohaptra et al. 2014) derived from multiple observations (Smiko & Piepho 2012) during the growing season. However, it was found that using NDVI to capture the effect of spot blotch early, during tillering and stem elongation, was ineffective due to the small difference in values between susceptible and resistant plants and the effects of variable plant densities and unknown influences (Kumar et al. 2016). Greenseeker™ has been used to capture NDVI values at times between anthesis and maturity that were predictive of the stay green trait (Christopher et al. 2014), suggesting that if limited measurements are taken consideration needs to be given to the particular trait and determine the best time of sensing.

2.5.2.2. The effect of canopy development on normalised difference vegetation index (NDVI)

Vegetation canopy can influence the accuracy of NDVI for predicting biomass. A linear relationship was found between biomass and NDVI value where vegetation canopy cover was in the range of 25 to 80% (Zhao 2003 cit. Ren et al. 2008). There was no relationship between biomass and NDVI where canopy cover was <15% or above 80% due to NDVI saturation (Zhao 2003 cit. Ren et al. 2008). On the other hand if biomass is too great, NDVI saturation occurs and the segregation for traits by NDVI may not be achieved. In one study, this occurred when the canopy closure was at 80% or more (Casadesús et al. 2007; Zhao 2003 cit. Ren et al. 2008). In another study, NDVI provided a good prediction of grain yield in drier or rain-fed conditions, but not in irrigated environments because of the denser canopies (Casadesús et al. 2007). In addition, NDVI values between 0.2 and 0.8 provide the best linear relationship (Ren et al. 2008). Additionally, vegetation indices will be influenced

by soil background in the early stages of plant growth, but will be less influenced by the soil background when the canopy covers the soil (Silleos et al. 2006). Therefore, sensing too early in crop development may not be an indicator of final wheat yield (Labus et al. 2002; Lee et al. 2010). Furthermore, sensing post-anthesis is prone to error if different cultivars senesce at different rates, unless the trait being studied is stay green in wheat (Christopher et al. 2014).

2.5.2.3. The effect of environmental influences on normalised difference vegetation index (NDVI)

Changes in environmental conditions, for example, air humidity, temperature, solar radiation and soil moisture content influence the NDVI value (Zhitao et al. 2014). Soils when wet have higher NDVI values than the same soils when dry (Jones et al. 2015). Therefore spatial variation in soil moisture levels may introduce error when comparing across or within fields. NDVI is also influenced by other effects such as previous crop residues (Jones et al. 2015). NDVI values are inversely related to increased row spacing and correlated with nitrogen rate in winter wheat (Lukina et al. 2008), and NDVI values are predictive to differing senescence rates when studying the stay green trait in wheat cultivars (Christopher et al. 2014). Furthermore, changes in soil physical properties can influence NDVI readings. Zhitao et al. (2014) found that soybean trials grown on sandy soil types impacted NDVI readings due to the change in the reflectance of the background soil. These effects need to be considered in the design of the experiments so that these interactions are minimised. For example, conducting experiments in the same field (limiting spatial effects like rainfall variation, soil type changes) and having similar soil conditions (plant available water, nutritional status) are required.

2.5.2.4. Operational constraints affecting use of normalised difference vegetation index (NDVI)

Wheat breeders screen many thousands of experimental lines for *P. thornei* tolerance each year. Limited seed (particularly for early breeding germplasm) for sowing and land availability can restrict plot size to a single row plot, or 3-row to 7-row plots. Previous field experiments by Thompson et al. (1999) found that single row plots had poorer expression of symptoms of intolerance to *P. thornei* compared with multi row plots, particularly for comparison of inter-row growth using the visual assessment scheme (Table 1). The effects of small plots on the sensitivity of NDVI are unknown. Greenseeker™ gave the most consistent results when the sensing head was between 93 and 122 cm above the canopy, and

maximum response from the sensor was with the sensor head in-line with the planted row (Martin et al. 2012). These findings may constrain Greenseeker™ to a particular plot size to avoid the influence of neighbouring plots.

2.6. Breeding superior wheat cultivars in Australia

Australia's history in breeding high performing wheat cultivars dates back to 1901 with the release of William Farrer's cultivar, Federation (Evans 1980). Investment is crucial in plant breeding programs (Chapman et al. 2104), and breeding, coupled with better crop management, has improved yields worldwide (Vandeleur & Gill 2004). In Australia, grower-funded research through state or public wheat boards and commercial breeding companies has improved yield of the nation's cultivars. For example, field experiments in the mid-north of South Australia tested 13 wheat cultivars that were released between 1958 and 2007 and found that yields increased by 18 kg per hectare per year of release (Sadras & Lawson 2013). Recently, wheat breeding was privatised and there are four breeding companies across the Australian wheat belt (GRDC 2011). A current major objective of wheat breeding programs, is to increase yield by 3% over the parent varieties, working on 0.5% increase per year on a 6 year varietal development program (Summers & Brown 2013). Therefore, in a ten-year breeding program, releasing a cultivar that yields 5% greater than its parent cultivars is ideal (Summers & Brown 2013). Yield is intrinsically linked to improved disease tolerance and resistance, and better agronomic properties.

Plant breeding alone is expensive (Chapman et al. 2014), but the lack of phenotypic accuracy has hindered the discovery and subsequent application of molecular markers (Araus & Cairns 2014). Furthermore, methods of back crossing, single seed descent and doubled haploid production used in breeding programs rely on accurate phenotyping for progeny selection (Tuberosa 2012). Phenotyping is not a new concept (Araus & Cairns 2014), and selecting plants with desirable traits has been occurring in the developing world (Miles & Pandey 2004). This method of selection continues with the modern day plant breeders in developing current commercial cultivars.

2.6.1. Breeding superior cultivars: the role of phenotyping in a breeding program

There is impetus to continually improve the selection capabilities of plant breeding programs in order to increase the yield potential of cultivars and meet the demands of society (Araus et al. 2008). Phenotyping is a quantitative measure of the growth and development governed

by the genetic composition of the plant in response to the environment (Walter et al. 2015). Phenotyping is pivotal for the identification of genes and their response to environment. Grain yield is controlled by many thousands of genes, linking the interaction of the genetics of the plant with the environment and management (Richards et al. 2010). Presently, a major focus of breeding programs is to improve yield, by increasing the water use efficiency (WUE) of cultivars (Furbank & Tester 2011). Phenotyping is recognised as a more efficient method for selecting cultivars with traits for drought adaption by incorporating the genetic and environmental interactions, than molecular marker methods (Richards et al. 2010). Richards et al. (2010) concluded that genetic gain is of utmost importance, and robust, high throughput phenotyping is the most effective way to achieve this for plant breeders.

Phenotyping is laborious (Furbank & Tester 2011), expensive and requires a high degree of training of personnel to ensure accurate results. Complications arise when phenotyping for drought tolerance if plant growth and function are affected by more than one stress, this either being abiotic or biotic (Tuberosa 2012). Consequently, many plant breeding companies only assess yield (Furbank & Tester 2011) and ignore other traits including leaf area, phenology, and tillering characteristics. A non-destructive phenotyping tool would be advantageous (Furbank & Tester 2011) to preserve genetic variability in segregating populations for population development. There is also a role for molecular markers in plant breeding (Young & Mudge 2002), although the success of molecular markers is dependent on good-quality phenotyping (Richards et al. 2010). Investing in novel high capacity technologies, such as NDVI that are able to accurately phenotype, is critical to improve the selection capabilities in breeding programs.

2.6.2. Aerial phenotyping platforms or unmanned aerial vehicles (UAV)

Aerial platforms are becoming increasingly popular for phenotyping field trials. Initially, crop duster or low flying aircraft were deployed (Harris & Haney 1973), but more recently unmanned aerial vehicles (UAV), or drones, have been used to rapidly capture phenotypic data using a variety of different sensing equipment (Araus & Cairns 2014). Aerial imaging can be an efficient platform to assess large breeding nurseries and field trials (Chapman et al. 2014). However, compatible software is needed to enhance rapid data acquisition if multiple camera shots are used to capture the entirety of the breeding nurseries (Shi et al. 2016) as data captured by NDVI is dependent on altitude. There is a tipping point between image quality, the stitching of multiple photo shots and efficiency. Improving software

capabilities will alleviate these problems and help UAVs become a staple tool for plant breeders.

2.6.3. Benefits of high throughput phenotyping platforms (HTPPs)

Training personnel and the use of technologies has facilitated the phenotypic study of biotic and abiotic plant traits (Granier & Vile 2014). Acquiring high quality data from field trials by automated collection systems will potentially accelerate plant breeders' selection capabilities and product development (Haghighattalab et al. 2016; Shi et al. 2016). Additionally, when data is collected rapidly in field experiments by HTPP, the effects of time of day on NDVI values (Crusiol et al. 2017; Zhitao et al. 2014) are minimised. High throughput phenotyping platforms can reduce labour and potentially the costs of phenotyping, while increasing rates of acquisition and increased understanding of plant traits.

2.7. Conclusion

The root-lesion nematode, *Pratylenchus thornei* is a problem in many countries and reduces the yield of intolerant wheat cultivars. To date, incorporating genetic tolerance and resistance into wheat cultivars is the most promising method to minimise yield loss and to reduce the population densities of *P. thornei* respectively. Wheat breeders are dependent on highly accurate and predictive phenotyping to select elite germplasm from within their programs. This review also explored NDVI measured by Greenseeker™ as an objective tool to assist breeders in their quest to select germplasm that is tolerant to *P. thornei.* Theoretically, NDVI should be suitable to measure the symptoms of intolerance to *P. thornei*. However, changes in crop canopy can also be attributed to other stressors such as drought or other diseases. To quantify tolerance to *P. thornei*, wheat cultivars would need to be phenotyped at a field site where the major constraint is *P. thornei*. Potentially, NDVI could be determined for many cultivars or experimental lines at such a site using UAV.

Phenotyping in breeding programs is costly and laborious, but breeders are heavily reliant on this process to improve wheat cultivars. Identifying new methods to alleviate these impositions will be beneficial for breeders. The application of technology in this study is a small step forward in the global picture to help secure adequate food supplies for an increasing world population, and in an agricultural environment that is constantly changing due to biotic and abiotic factors.

3. Chapter Three – Annals of Applied Biology Article

Chapter three of this thesis is formatted in its entirety according to the requirements of Annals of Applied Biology for submission for publication. This manuscript has been peer reviewed and accepted by the journal.

Robinson, NA, Sheedy, JG, MacDonald, BJ, Owen, KJ & Thompson, JP, 2019, 'Tolerance of wheat cultivars to root-lesion nematode (*Pratylenchus thornei*) assessed by Normalised Difference Vegetation Index (NDVI) is predictive of grain yield', *Annals of Applied Biology*, vol. 174, pp. 388–401, DOI: 10.1111/aab.12504

Accepted for publication – 21 February 2019.

Tolerance of wheat cultivars to root-lesion nematode (*Pratylenchus thornei*) assessed by Normalised Difference Vegetation Index (NDVI) is predictive of grain yield

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Running title: Predicting wheat tolerance to *P. thornei* by NDVI

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Abstract

Tolerant wheat cultivars yield well when sown in fields infested with the root-lesion nematode *Pratylenchus thornei*, which is present in 67% of fields in the subtropical grain region of eastern Australia. Wheat breeding programs require accurate phenotyping to select germplasm with superior tolerance to *P. thornei*. This study investigated Normalised Difference Vegetation Index (NDVI) as a phenotypic tool to predict the tolerance of wheat cultivars on low and high *P. thornei* population densities. Three, two-year field experiments used a resistant and a susceptible wheat cultivar in the first year to develop low and high *P. thornei* populations. In the second year, 36 wheat cultivars were sown on these plots. A NTech Greenseeker™ was used to determine the NDVI of each plot at regular times during the season and grain yield was measured at crop maturity. There was an inverse relationship between *P. thornei* population densities and the NDVI for intolerant wheat cultivars. Regression analysis showed a highly predictive response between the yield tolerance index and NDVI with R^2 ranging from 0.85 (n = 36) to 0.93 (n = 36) for the three experiments. The area under the disease progress curve (AUDPC) with respect to NDVI was highly predictive of yield tolerance (R^2 = 0.92; n = 36) when there were high populations (9091 *P. thornei*/kg), but not when populations were low (578 *P. thornei*/kg). Tolerant cultivars can be identified by NDVI when sown on soil containing high populations (>2500 *P. thornei*/kg) by measurement at approximately 1000 degree days (ºCd) after sowing. Greenseeker™ is a valuable tool for wheat breeders to select germplasm with tolerance of *P. thornei.*

Keywords

Normalised Difference Vegetation Index, NDVI, Greenseeker™, *Pratylenchus thornei* tolerance, yield loss, wheat

Introduction

The root-lesion nematode *Pratylenchus thornei* occurs in 67% of fields in the subtropical grain region of eastern Australia (Thompson *et al.,* 2010). This pest has forced many growers to grow tolerant wheat (*Triticum aestivum*) cultivars to minimise potential yield losses which can be up to 70% (Thompson *et al.,* 1999). Correct diagnoses of nematode populations, the use of tolerant wheat cultivars and rotations with resistant crops (Owen *et al.,* 2014) have reduced the impact of *P. thornei* from a potential loss of AUD$104 million/year to an actual loss of $38 million/year (Murray & Brennan 2009). Continued research and development is required to further reduce yield loss.

Many crops grown in this region of Australia are hosts of *P. thornei*. Both the major winter cereal, wheat, and the major winter pulse, chickpea (*Cicer arietinum*) (Unkovich *et al.,* 2009) are susceptible to *P. thornei* (Thompson *et al.,* 2000). *Pratylenchus thornei* causes necrosis of susceptible host plant roots (Nicol & Ortiz-Monasterio 2004). Lesioned roots are ineffective in acquiring water and nutrients from the soil (Thompson *et al.,* 2008, 2012; Whish *et al.,* 2014). This causes drought or nutrient deficiency symptoms of the above-ground plant biomass (Thompson *et al.,* 2012). Stunting, reduced tiller number and chlorosis of leaves decrease the photosynthetic leaf area of intolerant crops, thereby reducing grain yield (Whish *et al.,* 2014).

Wheat cultivars tolerant to *P. thornei* maintain yield, despite being challenged by the pathogen (Trudgill 1991; Thompson *et al.,* 2008). Wheat breeding has been able to increase the yield of locally adapted wheat cultivars when grown in *P. thornei*-infested fields by hybridising elite wheat cultivars with tolerant sources and selecting for the tolerance trait in field experiments (Thompson *et al.,* 1999). Plant breeding is a lengthy and costly procedure,

but investment in breeding is crucial to combat disease and pest pressure, adapt to market changes and increase yield (Chapman *et al.,* 2014).

Novel technologies are becoming increasingly adopted in plant breeding programs, thereby reducing the expense, time and subjectiveness of manually phenotyping constraints to production (Araus & Cairns 2014; Furbank & Tester 2011) and of sensing plant diseases (Mahlein *et al.,* 2012). The infestation of *P. thornei* of the roots of cereals and pulses causes symptoms of wilting, stunting and chlorosis of the plant tops (Thompson *et al*., 2008; Van Gundy *et al*., 1974), therefore new sensing methods could be used advantageously to discriminate tolerant genotypes of crops in the field. Normalised Difference Vegetation Index (NDVI) is a measurable variable which can be used for identifying superior genotypes based on vegetative greenness (Araus *et al.,* 2008; Christopher *et al.,* 2014, 2016). NDVI is the difference in reflectance of the near infrared (NIR) and red wavelengths as a proportion of their sum as calculated from Equation 1:

(Eq. 1) $$NDVI = (R_{NIR} - R_{Red})/(R_{NIR} + R_{Red})$$

where R_{NIR} is the reflectance of NIR radiation, and R_{Red} is the reflectance of visible red radiation (Verhulst & Govaerts 2010).

Greenseeker™ is a commercially available instrument to measure NDVI that is portable and has a built-in light source; it is efficient and non-destructive (Crusiol *et al.,* 2017). Measurements taken by Greenseeker can be predictive of grain yield (Walsh *et al.,* 2013) by providing a single measure of canopy greenness or healthiness (Araus *et al.,* 2008; Jansen *et al.,* 2014). However, NDVI does not discriminate between different traits if more than one trait influences greenness (Govaerts *et al.,* 2007). In addition, the most predictive sensing time of a particular trait is dependent on the trait to be investigated (Christopher *et al.,* 2014; Li *et al.,* 2008).

The aim of this study was to investigate the potential of NDVI to estimate the relative tolerance of adapted commercial bread wheat and durum wheat (*Triticum turgidum* ssp. *durum*) cultivars under low and high population densities of *P. thornei*. We hypothesised that NDVI could provide an index that integrates leaf yellowing, reduced tillering and reduced biomass associated with cultivar intolerance of *P. thornei*, and that this could be predictive of grain yield of various wheat cultivars. Multivariate methods of hierarchical classification and ordination, and area under disease progress curves (AUDPC), were used to examine the tolerance of cultivars based on NDVI readings throughout the growing season. Critical points for NDVI assessment were established by determining the correlation between NDVI at each sensing time and grain yield under high and low nematode population densities.

Materials and Methods

Field Site

Three field experiments were conducted at Formartin (27.46401ºS, 151.42616ºE), 70 km west of Toowoomba on the Darling Downs, Queensland, Australia, on farm land naturally infested with root-lesion nematodes, identified as *P. thornei* (Fortuner, 1977). The soil at the site is a self-mulching black Vertosol (Isbell 1996) of the Waco association (Beckman & Thompson, 1960). This deep cracking clay soil extends past the full crop rooting depth (1.8 m) and has a very high plant available water capacity (PAWC) (Hochman *et al.*, 2001). There is no supplementary irrigation at this site, with crop growth dependent on stored soil water and in-crop rainfall.

Experimental design – High and low *P. thornei* population experiments

Each experiment was a strip-plot design with three replicates in blocks. The establishment of the treatments occurred over two years with two wheat cultivars sown to pairs of seven row plots in the first year (Year 1) to produce different population densities of *P. thornei,* allowing 36 cultivars to be applied to pairs of adjacent plots with low and high *P. thornei* population densities in the second year (Year 2). Year 2 of Experiment 1 was in 2013 and Year 2 of Experiments 2 and 3 was in 2015. There was a 6-month or short fallow (SF) between the first and second crops for both Experiments 1 and 2, and an 18-month long fallow (LF) between the crops for Experiment 3. The fallow length for Experiment 3 was caused by insufficient rainfall for sowing in the 2014 winter season. Weeds were controlled by a non-selective herbicide (glyphosate 450 g/L) during the fallow periods and stubble was left standing.

Year 1: Establishing *P. thornei* population differentials

The wheat cultivars, QT8343 which is moderately-resistant to *P. thornei,* and Kennedy, which is susceptible (Thompson *et al.,* 1999) were sown in the first year to produce plots of soil with low and high *P. thornei* population densities. The plots were 1.75 m wide by 8 m long, with seven rows at 0.25 m spacing. The sowing rate was adjusted on seed weight and germination percentage of individual cultivars to provide 100 viable seeds per m^2. Urea (100 kg N/ha) drilled at 50 mm depth was applied approximately one month prior to sowing. Granulock StarterZ (Incitec Pivot, Australia) was drilled in the seed row at sowing at 40 kg/ha to provide 8 kg P/ha and 1 kg Zn/ha.

Year 2: Determination of *P. thornei* populations and plant available water

Experiment 1 was sampled approximately 2 days prior to sowing and Experiments 2 and 3 were sampled approximately 14 days after sowing (DAS) in Year 2 of each experiment where 18 plots of both high (after cv. Kennedy) and low (after QT8343) population density treatments from Year 1 in each replicate were soil sampled to determine initial *P. thornei* population densities. Two 43-mm diameter soil cores were taken on opposite sides of the middle row at equal distances along the plot using a hydraulic soil sampling machine with a push tube to a depth of 0.9 m. Each core was divided into three depth increments (0–0.3, 0.3–0.6, 0.6–0.9 m). For each depth increment, the soil from both cores was bulked together for each plot sampled. Samples were stored at 4°C and broken by hand into aggregates <10 mm, mixed together, and a 150-g sub-sample was extracted for nematodes for 48 hours at 22°C using the Whitehead tray method (Whitehead & Hemming, 1965). The nematodes were concentrated into 10–15 mL water using a 200 mm diameter sieve with a 20 µm aperture mesh and collected in a specimen tube. Nematodes were enumerated in a 1-mL Peters nematode counting slide (Chalex Corporation, Portland, Oregon, USA) under a compound microscope at x40 and x100 magnification. Nematodes were identified as *P. thornei* (Fortuner, 1977), *Merlinius brevidens* (Siddiqi, 1972) or as a composite of non-plant parasitic nematode species identified by the absence of a robust stylet. Another 100 g soil sub-sample was dried in a forced draught oven at 105°C for 48 hours to determine gravimetric soil moisture. Total *P. thornei* was expressed as number of nematodes/kg of oven dry soil for each of the three depth increments. Plant available water (PAW) for 0–0.9 m was calculated from the gravimetric soil moisture contents, bulk densities and wilting points at three depths in the soil profile as described in Reen *et al.,* (2014).

The minimum and maximum temperature were averaged each day and summed to provide a cumulative thermal time in degree days (°Cd) above a base temperature of 0°C (Richards *et al*. 2014). Temperature records from the Dalby Airport weather station (ID: 041522; 37 km NNW from trial site) from the time of sowing of each experiment were used.

Year 2: Assessing crop cultivar tolerance at high and low population densities of *P. thornei*

Thirty-one bread wheat cultivars and five durum wheat cultivars (Supplementary Table 1) adapted to Australia's subtropical grain region were grown in Year 2 on the high and low *P. thornei* treatments established in Year 1 in each experiment. The sowing rates for each cultivar, the plot dimensions and row spacing were the same as described for Year 1. For Experiment 1, urea (100 kg N/ha) drilled at 50 mm depth was applied approximately one month prior to sowing. For Experiments 2 and 3, urea (60 kg N/ha) was applied to the soil surface and incorporated into the soil during the sowing operation. Granulock StarterZ (Incitec Pivot, Australia) was applied to all Year 2 plots as per Year 1 application.

At maturity, plots were trimmed to 6.2 m long and harvested with a small plot combine harvester for grain yield. A 100-g subsample of grain from each plot was dried in a forced draught oven for 48 hours at 80°C to determine moisture content. Yield was expressed as kg/ha standardised at 12% grain moisture content.

Year 2: NDVI determination

Normalised Difference Vegetation Index readings were obtained using a Model 505 GreenSeeker™ hand-held optical sensor unit (NTech Industries, USA). Readings were taken at a consistent walking speed of approximately 4 km/h in the same orientation as the plant rows with the sensing-head approximately 0.8 m above the wheat rows (NTech Industries

Inc, 2004). The GreenSeeker™ trigger was depressed for approximately 4 seconds (obtaining ~40 readings per plot). An average NDVI value between 0 and 0.99 is calculated by the NTech software for each plot and recorded electronically. The NDVI readings were taken at approximately 7–14 day intervals as shown in Table 1. Total rainfall between sowing and the last NDVI time of sensing was 83.9 mm for Experiment 1 and 39.1 mm for Experiments 2 and 3 (Table 1).

Statistical analysis

Population densities of *P. thornei* in Year 2

Population densities of *P. thornei* and soil moisture in Year 2 from the two wheat cultivars grown in Year 1, averaged over the three soil depths, and plant available water summed over the three depths, were analysed for each experiment using a linear mixed model with the background (Year 1 cultivar) fitted as a fixed effect and design terms fitted as random effects. Design terms included replicate block, background strip and cultivar main plots both of which were nested within replicate block. Year 2 cultivar was not included in the model as the samples were taken prior to or at emergence to determine initial nematode population densities and soil water with little effect of year 2 cultivars on these parameters. To ensure homogeneity of variance, the population densities of *P. thornei* were transformed by ln (x+1) and mean values for each background were generated from the model as empirical Best Linear Unbiased Estimators. Significance of background effects were assessed using a Wald test with a significance level of α=0.05.

NDVI and grain yield in Year 2

Grain yield and NDVI values at each time of assessment were analysed using a linear mixed model. An environment term was defined as the combination of background (Year 1 cultivar), experiment and time of measurement (for NDVI only). Environment was fitted as a fixed effect and design terms were fitted as random effects. A factor-analytic model (Smith *et al.*, 2001) was included for the cultivar by environment interaction which allowed for heterogeneity of genetic variance between environments and heterogeneity of genetic covariance between pairs of environments. Cultivar was included as a random effect as the estimation procedure for random effects, best linear unbiased prediction (BLUP), results in more precise estimates of the true cultivar effects and it was of interest to investigate how the cultivar rankings changed between environments. Predicted yield and NDVI values for each cultivar x background combination in each experiment were generated as empirical BLUPs.

Residual maximum likelihood was used to estimate the variance parameters in the analyses of *P. thornei* population densities, yield and NDVI (Patterson & Thompson 1971). These analyses were undertaken using ASReml-R (Butler *et al.*, 2009) in the R software environment (R Core Team, 2016).

The area under the disease progress curve was calculated as the sum of trapezoids based on the average NDVI value between each pair of adjacent assessment time points (Madden *et al.*, 2007). The yield tolerance index (YTI) for a cultivar was calculated from its yield at the high population density as a percentage of its yield at the low population density. The NDVI tolerance index for each cultivar was calculated from the NDVI of that cultivar grown on the high *P. thornei* population density as a percentage of the NDVI of that cultivar grown on the low *P. thornei* population density at each NDVI sensing time.

Cluster analysis and principal component analysis

For each experiment, a data matrix was formed with objects being the 36 wheat cultivars and variates being the mean NDVI values as eBLUPs from the above analyses at each sensing time for both the low and high nematode population densities. A similarity matrix between the cultivars based on Euclidean distance was calculated from the data matrix of NDVI values. A hierarchical cluster analysis of the cultivars was produced using group average as the sorting strategy. A principal components analysis of the correlation matrix was conducted for each experiment using the same data matrix as for the cluster analysis. A multiple regression analysis using cultivar scores of the first three principal components as explanatory variates and tolerance index based on grain yield as response variate was conducted. These analyses were performed in Genstat 17th Edition (VSN International 2014).

Results

Nematode population density and plant available water at commencement of Year 2

Growing the susceptible wheat cv. Kennedy in Year 1 significantly increased *P. thornei* population densities compared with growing the moderately-resistant wheat cv. QT8343 for all three experiments (P<0.001, Table 2). The population density of *P. thornei* over the full soil profile of 0–0.9 m after growing cv. Kennedy was 3.5, 3.1 and 2.2 fold greater than after cv. QT8343 in Experiments 1, 2 and 3 respectively (Table 2). There was no significant difference (P<0.05) between growing wheat cv. Kennedy or cv. QT8343 in Year 1 on plant available water in the 0–0.9 m soil depth when sampled at Year 2 plant emergence for all experiments (Table 2).

Experiment 1

Cluster analysis of NDVI readings for Experiment 1

Eight groups of cultivars were delimited from the cluster analysis of Experiment 1 based on seven NDVI times of sensing, at low and high *P. thornei* population densities (Fig. 1a). The shapes of the NDVI response curves for the centroid of the eight groups are shown in Fig. 2. The maximum NDVI for group A was approximately 0.9 at both low and high nematode population densities, decreasing through the groups to become approximately 0.7 for low and 0.5 for high population densities for group H.

Principal component analysis for Experiment 1

Ordination of the 36 cultivars based on their scores along principal component 1 (PC1) and principal component 2 (PC2) from principal component analysis (PCA) of the NDVI data is given in Fig 5a. The latent vectors (loadings) were all positive for PC1 and of a similar magnitude for low and high *P. thornei* population densities for each time of sensing (Supplementary Table 2). The latent vectors for PC2 were strongly positive at the first NDVI time of sensing, then decreased through time to become negative at 106 DAS for both low and high *P. thornei* populations, with the low population having a stronger negative association. For PC3, the latent vectors on low *P. thornei* population density were negative, but were positive for high *P. thornei* population densities. The total amount of variance accounted for by PC 1, 2 and 3 was 98.7%, with PC1 = 81%, PC2 = 12% and PC3 = 3.5%.

Experiment 2

Cluster analysis of NDVI readings for Experiment 2

Eight groups of cultivars were delimited from the cluster analysis of Experiment 2 based on ten times of NDVI sensing, on low and high *P. thornei* population densities (Fig. 1b). The shapes of the NDVI response curves for the centroids of the eight groups are shown in Fig. 3. The maximum NDVI for group A was approximately 0.8 at both low and high nematode population densities, decreasing through the groups to become approximately 0.7 for low and 0.6 for high population densities for group H.

Principal component analysis for Experiment 2

Ordination of the 36 cultivars based on their scores along PC1 and PC2 from the PCA of NDVI data is given in Fig. 5b. The latent vectors were all positive for PC1, and of a similar magnitude for low and high *P. thornei* population densities for each time of sensing (Supplementary Table 3). A negative association occurred for latent vectors for PC2, initiating at 92 DAS for the low and 99 DAS for the high *P. thornei* populations, with the low population having a stronger negative association. For PC3, the latent vectors on the low population were all positive except for 127 DAS, compared to negative vectors for the high *P. thornei* population density, except 43 DAS. The total amount of variance accounted for by PC 1, 2 and 3 was 98.5%, with PC1 = 82.8%, PC2 = 13.5% and PC3 = 2.2%.

Experiment 3

Cluster analysis of NDVI readings for Experiment 3

Seven groups of cultivars were delimited from the cluster analysis of Experiment 3 based on ten NDVI times of sensing, at low and high *P. thornei* population densities (Fig. 1c). The shapes of the NDVI response curves for the centroids of the seven groups are shown in Fig

4. The maximum NDVI for group A was approximately 0.85 at both low and high nematode population densities. The maximum NDVI for group G was approximately 0.75 for low and high population densities.

Principal component analysis for Experiment 3

Ordination of the 36 cultivars based on their scores along PC1 and PC2 from PCA of the NDVI data is given in Fig. 5c. The latent vectors were all positive for PC1, and of a similar magnitude for low and high *P. thornei* population densities for each time of sensing (Supplementary Table 4). A negative association occurred for the latent vectors for PC2, starting at 85 DAS for the low and 99 DAS for the high *P. thornei* populations, with the low population having a stronger negative association. For PC3, the latent vectors on the low *P. thornei* population density were all negative except for 127 DAS, compared to positive vectors for the high *P. thornei* population density, except at 106 DAS. The total amount of variance accounted for by PC 1, 2 and 3 was 99.7%, with PC1 = 81%, PC2 = 16.4% and PC3 = 2.3%.

Multiple regression equations relating yield tolerance index to principal components of NDVI for all experiments

The scores for the first three principal components from the PCA of NDVI readings were highly predictive of yield tolerance index in multiple regression analyses of all three experiments as shown by the equations 2, 3 and 4:

(Eq. 2) *Experiment 1 Yield tolerance % = 86.2 + 4.85PC1 + 4.32PC2 + 10.79PC3,*

$$R^2 = 0.92, P < 0.001, d.f. = 32$$

(Eq. 3) *Experiment 2 Yield tolerance % = 84.6 + 2.39PC1 + 3.42PC2 – 12.04PC3,*

$$R^2 = 0.93, P < 0.001, d.f. = 32$$

(Eq. 4) *Experiment 3 Yield tolerance % = 93.2 + 0.96PC1 + 3.02PC2 + 4.16PC3,*

$$R^2 = 0.85, P < 0.001, d.f. = 32$$

Where PC1, PC2, PC3 = cultivar scores for the respective principal components

The residuals from the multiple regression analyses were distributed as Normal in all experiments. The relationship between the observed and predicted values of yield tolerance index followed a Y=X line in all three experiments (Supplementary Fig. 1).

To understand the relative influence of the three principal components on the prediction of yield tolerance index from the multiple regressions, the equations were solved using the actual minimum and maximum scores for the cultivars in the three respective experiments (Table 3). For experiments 1 and 2, PC1 had the greatest influence on predicted yield tolerance index and PC3 had the second greatest influence. For Experiment 3, the order of greatest influence on predicted yield tolerance index was PC2, then PC1 and then PC3.

Coefficient of determination (R^2) between grain yield and NDVI values at all times of sensing

For Experiment 1, the coefficient of determination between the yield tolerance index and NDVI tolerance index increased from 64 DAS ($P<0.001$, $R^2 = 0.80$) to 92 DAS ($P<0.001$, $R^2 = 0.92$), before then decreasing 126 DAS ($P<0.001$, $R^2 = 0.77$) (Table 4). Similar trends were observed when NDVI was measured on high and low *P. thornei* populations. However, the coefficients of determination were less for the low population than for the high population indicating that assessment at high population densities allowed better discrimination of cultivars on their tolerance to *P. thornei*.

For Experiment 2, the grain yield tolerance index was strongly related to the NDVI tolerance index between 43 and 127 DAS. The strongest significant relationships between the two indices was at 85 DAS ($P<0.001$, $R^2 = 0.90$) and the weakest was at 43 DAS ($P<0.001$, $R^2 = 0.62$). Between 57 DAS and 113 DAS the coefficients of determination ranged between

$R^2 = 0.84$ and $R^2 = 0.90$. For the low *P. thornei* population density, the strongest relationship occurred at 57 DAS, then decreased as the crop matured. On the high *P. thornei* population density, the strongest relationship occurred at 71 DAS, then decreased as the crop matured. The high *P. thornei* population density had a stronger relationship at all times of sensing than the low *P. thornei* population density.

For Experiment 3, the grain yield tolerance index was strongly related to the NDVI tolerance index at 43 and 127 DAS. The strongest and weakest relationship between the two indices was at 57 DAS ($P<0.001$, $R^2 = 0.86$) and 106 DAS ($P<0.001$, $R^2 = 0.49$) respectively. Between 57 and 85 DAS, the coefficients of determination were above 0.8. On the high *P. thornei* population density the strongest relationship occurred at 57 DAS, then decreased as the crop matured. For the low *P. thornei* population density the strongest relationship occurred at 43 and 57 DAS, then decreased as the crop matured. The high *P. thornei* population density had a stronger relationship at all times of sensing than the low *P. thornei* population density.

Area under disease progress curve (AUDPC) with respect to NDVI readings for all experiments

In all three experiments, the yield tolerance index (YTI) had highly significant relationships ($P<0.001$) with the AUDPC index with respect to NDVI (Table 5). AUDPC with respect to NDVI on high *P. thornei* population densities was significantly related ($P<0.001$) to YTI for all experiments. The strongest relationship was in Experiment 1 ($R^2=0.83$). AUDPC with respect to NDVI on low *P. thornei* population densities was significantly related ($P<0.001$) to YTI for Experiments 1 and 2. In Experiment 1, at high population densities of *P. thornei*, AUDPC with respect to NDVI had a stronger positive relationship with yield ($R^2 = 0.92$,

$P<0.001$) compared with the low population density ($R^2 = 0.54$, $P<0.001$). Likewise in Experiment 2, yield had a significant relationship with AUDPC at high nematode population density ($R^2 = 0.84$, $P<0.001$) than at the lower population density ($R^2 = 0.62$, $P<0.001$). Experiment 3 had a significant relationship ($R^2 = 0.35$, $P<0.001$) between yield and AUDPC measured by NDVI on high *P. thornei* population density, but not on the low population density.

Discussion

This is the first study to determine the suitability of NDVI as a predictor of wheat cultivar response to *P. thornei*. The results from i) classification and ordination of wheat cultivars into NDVI groupings, ii) multiple regression predictions of grain yield tolerance index from principal components of NDVI readings, and iii) coefficients of determination between grain yield and AUDPC of NDVI readings or individual sensing times, all support the use of NDVI as a predictive tool to assess the response of wheat cultivars to *P. thornei*. Thus NDVI can be used to assess tolerance in the vegetative stages without needing to wait for grain harvest. The advantage of NDVI is that large numbers of cultivars or lines in breeding nurseries can be screened objectively and non-destructively (Crusiol *et al.*, 2017). Additionally, only those lines that have tolerance to *P. thornei* could be selected and subsequently harvested for grain to use resources efficiently.

Our results showed that screening for tolerance to *P. thornei* by NDVI can be done effectively by setting up plots with low and high nematode population densities. The primary difference between the plots in our experiments was *P. thornei* density, as the plant available water at commencement of Year 2 was similar for the two nematode density treatments. This is important because when NDVI is used to measure the greenness of canopy coverage it can be influenced by confounding or multiple traits (Govaerts *et al.*, 2007; Jones *et al.*,

2015; Wang *et al*., 2016). Ideally, the low populations should have been zero as a control in order to determine the maximum potential of damaging populations of *P. thornei* on wheat yield, but because fumigant or chemical controls are not currently available and do not eliminate *P. thornei* from the soil profile, more than one resistant crop would have been required to further reduce populations (Owen *et al*., 2010; Thompson *et al*., 2010; Whish *et al*., 2014). The time and cost required to achieve this was not feasible for this study and is unlikely to be used in breeding programs. Intolerant groups of cultivars formed by cluster analysis were identified as having reduced NDVI values with these being less at high nematode population densities than at low population densities compared with more tolerant groups of cultivars. Grain tolerance index and grain yield at high nematode population densities (9091, 3018 and 1245 *P. thornei*/kg soil for Experiments 1, 2 and 3 respectively) were strongly related to NDVI. These relationships were still evident at the low population densities for Experiments 1 and 2 (2570 and 975 *P. thornei*/kg soil) but not so for Experiment 3 (578 *P. thornei*/kg soil). These results are in line with previously established thresholds for grain yield loss taken to be 2000 *P. thornei*/kg soil at any depth interval in the soil profile (Thompson *et al.*, 2010) or 1000 *P. thornei*/kg soil averaged over a soil profile of 0.9 m depth (derived from Fig 5b. Owen et al., 2014).

In our study we showed NDVI can be used to calculate AUDPC that is predictive of tolerance where populations of *P. thornei* were above 1000 *P. thornei*/kg. However, populations above 2500 *P. thornei*/kg soil provided a more robust discrimination of tolerance. A single value like the AUDPC is used to represent an interaction of host, pathogen, environment and time (Mohaptra *et al*., 2014). It is calculated from assessment of disease intensity over time. Although intense sampling is required to determine AUDPC, the use of unmanned aerial vehicles (UAV) can increase data capturing capacity. For example, UAV-based platforms measuring NDVI improved the speed of data acquisition

and with greater accuracy for studying drought adaptive traits of durum wheat cultivars compared to ground-based Greenseeker™ sensors (Condorelli *et al*., 2018). Furthermore, Shi *et al*., (2016) found that NDVI captured by UAV was strongly predictive of leaf area index and canopy cover, and could subsequently rank cultivars accordingly. Using UAV to determine AUDPC is a feasible option, particularly when an advantage of AUDPC is that the tolerance of a cultivar can be predicted when the density of *P. thornei* is low and less damaging, compared with the less intensive assessment at a critical time (discussed later) that requires higher *P. thornei* population densities.

Wheat cultivars with tolerance to *P. thornei* in the subtropical grain region of eastern Australia have been selected from breeding material by growing plants at high levels of *P. thornei* (Thompson *et al.,* 1999). Our results showed that NDVI was predictive of tolerance when experiments were grown on population densities greater than 2500 *P. thornei*/kg. Regionally, densities of *P. thornei* above 2000/kg occur in 31% fields, with these having a wide range of soil textures and pH (Thompson *et al.*, 2010). With numerous crops in the region being susceptible to *P. thornei* (Thompson *et al*., 2008), it is easier to increase populations, rather than to reduce populations below 1000/kg, and this is an advantage for research purposes. It is recommended that breeding programs that plan to use NDVI have a managed field site with uniformly distributed *P. thornei* maintained at high population densities of at least >2500/kg soil and preferably greater.

To further increase efficiency of screening, we can estimate a critical point of sensing by NDVI with this time point being used to take readings for prediction of the tolerance of a cultivar to *P. thornei*. When grown on the high population densities only, the predictive sensing window (when the R^2 from sensing time was greater or equal to 0.8) was 78–106 DAS (1021–1538°Cd) for Experiment 1, and was 57–85 DAS (695–1095°Cd) for Experiment 2. Wheat development is responsive to temperature (Slafer and Rawson 1994)

and can be described by a decimal growth code, i.e. Z (Zadoks *et al.*, 1974), which can be modelled from thermal time by the Agricultural Production Systems Simulator (APSIM) (Holzworth *et al.*, 2014). For 19 cultivars in our experiments, APSIM was used to determine the developmental stages at the beginning and the end of the predictive sensing windows for Experiments 1 and 2. For Experiment 1, the cultivars were between late stem elongation (Z37.2 ± 0.5 SEM) and the start of grain fill (Z70.3 ± 0.8) stages. For Experiment 2, the cultivars were between mid-tillering (Z25.0 ± 0) and early boot (Z41.6 ± 0.9) stages. In other studies, yield and NDVI have correlated well at similar stages of crop growth (Marti *et al.*, 2007), when NDVI values were between 0.2 and 0.8 (Ren *et al.*, 2008), and prior to complete canopy coverage (Casadesús *et al.*, 2007). It was from our two experiments, we estimated that a single time at approximately 1000°Cd thermal time for NDVI measurement is a practical predictive option to screen for tolerance, but cultivars must be tested on damaging populations of *P. thornei*, of at least 2500 *P. thornei*/kg soil.

Conclusion

Australian wheat breeders need to produce wheat cultivars with superior tolerance to *P. thornei* for growers in the subtropical grain region of eastern Australia. This imposes the need for wheat breeding programs to phenotype germplasm for tolerance accurately and on a high throughput scale. We demonstrated in this study that Greenseeker™ can accurately screen wheat germplasm for tolerance when grown on land with high *P. thornei* populations (greater than 2500 *P. thornei*/kg), and at one time of sensing at approximately 1000ºCd after sowing. With the development and availability of unmanned aerial vehicles (UAV), continued research to develop UAV as an aerial platform for NDVI assessment of *P. thornei* tolerance will provide a large-scale and rapid phenotyping tool for plant breeding programs.

Acknowledgements

The authors would like to thank the Grains Research and Development Corporation (GRDC) for co-investment in this study (Projects USQ00019 and DAQ00171) and Queensland Department of Agriculture and Fisheries for support through the USQ/QDAF Broadacre Grains Partnership. We also thank Timothy Clewett, Mei Wang and Michelle Thompson (USQ), Michael Mumford and James Henderson (QDAF), and Jeremy Whish (Commonwealth Scientific and Industrial Research Organisation) for their assistance. We thank Alec Gwynne and family for their valued cooperation and provision of farm land and rainfall records.

Conflicts of interest: none.

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Tables

Table 1 Times of assessment (days after sowing, DAS) of normalised difference vegetation index (NDVI) with corresponding thermal time and cumulative rainfall (mm) for each of the three experiments. Sowing date for Experiment 1 was 11 June, and Experiments 2 and 3 was 16 June.

	Experiment 1			Experiment 2		
DAS	Sensing time	Cumulative Thermal Time[a] (ºCd)	Cumulative rainfall (mm)[b]	Sensing time	Cumulative Thermal Time[a] (ºCd)	Cumulative rainfall (mm)[b]
43				NDVI-1	539	2.5
57				NDVI-2	695	2.5
64	NDVI-1	839	44.7			
71				NDVI-3	896	2.5
78	NDVI-2	1021	50.3	NDVI-4	985	23.9
85				NDVI-5	1095	33.3
86	NDVI-3	1159	50.3			
92	NDVI-4	1265	50.3	NDVI-6	1210	33.3
99				NDVI-7	1320	33.3
106	NDVI-5	1538	67.1	NDVI-8	1428	36.1
113				NDVI-9	1567	36.1
119	NDVI-6	1809	83.1			
126	NDVI-7	1963	83.9			
127				NDVI-10	1857	39.1

[a]Thermal time is the sum of the mean daily minimum and maximum temperatures above a base temperature of 0°C, recorded at the Dalby Airport weather station (37 km NNW from trial site) commencing at sowing.

[b]Cumulative rainfall (mm) is the amount of rainfall from sowing.

Table 2 Mean *Pratylenchus thornei* population densities and plant available water (PAW) to 0.9 m soil depth at the commencement of Year 2 after growing Year 1 wheat cvs. Kennedy and QT8343 for each experiment with 11 residual d.f.

Experiment	Year 1 cultivar	Year 2 designation	*P. thornei*/kg soil			High/low *P. thornei* ratio	PAW (mm)[c]
			ln(x+1)[a]	SED	BTM[b]		
1	Kennedy	High	9.12a	0.06	9091	3.53	158
	QT8343	Low	7.85b		2570		159
2	Kennedy	High	8.01a	0.11	3018	3.10	138
	QT8343	Low	6.88b		975		138
3	Kennedy	High	7.13a	0.10	1245	2.15	155
	QT8343	Low	6.36b		578		155

[a]Different letters denote significant differences within an experiment (P<0.001; Wald test)

[b]BTM, Back-transformed mean

[c] No significant difference within an experiment (P<0.05; Wald test)

Table 3 Sensitivity of prediction of yield tolerance index (%) by Principal Component 1 (PC1), Principal Component 2 (PC2) and Principal Component 3 (PC3) in a multiple regression model for three experiments. Yield tolerance index is yield of a cultivar at high nematode population density as a percentage of its yield at low nematode population density. Multiple regression equations were of the form y = *a* + *b*PC1 + *c*PC2 + *d*PC3 where y = yield tolerance index, PC = principal component score, *a* is the intercept (a constant), and *b*, *c* and *d* are coefficients; estimated values given with SE in parentheses.

	Experiment 1			Experiment 2			Experiment 3		
Intercept	86.2			84.6			93.2		
	(093)			(0.82)			(0.51)		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Coefficient	4.9	4.3	10.8	2.4	3.4	12.0	1.0	3.0	4.2
	(0.28)	(0.68)	(1.27)	(0.21)	(0.51)	(1.25)	(0.13)	(0.29)	(0.77)
Minimum score	-6.6	-1.8	-1.3	-10.7	-2.6	-1.4	-11.5	-3.3	-1.3
Maximum score	4.9	3.0	1.4	5.4	3.4	1.3	5.2	3.4	1.3
Range of effects on predicted yield tolerance index (%)									
Minimum	54.0	78.3	72.2	59.0	75.7	67.9	82.2	83.4	87.6
Maximum	110.2	99.2	101.6	97.6	96.2	100.0	98.2	103.6	98.7
Range	56.1	20.9	29.5	38.6	20.5	32.1	16.0	20.2	11.1
Total Range		106.4			91.2			47.3	

Table 4 Coefficient of determination (R^2) between grain yield and NDVI at various times of sensing (days after sowing, DAS), as either a tolerance index, or actual values on low and on high *P. thornei* population densities where n = 36 in all experiments. Index[a] derived from the relationship between NDVI tolerance index and yield tolerance index for each DAS for each experiment.

	Yield - NDVI coefficient of determination								
	Experiment 1			Experiment 2			Experiment 3		
DAS	Index[a]	Low	High	Index[a]	Low	High	Index[a]	Low	High
43	-	-	-	0.62^{***}	0.71^{***}	0.78^{***}	0.68^{***}	0.19^{**}	0.54^{***}
57	-	-	-	0.87^{***}	0.80^{***}	0.90^{***}	0.86^{***}	0.19^{**}	0.65^{***}
64	0.80^{***}	0.30^{***}	0.73^{***}	-	-	-	-	-	-
71	-	-	-	0.89^{***}	0.78^{***}	0.90^{***}	0.84^{***}	0.16^{**}	0.60^{***}
78	0.86^{***}	0.47^{***}	0.86^{***}	0.87^{***}	0.77^{***}	0.88^{***}	0.83^{***}	0.12^{*}	0.53^{***}
85	-	-	-	0.90^{***}	0.68^{***}	0.86^{***}	0.81^{***}	0.06^{NS}	0.39^{***}
86	0.91^{***}	0.54^{***}	0.92^{***}	-	-	-	-	-	-
92	0.92^{***}	0.53^{***}	0.92^{***}	0.84^{***}	0.54^{***}	0.77^{***}	0.67^{***}	0.03^{NS}	0.22^{**}
99				0.89^{***}	0.46^{***}	0.72^{***}	0.61^{***}	0.01^{NS}	0.08^{NS}
106	0.87^{***}	0.54^{***}	0.90^{***}	0.85^{***}	0.35^{***}	0.59^{***}	0.49^{***}	0.01^{NS}	0.03^{NS}
113	-	-	-	0.89^{***}	0.27^{***}	0.59^{***}	0.69^{***}	0.00^{NS}	0.07^{NS}
119	0.85^{***}	0.38^{***}	0.78^{***}	-	-	-	-	-	-
126	0.77^{***}	0.24^{**}	0.60^{***}	-	-	-	-	-	-
127	-	-	-	0.71^{***}	0.01^{NS}	0.11^{*}	0.78^{***}	0.00^{NS}	0.00^{NS}

$^{***}P<0.001$; $^{**}P<0.01$; $^{*}P<0.05$; ^{NS} non-significant *F*-tests; - no result

Table 5 Coefficient of determination (R^2) between area under disease progress curve (AUDPC) with respect to normalised difference vegetation index (NDVI) and yield tolerance index (YTI), grain yield on low *P. thornei* population density (GYL) and grain yield on high *P. thornei* population density (GYH) where n = 36 for all experiments.

	AUDPC with respect to NDVI								
	Experiment 1			Experiment 2			Experiment 3		
	Index	Low	High	Index	Low	High	Index	Low	High
YTI	0.92^{***}	0.49^{***}	0.83^{***}	0.92^{***}	0.41^{***}	0.73^{***}	0.85^{***}	0.12^{*}	0.41^{***}
GYL	-	0.54^{***}	-	-	0.62^{***}	-	-	0.04^{NS}	-
GYH	-	-	0.92^{***}	-	-	0.84^{***}	-	-	0.35^{***}

$^{***}P<0.001$; $^{*}P<0.05$; ^{NS} non-significant *F*-tests; - no result

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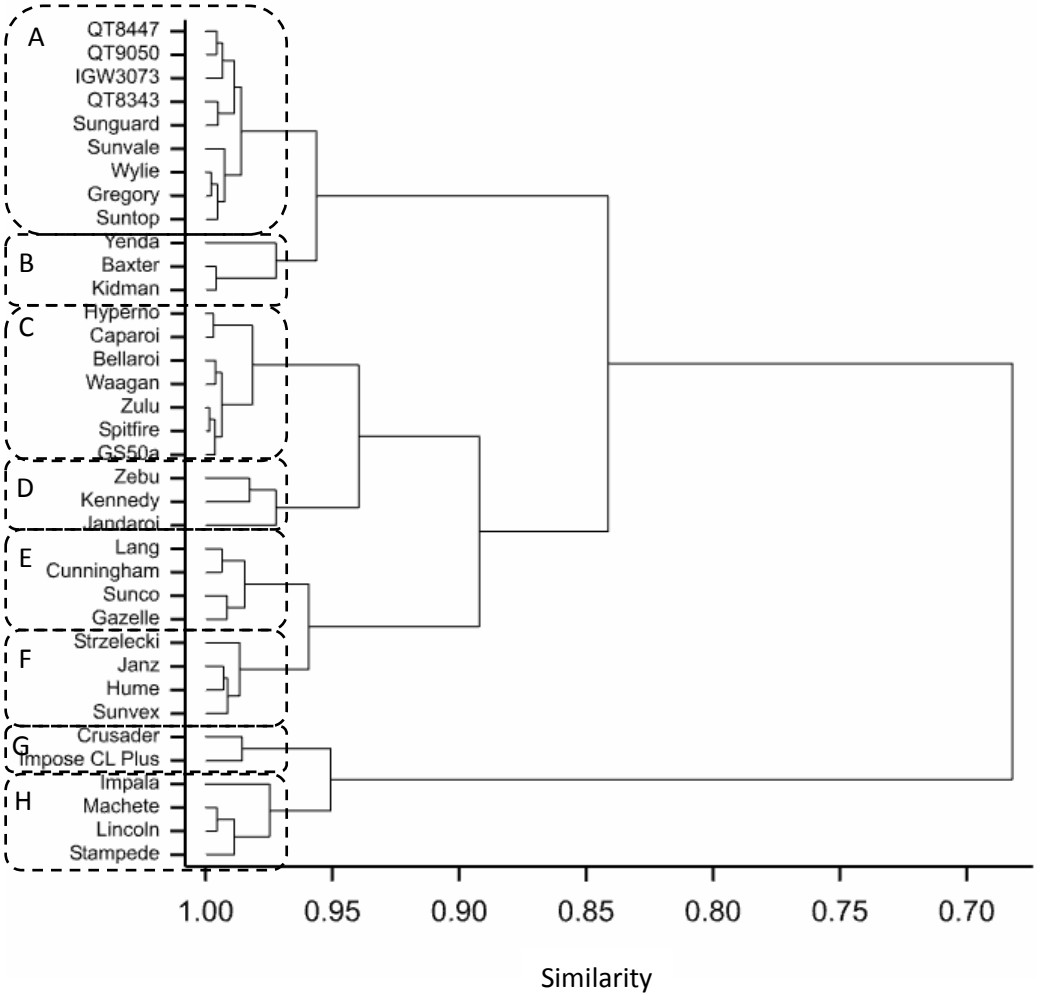
assessments in each of (b) Experiment 2 and (c) Experiment 3. The dashed lines surround cultivars in clusters identified in Fig. 1 dendrograms.

List of Supplementary Figures

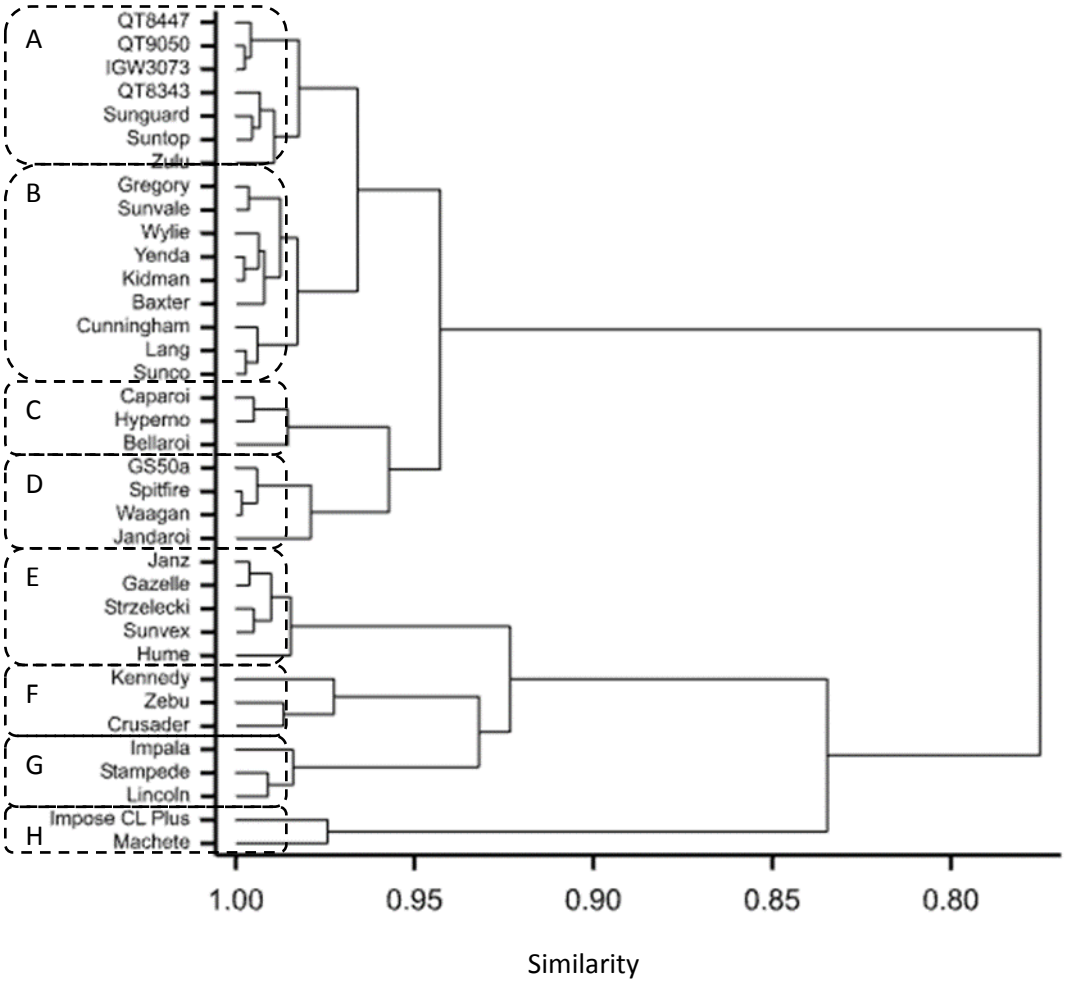
Supplementary Figure 1 Relationship between observed yield tolerance index (%) and fitted yield tolerance index (%) predicted from multiple regression analysis with explanatory variables being the scores of the first three principal components from analysis of NDVI readings of (a) Experiment 1, (b) Experiment 2 and (c) Experiment 3. Lines trace Y=X.

Figures

(a)


A
QT8447
QT9050
IGW3073
QT8343
Sunguard
Sunvale
Wylie
Gregory
Suntop
B
Yenda
Baxter
Kidman
C
Hyperno
Caparoi
Bellaroi
Waagan
Zulu
Spitfire
GS50a
D
Zebu
Kennedy
Jandaroi
E
Lang
Cunningham
Sunco
Gazelle
F
Strzelecki
Janz
Hume
Sunvex
G
Crusader
Impose CL Plus
H
Impala
Machete
Lincoln
Stampede
1.00
0.95
0.90
0.85
0.80
0.75
0.70
Similarity

(b)


A
QT8447
QT9050
IGW3073
QT8343
Sunguard
Suntop
Zulu
B
Gregory
Sunvale
Wylie
Yenda
Kidman
Baxter
Cunningham
Lang
Sunco
C
Caparoi
Hyperno
Bellaroi
D
GS50a
Spitfire
Waagan
Jandaroi
E
Janz
Gazelle
Strzelecki
Sunvex
Hume
F
Kennedy
Zebu
Crusader
G
Impala
Stampede
Lincoln
H
Impose CL Plus
Machete
1.00
0.95
0.90
0.85
0.80
Similarity

(c)

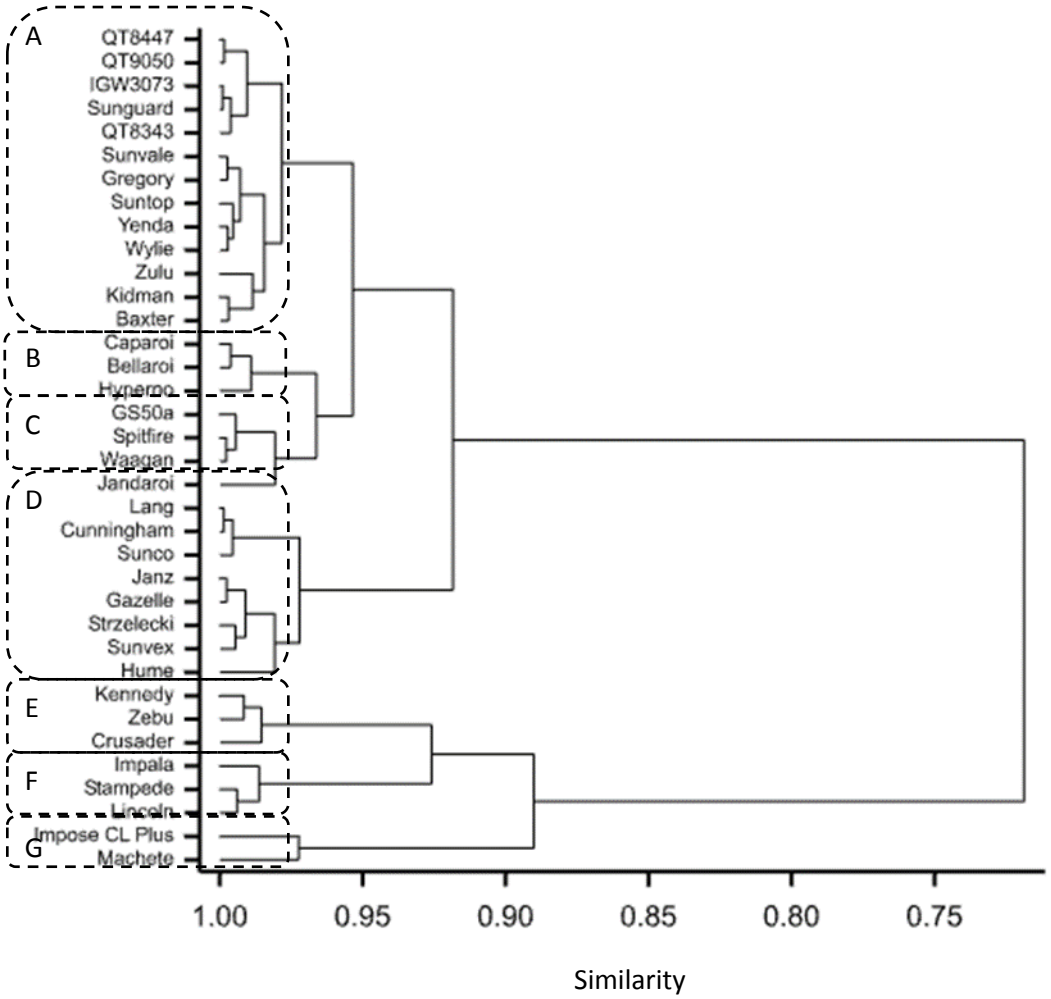


Figure 1 Dendrograms from cluster analysis of the 36 wheat cultivars grown at two nematode population densities, based on eBLUPs of seven normalised difference vegetation index (NDVI) assessments for (a) Experiment 1 and ten NDVI assessments for both (b) Experiment 2 and (c) Experiment 3. At similarity 0.97, eight groups (A-H) were formed for Experiments 1 and 2, and seven groups (A–G) for Experiment 3.

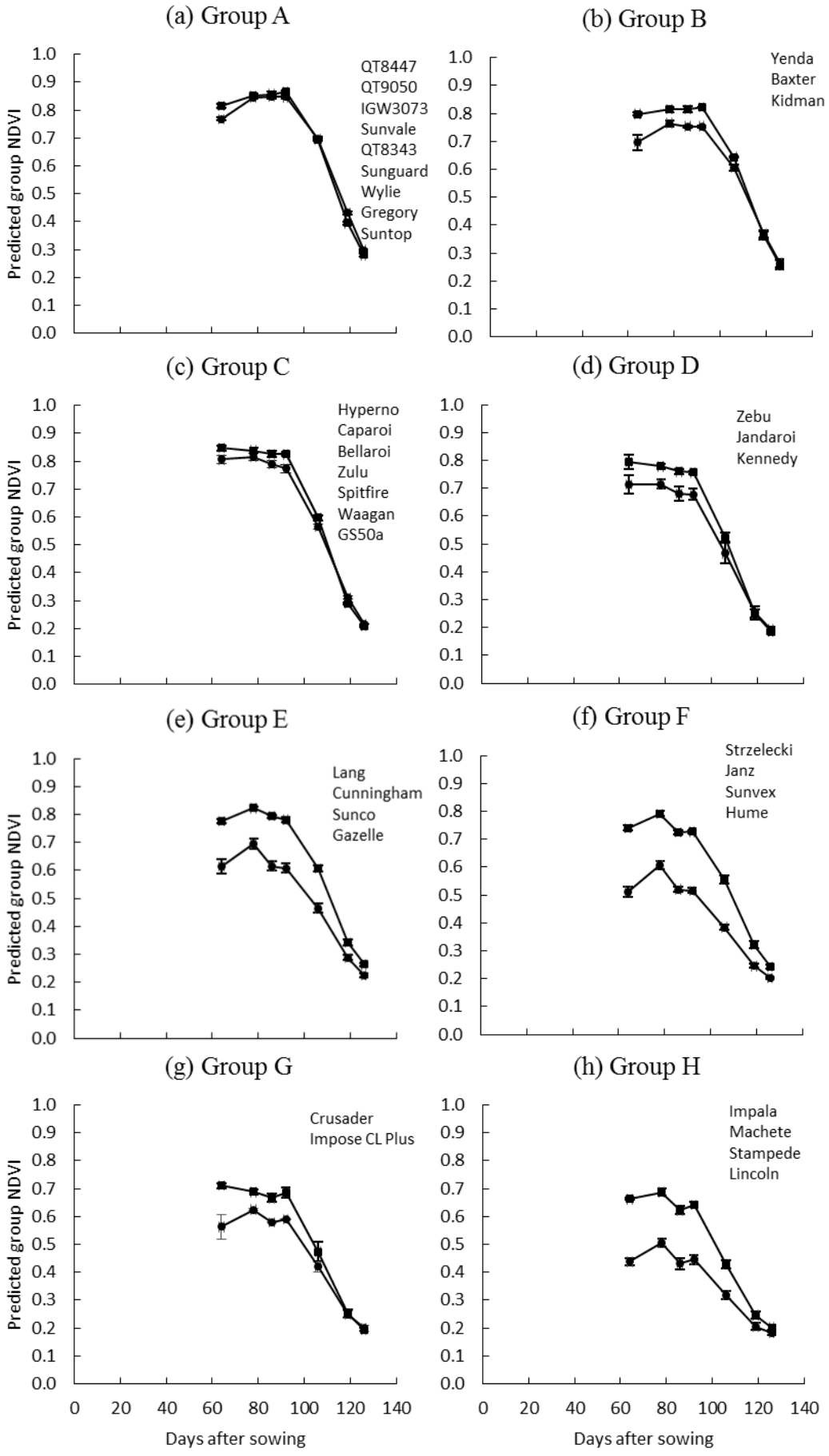

(a) Group A
QT8447
QT9050
IGW3073
Sunvale
QT8343
Sunguard
Wylie
Gregory
Suntop
(b) Group B
Yenda
Baxter
Kidman
(c) Group C
Hyperno
Caparoi
Bellaroi
Zulu
Spitfire
Waagan
GS50a
(d) Group D
Zebu
Jandaroi
Kennedy
(e) Group E
Lang
Cunningham
Sunco
Gazelle
(f) Group F
Strzelecki
Janz
Sunvex
Hume
(g) Group G
Crusader
Impose CL Plus
(h) Group H
Impala
Machete
Stampede
Lincoln
Predicted group NDVI
Days after sowing

Figure 2 Normalised difference vegetation index (NDVI) response curves at low (squares) and high (circles) *P. thornei* populations for the centroid of eight groups delimited by cluster analysis for Experiment 1. (a) Group A, (b) Group B, (c) Group C, (d) Group D, (e) Group E, (f) Group F, (g) Group G and (h) Group H. Bars on points represent ±SEM. Wheat cultivars in each group are displayed on each figure.

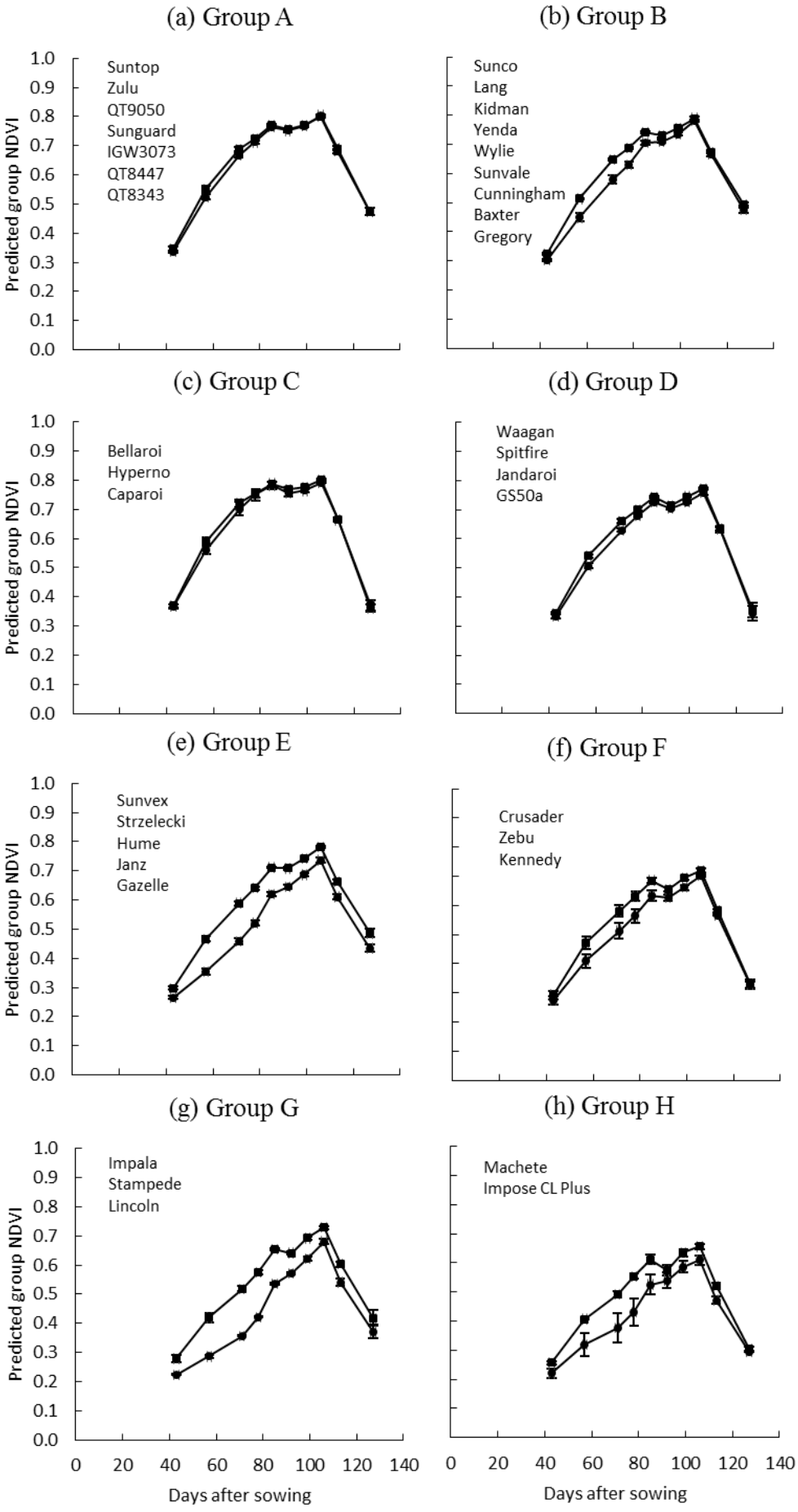

(a) Group A
Suntop
Zulu
QT9050
Sunguard
IGW3073
QT8447
QT8343
(b) Group B
Sunco
Lang
Kidman
Yenda
Wylie
Sunvale
Cunningham
Baxter
Gregory
(c) Group C
Bellaroi
Hyperno
Caparoi
(d) Group D
Waagan
Spitfire
Jandaroi
GS50a
(e) Group E
Sunvex
Strzelecki
Hume
Janz
Gazelle
(f) Group F
Crusader
Zebu
Kennedy
(g) Group G
Impala
Stampede
Lincoln
(h) Group H
Machete
Impose CL Plus
Predicted group NDVI
Days after sowing
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
20
40
60
80
100
120
140

Figure 3 Normalised difference vegetation index (NDVI) response curves at low (squares) and high (circles) *P. thornei* populations for the centroid of eight groups delimited by cluster analysis for Experiment 2. (a) Group A, (b) Group B, (c) Group C, (d) Group D, (e) Group E, (f) Group F, (g) Group G and (h) Group H. Bars on points represent ±SEM. Wheat cultivars in each group are displayed on each figure.

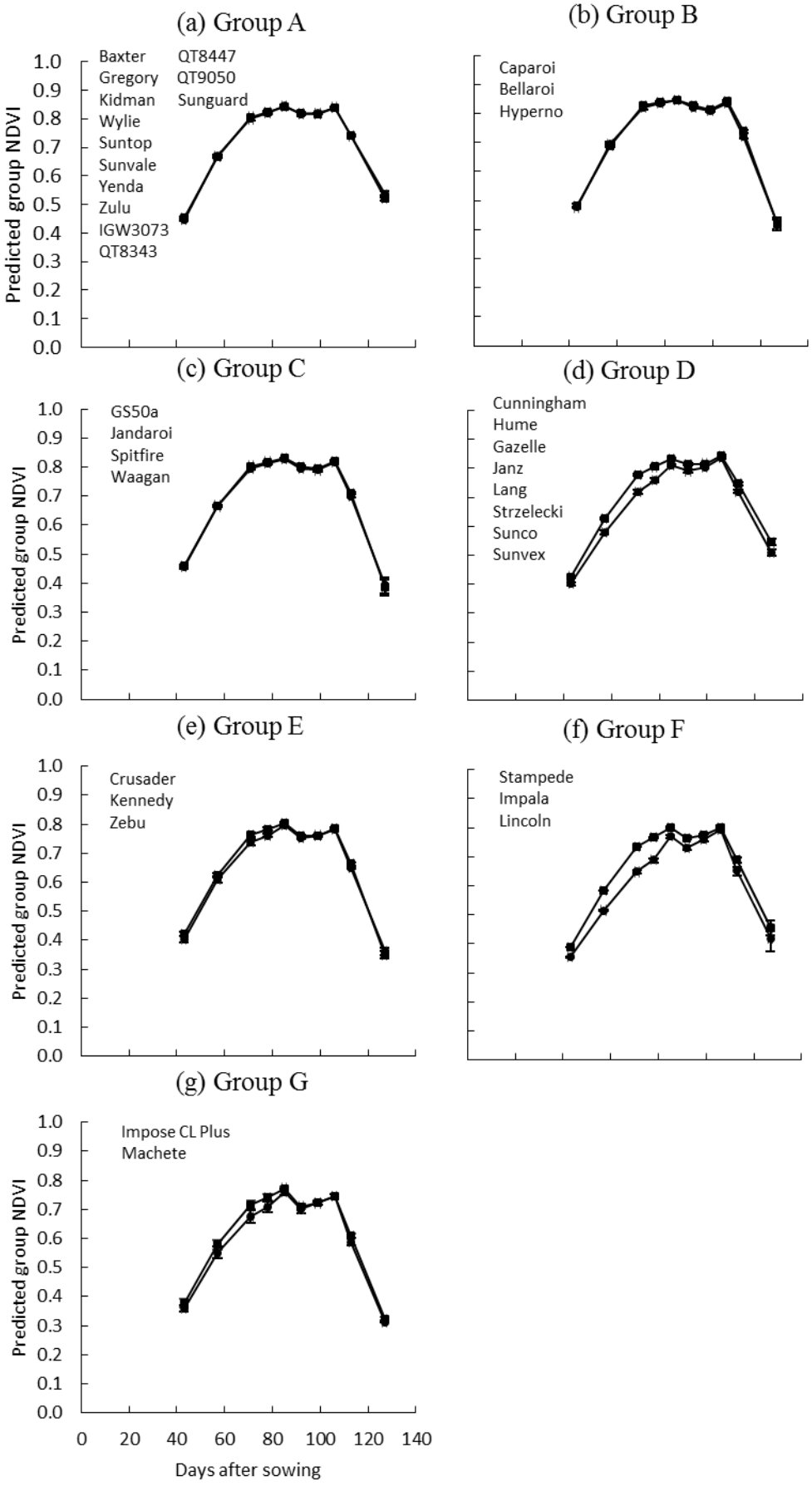
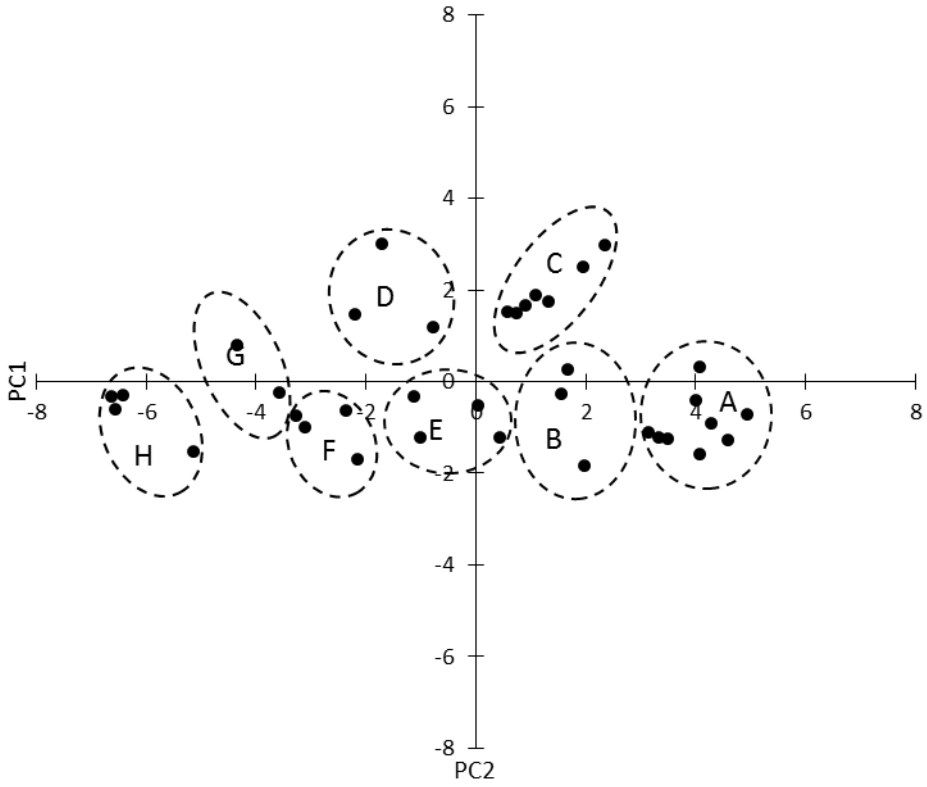
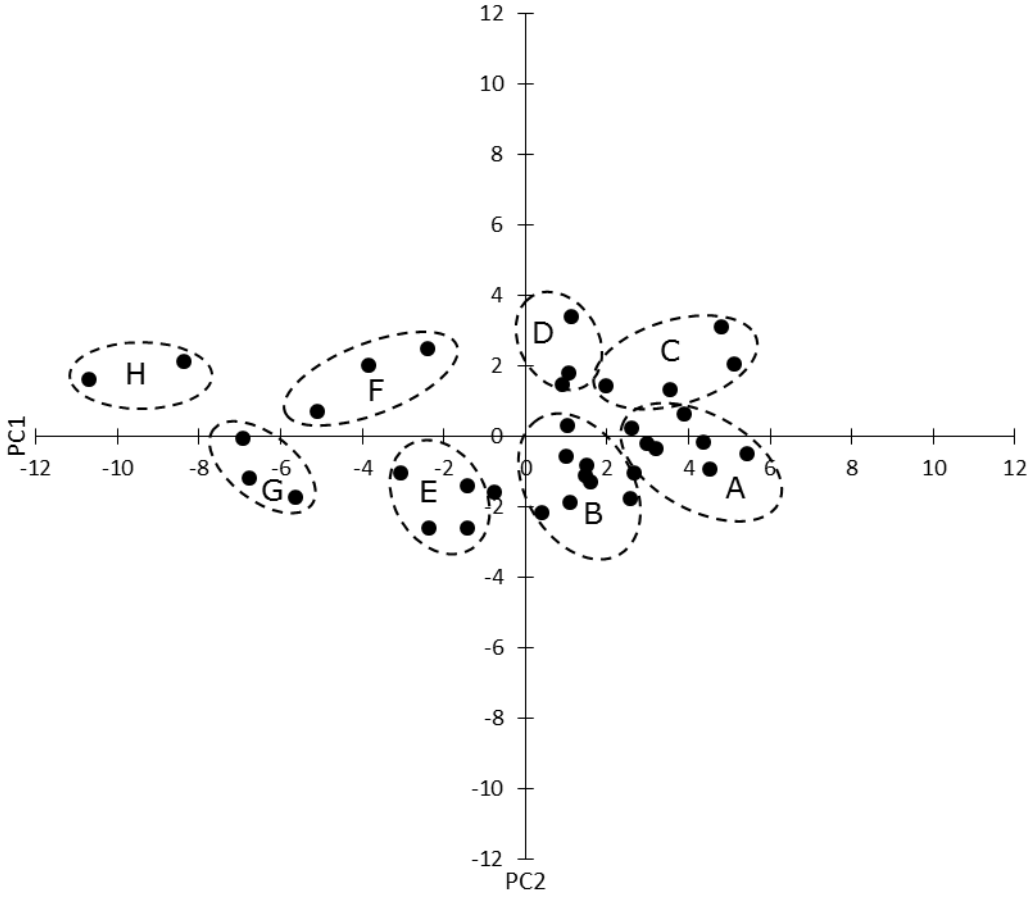

(a) Group A
Baxter
Gregory
Kidman
Wylie
Suntop
Sunvale
Yenda
Zulu
IGW3073
QT8343
QT8447
QT9050
Sunguard
(b) Group B
Caparoi
Bellaroi
Hyperno
(c) Group C
GS50a
Jandaroi
Spitfire
Waagan
(d) Group D
Cunningham
Hume
Gazelle
Janz
Lang
Strzelecki
Sunco
Sunvex
(e) Group E
Crusader
Kennedy
Zebu
(f) Group F
Stampede
Impala
Lincoln
(g) Group G
Impose CL Plus
Machete
Predicted group NDVI
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
20
40
60
80
100
120
140
Days after sowing

Figure 4 Normalised difference vegetation index (NDVI) response curves at low (squares) and high (circles) *P. thornei* populations for the centroid of eight groups delimited by cluster analysis for Experiment 3. (a) Group A, (b) Group B, (c) Group C, (d) Group D, (e) Group E, (f) Group F, and (g) Group G. Bars on points represent ±SEM. Wheat cultivars in each group are displayed on each figure.

(a)



(b)



(c)

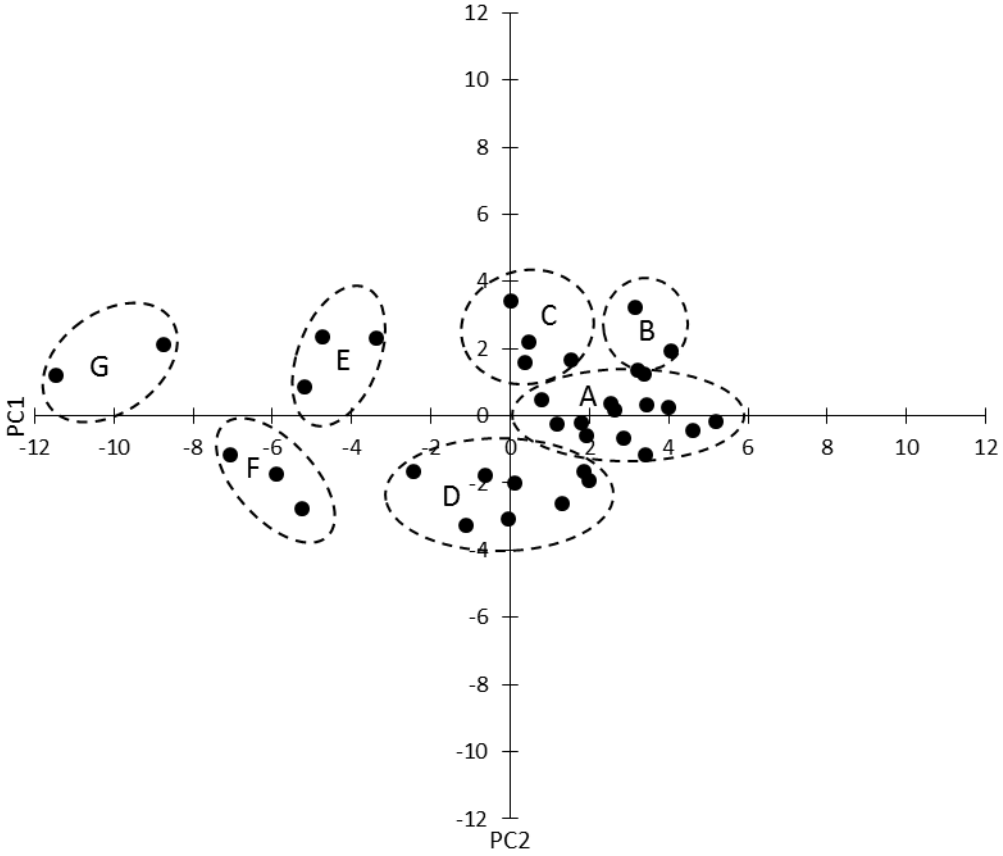


Figure 5 Ordination of 36 wheat cultivars on principal component 1 (PC1) and principal component 2 (PC2) obtained from principal component analysis of eBLUPs of seven NDVI assessments at two population densities of *P. thornei* in (a) Experiment 1, and ten NDVI assessments in each of (b) Experiment 2 and (c) Experiment 3. The dashed lines surround cultivars in clusters identified in Fig. 1 dendrograms.

Supplementary Table 1. The list of wheat cultivars and species name that were grown in Year 2 for Experiments 1, 2 and 3.

Cultivar	Type	Species
Baxter	Bread	*Triticum aestivum*
Bellaroi	Durum	*Triticum turgidum* ssp. *durum*
Caparoi	Durum	*Triticum turgidum* ssp. *durum*
Crusader	Bread	*Triticum aestivum*
Cunningham	Bread	*Triticum aestivum*
Gazelle	Bread	*Triticum aestivum*
Gregory	Bread	*Triticum aestivum*
GS50a	Bread	*Triticum aestivum*
Hume	Bread	*Triticum aestivum*
Hyperno	Durum	*Triticum turgidum* ssp. *durum*
IGW3073	Bread	*Triticum aestivum*
Impala	Bread	*Triticum aestivum*
Impose CL Plus	Bread	*Triticum aestivum*
Jandaroi	Durum	*Triticum turgidum* ssp. *durum*
Janz	Bread	*Triticum aestivum*
Kennedy	Bread	*Triticum aestivum*
Kidman	Bread	*Triticum aestivum*
Lang	Bread	*Triticum aestivum*
Lincoln	Bread	*Triticum aestivum*
Machete	Bread	*Triticum aestivum*
QT8343	Bread	*Triticum aestivum*
QT8447	Bread	*Triticum aestivum*
QT9050	Bread	*Triticum aestivum*
Spitfire	Bread	*Triticum aestivum*
Stampede	Bread	*Triticum aestivum*
Strzelecki	Bread	*Triticum aestivum*
Sunco	Bread	*Triticum aestivum*
Sunguard	Bread	*Triticum aestivum*
Suntop	Bread	*Triticum aestivum*
Sunvale	Bread	*Triticum aestivum*
Sunvex	Bread	*Triticum aestivum*
Waagan	Bread	*Triticum aestivum*
Wylie	Bread	*Triticum aestivum*
Yenda	Bread	*Triticum aestivum*
Zebu	Bread	*Triticum aestivum*
Zulu	Durum	*Triticum turgidum* ssp. *durum*

Supplementary Table 2 Latent vectors for low and high *P. thornei* population densities for days after sowing (DAS) and the percent variance accounted for (VAF%) by Principal Component 1 (PC1), Principal Component 2 (PC2) and Principal Component 3 (PC3) for Experiment 1.

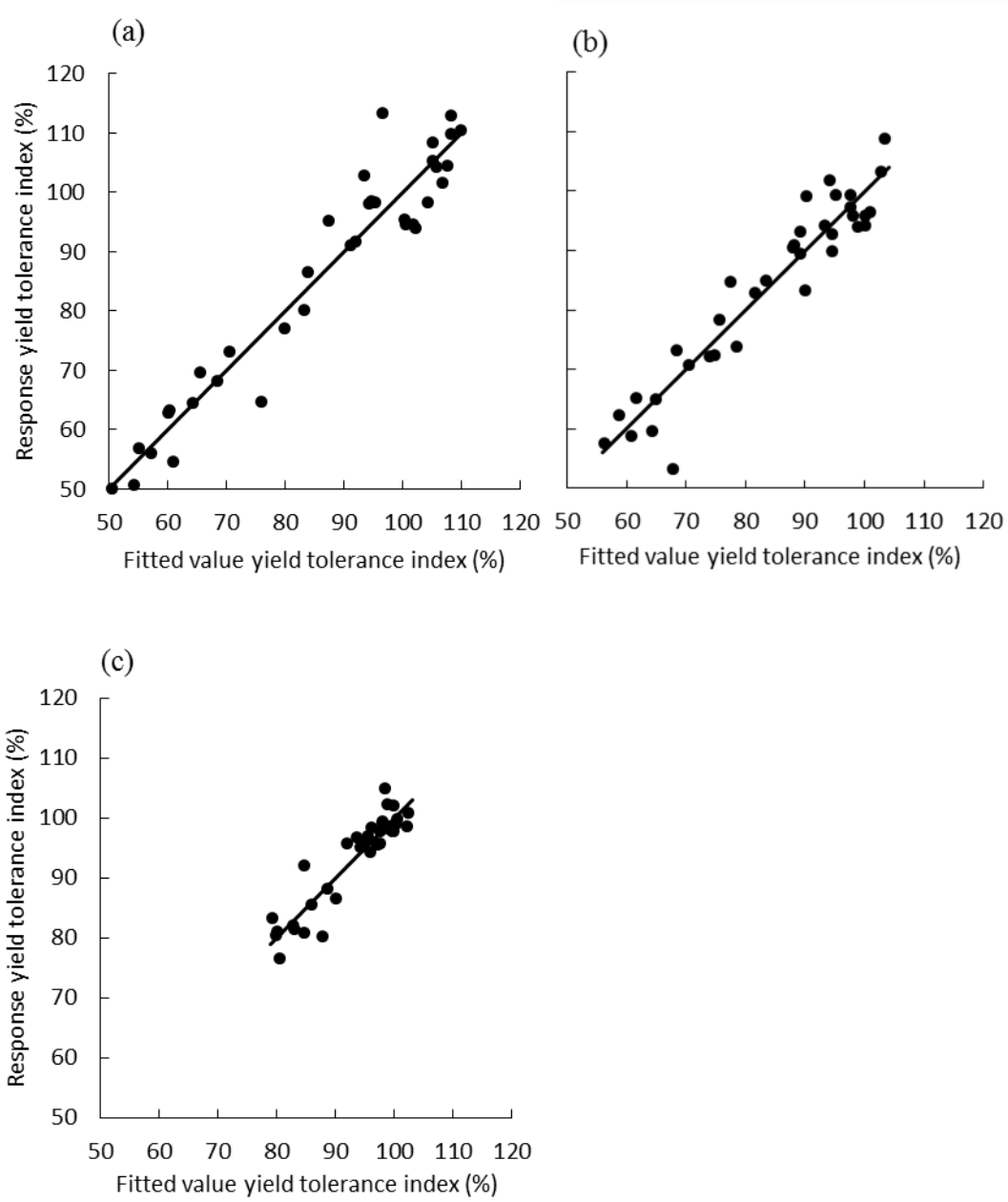
	PC1		PC2		PC3	
DAS	Low	High	Low	High	Low	High
64	0.25	0.25	0.36	0.35	-0.28	0.10
78	0.27	0.28	0.07	0.19	-0.55	0.10
86	0.29	0.28	0.09	0.17	-0.27	0.24
92	0.29	0.28	0.06	0.15	-0.12	0.28
106	0.28	0.29	-0.15	-0.02	-0.21	0.30
119	0.23	0.28	-0.44	-0.18	-0.12	0.31
126	0.19	0.26	-0.54	-0.31	-0.20	0.30
VAF%	81.0		13.7		4.0	

Supplementary Table 3 Latent vectors for low and high *P. thornei* population densities for days after sowing (DAS) and the percent variance accounted for (VAF%) by Principal Component 1 (PC1), Principal Component 2 (PC2) and Principal Component 3 (PC3) for Experiment 2.

	PC1		PC2		PC3	
DAS	Low	High	Low	High	Low	High
43	0.22	0.23	0.13	0.22	0.18	0.02
57	0.23	0.22	0.17	0.26	0.04	-0.27
71	0.24	0.23	0.10	0.20	0.03	-0.23
78	0.24	0.23	0.10	0.19	0.06	-0.18
85	0.24	0.24	0.01	0.13	0.21	-0.19
92	0.23	0.24	-0.09	0.03	0.32	-0.13
99	0.23	0.24	-0.15	-0.02	0.29	-0.12
106	0.22	0.24	-0.22	-0.11	0.39	-0.06
113	0.21	0.24	-0.31	-0.14	0.22	-0.19
127	0.10	0.13	-0.55	-0.46	-0.17	-0.50
VAF%	82.8		13.5		2.2	

Supplementary Table 4 Latent vectors for low and high *P. thornei* population densities for days after sowing (DAS) and the percent variance accounted for (VAF%) by Principal Component 1 (PC1), Principal Component 2 (PC2) and Principal Component 3 (PC3) for Experiment 3.

	PC1		PC2		PC3	
DAS	Low	High	Low	High	Low	High
43	0.22	0.21	0.24	0.27	-0.24	0.00
57	0.23	0.20	0.23	0.31	-0.02	0.27
71	0.24	0.21	0.16	0.28	-0.14	0.24
78	0.24	0.22	0.08	0.23	-0.17	0.22
85	0.25	0.24	-0.05	0.11	-0.12	0.24
92	0.24	0.25	-0.11	0.01	-0.25	0.05
99	0.23	0.24	-0.20	-0.14	-0.15	0.10
106	0.22	0.23	-0.21	-0.19	-0.31	-0.12
113	0.22	0.24	-0.24	-0.15	-0.22	0.06
127	0.14	0.17	-0.43	-0.34	0.32	0.53
VAF%	81.0		16.4		2.3	



Supplementary Figure 1 Relationship between observed yield tolerance index (%) and fitted yield tolerance index (%) predicted from multiple regression analysis with explanatory variables being the scores of the first three principal components from analysis of NDVI readings of (a) Experiment 1, (b) Experiment 2 and (c) Experiment 3. Lines trace Y=X.

4. Chapter Four – Conclusions

This chapter will explore the key findings of this study, and the future recommendations of using normalised difference vegetation index (NDVI) to assess the tolerance of wheat cultivars to *Pratylenchus thornei*. The future recommendations focuses on how NDVI can be used to assist breeders to select tolerant cultivars, and also addresses the scope of using unmanned aerial vehicle (UAV) and the need to determine whether NDVI would be a suitable tool to test other important crops for tolerance to *P. thornei.*

4.1. Future Recommendations and Conclusions

4.1.1. Key findings of this study

This is the first known study that demonstrated that NDVI can be used to accurately phenotype the tolerance of wheat cultivars to *P. thornei* (Chapter three; Robinson et al. 2019). There is need for novel technologies, like NDVI, to be made available to wheat breeders in order to select elite germplasm, and to reduce the bottleneck that phenotyping can impose on cultivar development. The use of NDVI to study many traits is well documented in the literature suggesting that it would also be suitable to study *P. thornei* tolerance of wheat cultivars, because the symptoms of intolerance in wheat include reduced canopy cover and leaf yellowing that would affect reflectance. However, these symptoms are similar to those associated with drought and nutritional stress caused by insufficient quantities in the soil rather, than inefficient root uptake as caused by nematodes (Whish et al. 2014). The success of this study can be attributed to two main aspects namely, Greenseeker™ and the dedicated *P. thornei* field site. Firstly, the Greenseeker™ is an objective, non-destructive tool that captures data rapidly and is relatively inexpensive and requires little operator experience and training to use successfully (Figure 4.1). Secondly, this research was undertaken at a dedicated experimental field site that has been developed for *P. thornei* research and has a long history in assisting wheat breeders to select tolerant wheat cultivars. The site is managed so that confounding factors such as nutritional deficiencies and other diseases are minimised, while also implementing the best management practices for cereal production following best regional practices.

Figure 4.1 The Greenseeker™ positioned above a plot assessing wheat for tolerance to *Pratylenchus thornei* at a dedicated field research site (Source: Stephen MacDonald, USQ).

4.1.1.1. Normalised difference vegetation index (NDVI) is influenced by the population density of *Pratylenchus thornei* when assessing the tolerance of wheat cultivars

A dedicated field site for *P. thornei* research that is managed through crop rotations to produce large, damaging populations is recommended to accurately predict the tolerance of wheat cultivars (Thompson et al. 1999). There was an inverse relationship between *P. thornei* population densities and NDVI for intolerant cultivars but not for tolerant cultivars (Robinson et al. 2019). The results from three experiments with differing population densities of *P. thornei* demonstrated that higher populations (>2500/kg soil) can be used to differentiate the levels of tolerance, particularly for the moderately-tolerant cultivars (Robinson et al. 2019). In Experiment 3 where nematode populations were <1000/kg soil, the most tolerant cultivars were clustered into one group rather than in two groups in Experiments 1 and 2 where *P. thornei* populations were greater (Robinson et al. 2019). Preparation of land with uniform *P. thornei* population densities and soil type and low incidence of other constraints such as disease throughout the experimental site is essential as NDVI is influenced by any constraint that affects plant biomass. This is an important consideration for plant breeding companies so that superior tolerance levels are identified, and that only elite tolerant germplasm are advanced in breeding programs.

4.1.1.2. Indices and area under disease progress curves (AUDPC) increased the window of prediction for tolerance

Developing tolerance indices and calculating AUDPC with respect to NDVI, increased the window of prediction and the ability to predict the tolerance of wheat cultivars when the population densities of *P. thornei* are low (Robinson et al. 2019). In this case, applying indices and AUDPC with respect to NDVI provided valuable results for discrimination of the tolerance of wheat cultivars. Although indices and AUDPC with respect to NDVI were predictive of tolerance, their application may be more applicable in a research capacity because of the requirement for regular sensing over an extended period. Extensive sensing is often unfeasible in breeding programs due to the large size of screening nurseries or field experiments that are remotely located. However, high throughput phenotyping platforms that are able to screen multiple times across large numbers of cultivars may mean that determining AUDPC is more practicable for obtaining NDVI values than with the Greenseeker™.

4.1.1.3. A single time of sensing on high *P. thornei* populations was sufficient to differentiate between tolerance levels

A single time of sensing using NDVI was sufficient to differentiate *P. thornei* tolerance levels when wheat cultivars are gown on evenly high populations (Robinson et al. 2019). This is particularly important for wheat breeding programs that are selecting cultivars with improved levels of tolerance. Breeding programs are often constrained for time and resources, and rapidly acquiring phenotypic data is essential. A single NDVI reading on high *P. thornei* populations (>2500/kg soil) gave an accurate prediction of the tolerance of wheat cultivars (Robinson et al. 2019). A single reading at approximately 1000°Cd is sufficient to distinguish cultivars on their levels of tolerance to *P. thornei* (Robinson et al. 2019).

4.2. Future Recommendations

This is the first study of the application of NDVI to detect the tolerance of wheat cultivars to *P. thornei*. This study is based on 216 plots for each experiment, but for breeding companies, experiments may comprise thousands of plots (Chapman et al. 2014). Further research is required to determine the minimum plot size on which NDVI can be used to accurately predict tolerance. This is important particularly on early generation germplasm that may constrain plot dimensions due to limited seed for sowing or where there is limited land availability.

Unmanned aerial vehicles (UAV) can carry multiple types of cameras with programmable flight patterns, large field experiments with many cultivars can be efficiently measured for NDVI. Research is required to ground truth Greenseeker™ with NDVI acquired by UAV for *P. thornei* tolerance, and how well this correlates with grain yield. Condorelli et al. (2018) found that UAV-based platforms gather rapid, detailed NDVI measurements that were able to accurately identify specific QTL variation associated with drought tolerance, and of higher accuracy compared to ground based Greenseeker™ measurements. In addition, there are novel technologies other than NDVI that may be able to identify *P. thornei* tolerance. Thermal imaging, multispectral and hyperspectral cameras can also be used on UAV-based platforms (Araus & Cairns 2014). These technologies are available commercially and have been used to study other abiotic and biotic stresses of crops.

Pratylenchus neglectus co-exists with *P. thornei* in 27% of fields in the subtropical grain region of eastern Australia (Robinson et al., 2014; Thompson et al., 2010). In the Eyre Peninsula of South Australia, *P. neglectus* reduced the yield of intolerant wheat cultivars by 27% (Taylor et al., 1999) and in the subtropical grain region of Australia, ~70% of the 44 wheat cultivars recommended have a medium–high risk of yield loss (Lush 2018). Therefore, there is need for commercial cultivars that are tolerant to both *P. thornei* and *P. neglectus*. Knowing that NDVI is capable of predicting tolerance to *P. thornei* raises the question of whether this technology can also be applied to phenotype cultivars where both species infest the same field. In addition, the subtropical grain region of eastern Australia also grows other crops that have varying levels of tolerance amongst their cultivars including chickpea (Reen et al. 2014; Rodda et al. 2016; Thompson et al. 2008) and mung bean (*Vigna radiata*) (Owen et al. 2014; Thompson et al. 2008). Research is required to determine if NDVI could be used to predict tolerance in these other crops.

4.3. Conclusion

This study demonstrated that NDVI can be used to predict the tolerance of wheat cultivars to *P. thornei.* There is an opportunity for wheat breeders to use NDVI as a tool in their programs for selection of tolerance of cultivars and lines to *P. thornei* provided the experimental field site has damaging populations of *P. thornei* with minimal influence from other diseases or nutritional and environmental stresses. Greenseeker™ is readily available from NTech and comes fully equipped to readily measure NDVI. Smaller–sized instruments are also available that capture NDVI. There is also scope for this technology is to be mounted

to UAVs or for multiple sensing units to be attached to field equipment thereby increasing the potential rate of data capture. A combination of breeding for genetic tolerance and selecting tolerance using NDVI will help wheat breeders develop cultivars that do not lose yield when grown in fields with damaging levels of *P. thornei*.

References

The list of references below relates to the literature cited to prepare Chapters one, two and four of this thesis. An additional reference list is provided on page 52 of this thesis and this list relates to the literature cited to prepare the journal article that constitutes Chapter three of this thesis.

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