final report

Project code: B.PSP.0005

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Date published: 30 August 2017

PUBLISHED BY
Meat and Livestock Australia Limited
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NORTH SYDNEY NSW 2059

Managing soil-borne root disease in sub-clover pastures

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Soilborne root disease has long been suspected to be the main cause of poor productivity and lack of persistence of subterranean clover pastures across southern Australia. Tests across south-west, southern and south-east Australia – home to 29 million hectares of sub-clover pasture – found almost all samples suffered root disease. More than 80% of samples suffered extreme levels of disease. After an initial three years of glasshouse and plot trials, during 2015 and 2016 research moved to large-scale field trials carried out at 16 field trial sites (two in each state each year — WA, SA, Vic, NSW). In summary, the most cost-effective on-farm chemical treatments and cultural practices for control of root disease in sub-clover pastures include: (i), soil cultivation to reduce pathogens and subsequent root disease impact on productivity for several years; (ii), when registered, fungicide seed coating prior to replanting, or fungicide sprays on regenerated pastures, particularly fungicides that boost plant immunity; (iii), improving soil and plant nutrition to enable better root and shoot growth even when disease is severe; (iv), choosing varieties that perform best in your area; (v), sowing a mixture of clovers can be a good insurance policy; and, (vi), use a rotational grazing system that allows more plant growth that in turn improves root development even where disease is severe.

While no silver bullet was discovered to cure soil-borne diseases, the research reinforced that long-term success will rely on identifying resistant and field-tolerant sub-clover varieties – which is likely to boost productivity up to 4–5 fold. In the interim, focussing on pasture health first and foremost is the best approach — healthy plants best tolerate disease. To this end, this project has identified a range of flexible management options producers can employ to minimise the impact while they wait for resistant varieties to be delivered to market. In addition, producers now have access to rapid diagnostic testing through the Predicta B test to help producers establish the main causes of root disease on their property and support strategic management decisions. Overall outcomes for producers from this project, across southern Australia for farming systems involving sub-clover pastures, include: availability of effective, practical and flexible chemical and cultural options to reduce the losses during autumn-winter from soil-borne root disease; availability of meaningful Predicta-B tests to predict pathogens and their incidence; knowledge of true losses from soilborne pathogens; and an understanding of the influence of environmental factors on root disease epidemics. It is clear from these studies that while locating and developing further improved host resistance offers the best long-term and most cost-effective way to curtail future losses from damping-off and root disease occurring in subterranean clover, this critical benefit for meat and other livestock producers will only occur if there is new funding support for such an approach to be delivered.

Pathology skills and expertise have been greatly enhanced and consolidated, particularly in relation to the technical and knowledge capacity on pasture plant pathology - soil biology. This skills have now been secured for current and, providing there is ongoing industry support, future pathology requirements of the livestock industry.

Executive summary

This research was undertaken to increase feed productivity and sustainability for producers by targeting improved management practices associated with grazing systems that effectively manage soil-borne diseases to enhance production efficiency and profitability for all livestock producers across southern Australia. Severe pre- and post-emergence damping-off and root disease in subterranean clover caused by *Pythium, Phytophthora, Aphanomyces,* and *Rhizoctonia*, plus a range of other pathogens such as *Fusarium*, *Phoma* and *Cylindrocarpon*, together constituted the over-riding biotic constraints to productive subterranean clover pastures across southern Australia. It was evident that many subterranean clover pastures have and continue to fail to persist or be productive, particularly in high rainfall regions >500mm in situations where annual losses up to 40-50% occur. Further, as soil-borne pathogens operate in various interacting combinations and complexes with each other to maximise damage to plants, understanding these interactions was critical both to interpreting molecular DNA tests and development of successful control strategies. There was also a need for further quantification of impact of soil-borne diseases to address the need for a more relevant/recent reassessment of incidence and impacts (losses and economic importance) of major soil-borne diseases in subterranean clover across southern Australia. Such information is fundamental both to defining regions and situations of highest priority for management intervention and research priorities for subterranean clover pastures. Wide-ranging field, glasshouse, controlled environment and laboratory investigations were undertaken.

The **causes, losses and impacts of soilborne root disease studies** found that the main pathogens, *Phytophthora, Pythium*, *Rhizoctonia* and *Aphanomyces,* generally occurred as complexes of 2-4 different pathogens. In terms of overall losses, soilborne pathogens reduced germination by up to 70%; reduced root systems up to 90%; reduced shoot systems up to 85% and were the overriding reason for why subterranean clover in severely affected pastures does not persist. Individually, *Pythium* reduced germination by up to 60% and up to a 50% loss in plant productivity; *Phytophthora* reduced germination by up to 25% and caused a 4.5 fold loss in plant productivity; *Rhizoctonia* reduced germination by up to 90% and caused up to 75-80% loss in shoot and root production; *Aphanomyces* reduced germination by up to 14% and caused up to 50-55% loss in shoot and root systems; and when there were two or more pathogens, losses up to 100% germination failure and total loss of root and shoot production occurred. Fungicide seed treatments increased germination by up to 30%; fungicide spray treatments reduced root disease up to 30%; addition of a complete fertilizer application increased shoot productivity up to 1.5 fold; cultivation increased productivity up to 75%; and, most spectacularly, more disease-resistant or disease-tolerant varieties showed increased plant productivity of up to 4-5 fold. 'Environmental factors' such as soil moisture, type, temperature and nutrition determined severity and impact of soilborne root diseases; but the specific roles of individual factors varied depending upon the prevailing pathogen(s) and as detailed later in this report.

Seed dressing and spray fungicides studies against *Pythium irregulare* and *Aphanomyces trifolii* showed the most promising fungicide treatment was using Metalaxyl and this holds overall the best prospects for control of soilborne disease caused by oomycetes. Phosphorous acid (Phos-Jet) was the best spray chemical for *Aphanomyces trifolii* root disease. While there were no completely effective spray chemicals against *Pythium irregulare* or *Rhizoctonia solani* root diseases; in contrast, some farmer test strips [e.g., with phosphorous acid (Phos-Jet)] have been very encouraging and on occasions given spectacular increases in subterranean clover productivity.

Species composition studies on the effects of the relative proportions of subterranean clover to annual ryegrass showed that the levels of tap and lateral root disease on subterranean clover and also their shoot dry weights were affected by the different sward compositions. In particular, tap

and lateral root disease was most severe when subterranean clover percent composition was as low (e.g., 20% subterranean clover and 80% ryegrass). When subterranean clover composition was 100% (i.e., no ryegrass at all), shoot dry weight per plant for subterranean clover was the greatest and the level of tap and lateral root disease much less. This is the first study to demonstrate the reasons for why, as observed in the field, the level of root disease greatly increases and the rate of decline in deteriorating pastures rapidly accelerates as the subterranean clover content diminishes.

Cultivation studies in soil cores from five farms demonstrated the significant role of cultivation in ameliorating severe tap and lateral root disease severity and in promoting dry root and shoot weights. Tap and lateral root disease was significantly less severe in cultivated soils than in non-cultivated soils and dry root and shoot weights were significantly greater in cultivated than in non-cultivated farmer soils.

Field studies on cultivation showed that cultivation greatly reduces tap and lateral root disease and significantly increases germination, nodulation, root and shoot dry weights across three subterranean clover varieties. Tap and lateral root disease were strongly negatively correlated with nodulation. Cultivation offers significant prospects not only for utilization as an effective option to control root disease but also for significantly improving nodulation.

Simulated grazing studies showed that grazing, particularly continuous grazing, leads to more severe tap and lateral root disease, poorer nodulation and smaller plants in terms of both roots and shoots. Tap and lateral root disease were most severe, nodulation poorest and dry root and shoot weights lowest when subterranean clover was under intensive grazing in the presence of and affected by *Pythium irregulare*. Reducing grazing pressure offers potential for significantly increasing subterranean clover pasture productivity during the critical autumn feed-gap period from decreased root disease and increased nodulation associated with less intensive grazing of root-rot-affected subterranean clover pastures.

Studies on the influence of rhizobium on subterranean clover root disease showed that rhizobium application via seed or as a granule treatment significantly increased nodulation. However, rhizobium application did not increase either germination rate or shoot dry weight; and when applied as seed treatment it actually decreased both germination rate and shoot dry weight. The presence of the root rot pathogens *Pythium irregulare* and *Rhizoctonia solani* significantly reduced nodulation indices, but the extent of this depended upon the pathogen and the subterranean clover variety.

Studies on *Phytophthora* interactions with environmental factors showed seedling emergence was significantly affected by moisture, soil type, temperature and cultivar. The level of rotting of tap and lateral roots was significantly affected by nutrition, soil type, temperature and cultivar. There were significant interactions involving temperature, moisture, soil type and cultivar, with cultivar resistance, high moisture, high or medium temperature, high nutrition and sand soil all contributing towards less pre-emergence damping-off and tap and lateral root disease and to greater clover productivity. Subterranean clover cultivar host resistance was critical for reducing disease severity and increasing productivity even when favorable environmental conditions for severe disease occurred. In the presence of *Phytophthora clandestina,* the most resistant cultivar, Seaton Park, performed best under a high temperature, high nutrition and high moisture combination, but showed lower productivity under conditions of low nutrition or lower temperature, even when moisture level was high. In contrast, less resistant Riverina and Meteora had less disease and greater productivity under low moisture conditions less favorable for *Phytophthora clandestina*. Findings reflect field observations that pre-emergence damping-off and root disease from *Phytophthora clandestina* in subterranean clover is particularly severe when moisture level is high.

Studies on *Aphanomyces* interactions with environmental factors showed how environmental explanatory variables (viz. temperature, soil type, moisture, nutrition) as well as variety, influence the severity of disease and consequent forage persistence and productivity. Relationships were modelled using linear and generalised linear models and boosted regression trees. Linear modelling highlighted complex relationships between environmental variables and each dependent variable (emergence, tap and lateral root disease, dry shoot and root weight). All environmental variables produced significant interaction and/or main effects within each dependent variable. Boosted regression trees supported the complex nature of relationships in linear models, with temperature and either soil or variety most, and nutrition least, influential. Heat maps showed higher disease severities for low temperatures. Lowest tap root disease severities were under high temperatures, while lowest lateral root disease severities were under medium or high temperatures, low moisture, and in sand-based soil. This is the first study to apply these modelling approaches to understanding complex interactions between environmental factors with a soilborne forage legume disease. Modelling provided new understanding into how fluctuating soil moisture, temperature, and soil type and variety, determine the extent of damping-off and disease severity and consequent subterranean clover forage persistence and productivity. Studies highlighted how warming temperatures and drying climate associated with climate change will likely reduce the future impact and importance of this and other soilborne oomycete diseases of forage legumes favoured by cold temperatures and wet and waterlogged conditions.

The **studies on *Rhizoctonia* interactions with environmental factors** showed environmental factors significantly affected the severity of damping-off, root disease and root and shoot productivity. Damping-off was at or close to 100% at the two cooler of the three temperature regimes. At the warmest of the three temperature regimes (22/17°C), significant numbers of subterranean clover plants germinated and survived, and germination, dry root and shoot weights all increased conversely with decreased tap and lateral root disease under higher moisture, better nutrition and under 'heavier' soil conditions. Findings demonstrate how variations in environmental factors like temperature in particular, but also soil type, nutrition, moisture, individually and interacting, have profound effects on the expression and severity of Rhizoctonia damping-off and root disease and the consequent productivity of subterranean clover forages. Findings explain the severe devastation to subterranean clover pastures observed in the presence of this pathogen when cool seasonal conditions occur across the critical autumn feed-gap period; especially under relatively drier soil conditions and in nutritionally impoverished sandy soils where there is little competition from other soil microbes.

Studies on *Pythium* interactions with environmental factors showed how environmental factors (viz. temperature, soil type, moisture and nutrition) as well as variety, influence the severity of damping-off and root disease as well as productivity in subterranean clover under challenge by this pathogen. Relationships were statistically modelled using linear and generalised linear models and boosted regression trees. Modelling found complex relationships between explanatory variables and the extent of Pythium damping-off and root rot. Linear modelling identified high-level (4 or 5-way) significant interactions for each response variable (dry shoot and root weight, emergence, tap and lateral root disease index). Furthermore, all explanatory variables (temperature, soil, moisture, nutrition, variety) were found significant as part of some interaction within these models. A significant five-way interaction between all explanatory variables was found for both dry shoot and root dry weights, and a four way interaction between temperature, soil, moisture, and nutrition was found for both tap and lateral root disease index. A second approach to modelling using boosted regression trees provided support for and helped clarify the complex nature of the relationships found in linear models. All explanatory variables showed at least 5% relative influence on each of the five dependent variables. All models indicated differences due to soil type, with the sand-based soil having either higher weights, greater emergence, or lower disease indices; while lowest weights and

less emergence, as well as higher disease indices, were found for loam soil and low temperature. There was more severe tap and lateral root rot disease in higher moisture situations.

Studies on host resistance showed that varieties Guildford-D, Campeda, Urana, and Antas, along with Dalkeith, Riverina and York are the first varieties identified with resistance to *Aphanomyces trifolii*. However, there were strong indications of multiple pathogen races with different host genes controlling resistance to damping-off *vs* tap *vs* lateral root disease from *Aphanomyces trifolii*.

DNA qPCR assays have been designed by SARDI as part of this project for the specific detection of *Aphanomyces trifolii*. The one with better sensitivity for the detection of *Aphanomyces trifolii* and that did not detect the related species *Aphanomyces euteiches* has been successfully developed and field validated such that meaningful application of DNA Predicta B test findings is now possible for the first time for subterranean clover pastures across Australia. *Aphanomyces trifolii* clearly constitutes a much more widespread and serious threat to subterranean clover productivity than previously thought and DNA tests showed a strong relationship between amount of DNA detected and level of disease severity by *Aphanomyces trifolii*. However, until this project, this is not the case for other soilborne diseases of pastures as there previously was no comparative data on the interpretation of DNA test results where environmental factors vary widely between different locations and situations across southern Australia. Definition of relationships between the expression of disease on plants with the associations of particular soil-borne pathogens and how environmental influences determine the outcomes of these interactions and relationships is essential both to define both the different symptom expressions on roots and the relative impacts of different individual pathogens and pathogen complexes in different regions and across different seasons; and is the only sound basis for interpreting molecular DNA tests for producers.

Critical evaluation studies of DNA *versus* disease levels in relation to *Pythium* and *Rhizoctonia*, showed that the quantity of either pathogen in the soil [expressed as DNA weight (pg/g soil)] when using subterranean clover composition at 20% and 100% subterranean clover by "Predicta B" significantly differed in relation to differences in pasture composition, pathogen, subterranean clover variety, and also their interactions. For example, tap and lateral root disease indices and DNA weight were lower in 100% than 20% subterranean clover composition for all tested varieties. These relationships were variety-dependent, for example, Seaton Park and Riverina suffered lest *Pythium* root rot and contained least *Pythium* DNA weight at 10% subterranean clover than at 100% subterranean clover composition. However, it was encouraging that both *Pythium* and *Rhizoctonia* DNA weights were positively correlated with tap and lateral root disease. Further, while it was particularly encouraging that "Predicta B" tests gave about 70 to 80% indication for *Pythium* root disease it was only 40% for *Rhizoctonia*. The percent subterranean clover in the pasture also strongly influenced the level of relevance of the Predicta B tests. For example, at 10% subterranean clover, root rot disease indices were only about 10-15% when DNA weight was around 500 pg /g soil for *Pythium* but root rot disease indices were 60-80% when DNA weight was only about 100pg /g soil for *Rhizoctonia*. Therefore, it is clear not only that for the level of root disease from *Rhizoctonia* is determined by other factors than just its Predicta B DNA weight, but that even a relatively low 100pg DNA weight is sufficient for *Rhizoctonia* to cause severe root rot.

National Field trials across both 2015 and 2016 showed that the main pathogens were *Phytophthora clandestina, Pythium* spp., especially *P. irregulare, Rhizoctonia solani* and *Aphanomyces trifolii*; generally complexes of 2-4 different pathogens; that soilborne pathogens reduce germination by up to 70%, reduce root systems up to 90%, reduce shoot systems up to 85%, and that subterranean clover in severely affected pastures does not persist, particularly where there are two or more pathogens as losses up to 100% germination failure and total loss of root and shoot production can occur in such situations. Fungicide seed treatments increased germination by up to

30%; fungicide spray treatments reduced root disease up to 30%; complete fertilizer application increased shoot productivity up to 1.5 fold; cultivation increased productivity up to 75%; and, finally and most importantly, more disease-resistant or disease-tolerant varieties increased plant productivity up to 4-5 fold. Clearly, identification of cultivar resistances to each of the pathogens offers the best and most cost-effective, long term, and economically feasible means of managing damping-off and root diseases in subterranean clover across southern Australia. However, to achieve this universally for producers' on-farm, resistances and tolerances first need to be identified across subterranean clover varieties and in naturally occurring ecotypes to each of the pathogens and will only be achieved in the future if there is future funding support for the disease screening work that needs to be undertaken to ensure this, otherwise producers will never capture this most outstanding of potential productivity improvement benefits.

Pathology skills and expertise for pastures currently, and if there is ongoing industry support into the future, has been secured by the appointment of a post-doctoral researcher (Dr Ming Pei You) to this project from its commencement. This appointment and training has enhanced and consolidated the technical and knowledge capacity on pasture plant pathology - soil biology available for current and potentially future research and extension in southern Australian pasture systems. In addition to this MLA project, Dr You also closely collaborated and work closely with two Producer Site Research Projects in South Australia and one in Western Australia, attended MLA and many other producers field days and project meetings. The outcome is a highly skilled and knowledgeable pastures plant pathologist to meet both current and, if ongoing funding support is available, also future pathology requirements of the livestock industry.

In summary, the most cost-effective on-farm chemical treatments and cultural practices for control of root disease in sub-clover pastures, developed from this MLA research project, include: cultivating soil to reduce pathogens and subsequent root disease impact on productivity for several years; applying a registered fungicide seed coating prior to replanting, or fungicide sprays on regenerated pastures; ensuring adequate soil and plant nutrition, through strategic fertiliser management, to enable better root and shoot growth even when disease is severe; choosing varieties that perform best in your area; and, using a rotational grazing system that allows more plant growth and in turn improves root development, even where disease is severe. In terms of the future, it is clear from these studies that locating and developing further improved host resistance offers the best long-term and most cost-effective way to curtail future losses from damping-off and root disease occurring in subterranean clover. However, this critical benefit for meat and other livestock producers will only occur if there is new funding support for such an approach to be delivered to producers.

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1 Background

1.1 Why was this work undertaken

A Soil Biology Workshop held in February 2012 highlighted both the need and opportunities to curtail current severe losses from soil-borne diseases such that legume based pasture productivity across southern Australia is greatly increased. It was agreed at this workshop that this would best be achieved by focusing on the managing soil-borne diseases in the main legume component, subterranean clover, and hence the focus on subterranean clover in this application. This proposal addresses the producer outcome to "Increase feed productivity and sustainability". Specifically, this project targets improved management practices associated with grazing systems that effectively manage soil-borne diseases to enhance production efficiency and profitability for all livestock producers across southern Australia.

Pre-emergence damping-off in subterranean clover, such as caused by *Pythium irregulare,* and pre- and post-emergence damping-off combined with severe root disease in subterranean clover pastures, such as caused by *Phytophthora clandestina,* constitute the over-riding biotic constraints to productive subterranean clover pastures across southern Australia. A range of other pathogens are also very important and damaging, including one or more species of *Aphanomyces, Fusarium*, *Phoma* and *Cylindrocarpon*, with *Aphanomyces trifolii* recently being highlighted as pathogen of corresponding importance to *Pythium* and *Phytophthora* (O'Rourke et al. 2010). However, soil-borne pathogens operate in various interacting combinations and complexes with each other to maximise damage to plants, and understanding these interactions is critical both to interpreting molecular DNA tests and development of successful control strategies.

There was also a need for further quantification of impact of soil-borne diseases. Except for the studies by Simpson et al. (2011) and O'Rourke et al. (2009), the impact and related causes of severe disease have been largely ignored for some decades. This is despite field observations indicating that the situation in terms of relevant pathogens (e.g., addition of *Aphanomyces trifolii*) and disease expression has changed dramatically over recent decades highlighting the need for a more relevant/recent reassessment of incidence and impacts (losses and economic importance) of major soil-borne diseases in subterranean clover across southern Australia. Such information is fundamental both to defining regions and situations of highest priority for management intervention and research priorities for subterranean clover pastures. It is evident that many subterranean clover pastures have and continue to fail to persist or be productive, particularly in high rainfall regions >500mm in situations where annual losses of 40-50% or more occur.

Further, and prior to this project, there clearly were opportunities for managing soil-borne diseases from altered farm practices, as follows in relation to application of chemicals, changes in cultural practices and from altering plant nutrition and these are briefly outlined as follows.

Chemicals: There exist significant but rarely exploited opportunities for utilizing low cost chemical seed treatments to ensure successful stand establishment when sowing pastures (see MLA reports of Barbetti et al. 2006b and Barbetti and Jones 2011; Barbetti et al. reviews of 2006a, 2007). For example, Taylor et al. (1985c) demonstrated increases in Victoria from disease control by fungicides of nearly 60% in late autumn and more than 95% in mid-spring in subterranean clover herbage production. There are several cheap potential fungicide treatments that target a wide range of soil-borne pathogens associated with damping-off that would be appealing for widespread uptake and usage by producers. These not only include simple and cheap fungicidal seed treatments that could ensure successful stand establishment of pasture legumes, but also foliar applications to protect roots in established seedlings, together ensuring and/or re-establishing pastures that persist. If

successful, benefits would be extensive particularly in the high rainfall regions (>500mm), where pre- and post-emergence damping-off by *Pythium* and *Phytophthora* alone currently result in pastures sometimes failing to persist or be productive and where annual losses of 40-45% or more occur in the most disease prone areas.

Farm cultural practices: There are several potential yet rarely exploited opportunities for utilizing manipulation of cultural practices for soil-borne disease management in pasture legumes (see MLA reports of Barbetti et al. 2006b; Barbetti and Jones 2011; Barbetti et al. reviews of 2006a, 2007). While the low level of utilisation of cultural management measures by meat producers arises partly from lack of awareness of their benefits, this is also partly a consequence of additional measures not having been identified or evaluated for their potential benefits. This is despite historical information clearly suggesting roles for practices such as controlled grazing (e.g., can reduce losses from *Phytophthora* root rot in subterranean clover by up to 55%) and soil disturbance (e.g., can greatly reduce levels of damping-off and root disease in subterranean clover for up to three years).

Nutrition: Soil fertility affects the severity of disease on subterranean clover, by influencing root physiology and host resistance. Better utilization of applied fertilizer and vastly improved management of root diseases of subterranean clover have been demonstrated from utilising nutrient amendments. There is significant potential for improved management of major soil-borne pathogens of subterranean clover from correction of common nutrient deficiencies. This is highlighted by current research at UWA that demonstrated reductions in root disease of the order of 27-45% were obtainable from improving the nutritional status of soils under subterranean clover pastures. There are significant opportunities for such practices to be integrated into a sustainable approach to successfully manage root disease in subterranean clover pastures (O'Rourke et al., unpubl.).

In addition, environnemental influences on soilborne pasture diseases, such as rainfall (soil moisture) and soil temperature have a marked effect on both the root disease severity of subterranean clover from individual pathogens and on the interactions that occur between different root pathogens. Understanding differences in root disease symptoms and severity that occur between different areas across southern Australia as a consequence of fluctuating soil temperature and moisture conditions (particularly from influence of climate change) is fundamental to relevant interpretation of molecular 'Predicta B' DNA tests. Environmental differences across southern Australia determine distribution of the most important soil-borne subterranean clover pathogens such *Phytophthora* and its races (You et al. 2006) and *Pythium* (MJ Barbetti unpubl.). Environmental factors are major factors affecting both the root disease severity in subterranean clover from individual pathogens and the complex interactions that occur between the different root pathogens.

So, why address soil-borne disease constraints to pasture productivity? Mitigating soil biological constraints to pasture productivity will result in very significant increases in pasture persistence, pasture production both in quantity and reliability of feed production, and nitrogen fixation. Further, Barbetti (2007) highlighted that reducing the impacts of soil-borne disease enhances efficient utilisation of pasture inputs; pasture persistence; pasture palatability; plant nutritional value; seed set and viability; and pasture composition in favour of legumes and enhances resilience to grazing. Please note that while it is acknowledged that a desirable long term approach to minimise losses is by understanding and deploying the mechanisms by which pasture plants resist infection and colonisation of plant roots, and by improving our understanding of the genetics of resistance in desirable pasture species, such an approach was a much longer term proposition and outside the scope of this project.

Finally, this project also offered the opportunity to secure future pathology skills for pastures as, at that time, Dr Barbetti was the last remaining full time pasture plant pathologist in Australia with recognised expertise in soil-borne pasture diseases and with combined competency skills across the range of fungal and oomycete soil-borne pathogens involved. This project offered the unique opportunity to train a new pasture pathologist by way of a position as the main researcher on this project. In this way, existing pasture pathology knowledge skills could be captured and transferred in the most cost-effective way (i.e., no additional cost to MLA).

1.2 Significance for industry

Crucially, meat producers inherently face critical feed shortage in across the autumn-winter months, coinciding with the main soil-borne pathogens attack and consequent damage from massive pre- and post-emergence damping-off and seedling root disease, inflicting well documented losses 40-45% or more in the most disease prone areas; consisting up to 30-90% of seedlings failing to emerge, 10-40% of surviving seedlings succumbing to post-emergence, and with a 35-70% reduction in growth of survivors (see Barbetti et al. 2005, Barbetti and Jones 2011, Barbetti et al. reviews of 2005, 2007 and MLA report of 2005 by Barbetti et al.). This feed shortage is likely the major constraint to increasing livestock production and profitability. Any increase in extra carrying capacity developed during this autumn-winter period from improved disease management will not only improve overall carrying capacity but also reduce the current high degree of uncertainty faced by meat producers in being assured of sufficient feed over this period. Perhaps of greatest significance is the well documented rapid deterioration and lack of persistence of subterranean clover pastures across southern Australia following attack by soil-borne pathogens (Johnstone and Barbetti 1986; Barbetti et al. 2005, Barbetti and Jones 2011, Barbetti et al. reviews of 2006a, 2007 and MLA report of 2006b by Barbetti et al.). The combination of soil-borne disease particularly targeting and restricting seedling emergence and growth, along with poor performance of surviving plants throughout the remainder of the season (O'Rourke et al. 2009), results in severe decline in seed banks depriving pastures of the ability to persist, further increasing both the risks and opportunity costs and removing any incentive to invest in pasture improvement.

Importantly, Simpson et al. (2011) used field-based plant bioassays at 17 locations across southern Australia to show that 9-93% of subterranean clover seedlings failed to emerge at 14 locations, and with post-emergence losses ranging up to 32% and moderate to severe root disease on any surviving plants at all sites. Prior to that research, there were many studies to demonstrate the impact of soil-borne diseases across southern Australia where root rot has seriously adversely affected the production from subterranean clover pastures over at least the past five decades (e.g., Johnstone and Barbetti 1986; Barbetti et al. 2005, Barbetti and Jones 2011, Barbetti et al. reviews of 2006a, 2007 and MLA report of 2006b by Barbetti et al.). Conservatively, the combined annual losses from major soil-borne diseases of legume based pastures across southern Australia likely exceed $400 m annually, the majority of this being in relation to subterranean clover pastures (Barbetti, unpubl.).

1.3 Overarching aims

This project is built upon a solid national focus, one that will provide the critical knowledge that meat producers, extension consultants, agronomists and researchers need to manage soil-borne diseases in subterranean clover pastures and in order for them to be able to increase their sustainability, productivity and profitability. It will also eliminate the current production uncertainly, by providing producers with a range of management options that ensure certainty of success in managing soil-borne disease threats. This will not only ensure sustainable pastures that are reliable and persist, but that they have high productivity across the critical autumn-winter period. This will also ensure ongoing productivity from having pastures that not only persist throughout an individual season but across multiple years. In particular, it will provide the practical pasture management techniques, giving meat producers flexible options to curtail current losses in pasture productivity caused by soil-borne root pathogens and thereby increase farm profitability with existing pastures. Finally, this project aimed to ensure that the necessary pathology skills and expertise for dealing with soil-borne disease threats to the feedbase are maintained for future generations of producers.

The overarching aims of this project were to:

- Determine the farm management factors that influence the expression of soil-borne root diseases in subterranean clover pastures.
- Evaluate practical management techniques aimed at reducing the pasture productivity loss during autumn-winter induced by soil-borne root disease.
- Characterise the relationships between the expression of disease and the associations of soil-borne pathogens with affected plant roots.
- Broaden the quantification of pasture productivity constraints induced by pasture soil-borne root pathogens across agro-ecological zones.
- Develop molecular assays to fill gaps in experimental tools needed to adequately conduct research into, and monitor, soil borne diseases in pastures.

2 Project objectives

2.1 Specific objectives of this project

1. Determine the farm management factors that influence the expression of soil-borne root diseases in subterranean clover pastures.
2. Evaluate practical management techniques aimed at reducing the pasture productivity loss during autumn-winter induced by soil-borne root disease.
3. Characterise the relationships between the expression of disease and the associations of soil-borne pathogens with affected plant roots.
4. Broaden the quantification of pasture productivity constraints induced by pasture soil-borne root pathogens across agro-ecological zones.
5. Develop molecular assays to fill gaps in experimental tools needed to adequately conduct research into, and monitor, soil borne diseases in pastures.
6. Secure pathology skills and expertise for pastures into the future.

3 Methodologies

3.1 Glasshouse studies

3.1.1 Fungicide seed dressings

Fungicides were diluted to the recommended application concentration. Seeds were coated by placing into a glass beaker and thoroughly mixing to coat seeds evenly. All application rates chosen were the application rates recommended by the manufacturer for similar pathogens on other crop species as, except for metalaxyl which is registered as Apron, none of these test chemical treatments are registered for use on pasture legumes. Details of chemical tested are shown in Table 1 below. 1g of seeds required 300 µl of solution to wet/coat thoroughly, such that each fungicide was constituted to a total volume of 300 µl (using distilled water as needed for dilution).

Table 1. Fungicides and their application rates.

Fungicide Name	*Application rate*	*Amount per g seeds*
Dividend (active ingredients: 92 g/L Difenoconazole + 23 g/L Metalaxyl-M)	65ml/100kg	0.65µl
Jockey (active ingredient: Fluquinconazole 167g/L a.i.)	4.5L/ton	4.5 µl
Metalaxyl （350g/L）	5ml a.i./kg	14.3µl
Phos-Inject 200 (200g Phosphorous acid/L）	10g a.i./L	15 µl
Previcur (active ingredient: Propamocarb 600g a.i./L)	7.2g/kg	12 µl
Rancona Dimension (active ingredients: Ipconazole + Metalaxyl + N-Methyl-2-pyrrolidone)	160ml/100kg	1.6 µl
Rovral (active ingredient: iprodione 250g a.i./L)	800ml/ton	0.8 µl
Syn-A16874F (active ingredients: Sedaxane + Difenoconazole + Metalaxyl)	90ml/100kg	0.9 µl
Thiram 800	1g a.i./kg	1.25 mg

Four varieties of subterranean clover were used in these tests, viz. Woogenellup, Riverina, Seaton Park and Meteora. A single isolate was used for *P. irregulare* but a mixture of different isolates of varying pathogenicity of *Aphanomyces trifolii* were used (to avoid any pathotype or race interactions across different varieties as there had been suggestions from earlier work by O'Rourke 2009). In total there were 10 treatments (9 fungicide treatments plus a nil treatment control comparison) x 2 pathogen treatments (*A. trifolii* and a nil control comparison) x 4 clover varieties x 8 replications; totalling 640 pots arranged in a complete randomized design. The experiment was conducted in a controlled environment room maintained at 12/12 dark/light and at a temperature of 13/17^{o}C, respectively.

Inoculum of *P. irregulare* or *A. trifolii* was prepared using sterile millet seeds (*Panicum miliaceum*); by soaking 200g millet seeds in de-ionised water in a 1 L flask for 12 h, excess water drained and then autoclaved at 121°C for 20 minutes on three consecutive days. The sterile millet seeds in the flask were inoculated with 1cm squares of colonies of *P. irregulare* or *A. trifolii* on agar cut from the leading edge of colonies. Using the methods of O'Rourke 2009 and O' Rourke *et al.* 2012, inoculated millet seeds were then incubated on a laboratory bench at 22°C for 14 days and shaken vigorously every second day to ensure even pathogen colonisation of the millet seeds.

The fungal colonised millet seeds were applied at a rate of 0.5% (w/w air dried soil) as a layer 4 cm beneath the soil surface. Clover seeds were sown into pasteurised UWA Potting mix at 2 cm depth in 6 x 6 cm square pots, with 5 seeds per pot. The pasteurised soil mix was prepared using aerated steam for 90 minutes at 70^{0}C. As varieties tested can vary in relation to germination rate, all disease

affects in relation to seedling survival were assessed against respective 'nil disease' controls. Pots are watered daily with de-ionised water to field capacity.

Emergence rate was recorded one week after sowing. Four weeks after sowing, the plants were removed and the roots washed free of soil under running tap water. Tap and lateral roots were rated individually for their disease severity using a 0 to 5 scale modified from Barbetti and MacNish (1984) where: 0, = root completely healthy; 1, = slight root rot (not exceeding 25% of root tissue affected by root rot); 2, = root rot >25-50% of root tissue affected; 3, = root rot >50 - 75%; 4, = severe root rot >75%; 5, = root rot off, plant died.

3.1.2 Fungicide spray treatments

Prior to conducting experiments looking at the effect of foliar fungicide sprays on subterranean clover damping-off and root rot caused by *P. irregulare* or *A. trifolii* or *P. clandestina* potential spray fungicide treatments for control of seedling damping-off and root disease, four fungicide spray treatments (and a nil treatment control) were highlighted as having the best potential benefits. These were:

- Phos-Inject 200 (200g phosphorous acid/L, application rate 10g a.i./L);
- Metalaxyl (350g/L, application rate 5ml a.i./kg or L);
- Propamocarb (600g a.i./L, application rate 7.2g/kg seed or L);
- Rovral (250g a.i./L, application rate 800ml/ton or 0.8ml/L)
  (Along with a 'nil disease' comparison treatment with deionised water).

Testing with these fungicide treatments involved four subterranean clover varieties [viz. Meteora, Riverina, Seaton Park and Woogenellup]. Pasteurized UWA potting mix was used for growing subterranean clover. Each pathogen was colonized on sterilized millet seed as inoculum was applied at a rate of 0.5% (w/w air dried soil) in a layer 4 cm beneath the soil surface. Clover seeds were sown into inoculated potting mix 2 cm depth in 6 x 6 cm square pots, 5 seeds per pot. Pots were watered daily to field capacity. Fungicide treatments were applied as foliar sprays on fully expanded cotyledons (about one and ha weeks after sowing), with harvesting of plants for assessment of disease levels and plant productivity made four weeks later and as described above. Roots were washed under running tap water and root disease levels were recorded. Plant shoots and roots were separated and oven dried at 60°C for 3 days, then shoot and root dry weights were recorded.

3.2 Cultural control treatments

3.2.1 Cultivation – glasshouse

Intact soil cores (6 cm in diameter x 10 cm depth) were collected from subterranean clover pastures on 5 farms widely dispersed across south west of Western Australia, a region known for its inherent severe soilborne root disease, and areas known to have severe root disease in other surveys (e.g., Ma *et al*., 2008) were targeted. Cores were maintained for approximately 2 weeks in a controlled environment room held at 13℃/18℃ (night/day) under 12hr/12hr (day/night) light cycle but allowed to dry out to kill any growing plants. Soil in cores to be used in simulated cultivation treatments was then mixed by hand in each core using a steel knife to a depth of 5 cm to simulate cultivation treatment while the remaining cores were kept intact and undisturbed for uncultivated comparison treatments. Five scarified subterranean clover seeds were sown into each core. Subterranean clover varieties Meteora, Riverina, Seaton Park and Woogenellup were used. Plants were maintained at the same 13℃/18℃ conditions as this mimic temperatures commonly seen in the field in Western Australia during May–August when root disease is prevalent in subterranean clover pastures (Barbetti, 1991).

Plants were harvested at 4 weeks after sowing, roots washed clean, and assessed for disease severity on tap and lateral roots, nodulation index and dry root and shoot weights. In all cases, plants were floated in shallow trays of deionised water and both tap and lateral roots were visually scored independently using a modified scoring system described and used earlier by Wong *et al*. (1984). This assessment scale contained six disease severity categories: score 0 = root healthy, no discolouration; 1= < 25% of root brown, no significant lesions; 2 = 25- < 50% of root brown, lesions towards base of tap root; 3 = 50- < 75% root brown, lesions mid tap root; 4 = ≥ 75% root affected, significant lesions towards crown; 5 = plant dead and/or root system completely rotted off. The number of plants in each disease severity category was recorded. Then, all disease rating scores were transferred to a tap or lateral "Percent Disease Index (PDI)" based on (McKinney, 1923) where tap or lateral root PDI = (sum of all numerical grades) × 100 ÷ (total number of plants scored × maximum rating score).

At the same time, plants were also assessed for level of nodulation on roots; using a modified rating scheme from Corbin *et al*. (1977) using a 0 to 5 scale (*5*) where: 0 = no nodules on the crown or elsewhere; 1 = no nodules on the crown with a few (1-10) elsewhere; 2 = a few crown nodules but no nodules elsewhere, or no crown nodules >10 nodules elsewhere; 3 = many crown nodules (>10) but no nodules elsewhere; or no crown nodules but man nodules elsewhere; 4 = many crown nodules with a few nodules elsewhere; 5 = many crown nodules with many nodules elsewhere. All nodulation rating scores were transferred to a "Percent Nodulation Index (PNI)" based on (McKinney, 1923) where PNI = (sum of all numerical grades) × 100 ÷ (total number of plants scored × maximum rating score).

Germination rate (%) was also calculated at harvest as a percentage of viable seeds sown. Shoots and roots from each pot were separated and dried at 60°C in a drying oven in separate paper bags for 3 days then dry shoot/and root weights were recorded and calculated as mg/plant.

Treatments were replicated four times and arranged in a randomized block design and the whole experiment fully repeated once. For the controlled environment field core studies, analysis of variance (ANOVA) was conducted using GenStat® (14th edition, Lawes Agricultural Trust). Normality of data and homogeneity of variances from each experiment were tested before conducting analyses. Data from the original and the repeat experiments using field cores were not significantly different (P>0.05) using a *t*-test. Therefore, data from the original and repeat field core experiments were combined and re-analyzed together. Data on emergence rate, disease index of tap roots, disease index of lateral roots, root dry weight and shoot dry weight of seedlings from each experiment using field cores had a normal distribution and similar variation. All treatment comparisons between treatments were made using Fisher's protected least significant differences (LSD) at P=0.05. GenStat® was also used to test the significance of correlation co-efficients between the parameters assessed and the different treatments utilized in the field trials.

3.2.2 Cultivation – field

Two separate but related field experiments were set up in May 2013 approximately 15 km apart on separate farms in a region historically known to have severe damping-off and root disease in subterranean clover pastures. The first site was at Wagerup (-32.927736, 115.919586) 100 km south of Perth Western Australia, and the second at Coolup (-32.758534, 115.825976) 90 km south of Perth. The Wagerup site was a low productivity pasture that had been uncultivated and unrenovated for more than 15 years while the Coolup site was a highly productive pasture that had been regularly renovated and resown predominantly to Italian rye grasses every few years. There was low incidence of subterranean clover at either site and at both sites soils were a highly weathered acid sandy duplex soil. No field cores from these two field sites were included for the detailed 'soil cores under controlled environment conditions' as described in the previous paragraph above. There were three

treatments, viz. uncultivated/unfumigated' cultivated/unfumigated, and cultivated/fumigated. Three varieties were used, viz. Seaton Park, Riverina and Woogenellup. Varieties were selected based on previous reports of Meteora and Riverina being moderately resistant, Seaton Park being resistant and Woogenellup susceptible to *P. clandestina*; reports of Seaton Park, Meteora and Riverina being resistant while Woogenellup has moderate resistance to *F. avenaceum;* and reports of Riverina and Seaton Park being moderately resistant but Meteora and Woogenellup moderately susceptible to *P. irregulare* (You *et al* 2005a, 2005b and 2005c). Further, Woogenellup was also chosen because it has been widely utilized as the universal susceptible comparison in root disease studies. Treatments were replicated three times and arranged in a randomized block design. Cultivation treatment was applied using a tractor-driven rotary hoe with several passes to thoroughly break up and mix soil layers to a depth of approximately 10 cm. Fumigation treatment was applied to cultivated soil on 2nd May 2013 using Metham sodium at a rate of 20L/140 m^2 using commercial application equipment. Fumigation was included to provide the ultimate measure against which any cultivation effects *per se* could be gauged. Cultivation and fumigation treatments were applied approximately 2 weeks prior to sowing to allow for fumigant dispersal before sowing. Both field sites were sown on 16th May 2013, with each treatment consisting of a lot of 100 seeds sown into a groove 10-15 mm deep in each 1 m single row; then covering seed and then pressing down the soil for a firm seed bed. Subterranean clover was inoculated with commercial *Rhizobium* inoculant at approximately twice the recommended rate using a watering can. Seedling germination counts were made on 29th May 2013 and at this same time, plants in each row were dug up, washed and assessed for levels of tap and lateral root disease. Plant root and shoot dry weights were taken after drying in an oven at 60°C for three days. Examination of root samples confirmed a very high incidence and predominance of the soilborne pathogen *P. irregulare* at both field sites.

Data from both the Wagerup and Coolup field sites was combined into a single data set for statistical analyses comparing different treatments by ANOVA (e.g., cultivation at Wagerup as one treatment and cultivation at Coolup as another treatment). In this way, analyses not only indicated common sense treatment comparisons (e.g., cultivation and no cultivation) but also accommodated site differences. These data analysis outcomes were also confirmed by "Accumulated Analysis of Variance" and "Predictions from Regression Model" by **"**Analysis of an Unbalanced Design" using the regression model in GenStat® (14th edition, Lawes Agricultural Trust) that takes into account the "incomplete block effect" (i.e., two sites considered as two blocks) (Hector *et.al*., 2010, Holden *et.al*., 2015 and Shaw and Thomas, 1993). All treatment comparisons between treatments were made using Fisher's protected least significant differences (LSD) at *P*=0.05. GenStat® was also used to test the significance of correlation co-efficients between the parameters assessed and the different treatments utilized in the field trials.

3.2.3 Cultural control treatments – species composition (glasshouse)

Subterranean clover varieties Meteora, Riverina, Seaton Park and Woogenellup, along with ryegrass (*Lolium rigidum*) were used for this study. *R.* solani AG8 and *P. irregulare* were used across various subterranean clover/ryegrass compositions of 20%, 40%, 60%, 80% and 100% subterranean clover. General experimental conditions and methodologies, including disease assessments, were as outlined above for studies relating to the pathogen *Pythium irregulare*. In brief, studies were carried out under environmental controlled conditions of 18°C/13°C (day/night) and 12hr/12hr (day/night) cycle. *R.* solani AG8 was inoculated two weeks prior to sowing and *P. irregulare* was inoculated at the time of sowing. All plants were harvested at 4 weeks after sowing; washed clean; and assessed for disease severity (expressed as tap and lateral root disease indices), nodulation index and dry root and shoot weights. In addition, the impact of pasture composition on soil-borne *Pythium* and *Rhizoctonia* root rot diseases and quantity of pathogen in the soil (expressed as DNA weight) was assessed to understand the relationships between DNA levels and expression of disease. To

understand this, subterranean clover composition at 20% and 100% were tested for *Pythium* and *Rhizoctonia* DNA (pg/g soil) weight by commercial "Predicta B."

3.2.4 Cultural control treatments – simulated grazing (glasshouse)

Annual subterranean clover varieties Meteora, Riverina, Seaton Park and Woogenellup were used for this study; with plants grown in a controlled environmental room at 13°C/18°C (night/day) under 12hr/12hr (day/night) light cycle. Root rot pathogen *P. irregulare* was used for inoculation. Nine simulated grazing (cutting) treatments were applied at the following stages: a) mono-foliate leaf (1-leaf), b) two trifoliate leaves (2-leaves), c) four trifoliate leaves (4-leaves), d) eight trifoliate leaves (8-leaves), e) twelve trifoliate leaves (12-leaves), f) sixteen trifoliate leaves (16-leaves) and then left un-grazed for above (a to f); g) retained first two trifoliate leaves and cut the remainder at every grazing stage (2-kept); h) all leaf stages from mono-foliate leaf till 16 leaves (cut all) and i) no grazing (nil). Plants were harvested two weeks after the last simulated grazing (16-leaves). Roots were washed clean and assessed for disease severity on tap and lateral roots (% disease index), nodulation (% nodulation index), and dry root and shoot weights recorded. Germination rate was also calculated at harvest to confirm that there was no loss in plant numbers from the simulated grazing treatments.

3.2.5 Cultural control treatments – rhizobium (glasshouse)

Rhizobium Group C Alosca® was used as source of rhizobium for inoculation. Seed and granule treatments were used. For seed treatment, rhizobium was dissolved in water and mixed with seed at the rate of 100 seeds/g of inoculant. Granule treatment was applied when seed was sown by placing a single Alosca granule with each seed at the rate of 0.61g/m^2. Root rot pathogens *P. irregulare* and *R. solani* were inoculated into soil before sowing. Other experiment conditions were the same as above. In brief, plants were grown in a controlled environment room held at 13°C/18°C (night/day) under 12hr/12hr (day/night) light cycle. Plants were harvested at four weeks after sowing; roots washed clean; and tap and lateral root disease, nodulation and dry root and shoot weights assessed as described earlier. Germination rate was recorded at the time of harvesting.

3.3 Environmental interactions with soilborne diseases

3.3.1 Environmental interactions – *Pythium*

Subterranean clover varieties, temperature regimes, moisture levels, soil types and treatments
Varieties Riverina, Seaton Park and Woogenellup were used. Seaton Park is known to be highly susceptible to Pythium damping-off and root disease caused by *P. irregulare*, while Woogenellup is moderately susceptible and Riverina moderately resistant (Nichols et al., 2014). Further, Woogenellup is utilized as the universal susceptible control comparison for all soilborne root disease studies on subterranean clover (Barbetti et al. 2006b, 2007). Subterranean clover seeds were surface sterilised in 70% ethanol for 30 sec to remove any seed pathogen contamination; then scarified lightly with sandpaper to break dormancy and increase germination and then sown at 5 seeds per pot at a depth of 10 mm. Pots were 9 cm × 9 cm square pots × 10 cm depth.

Experiments were conducted in three separate controlled environment rooms with temperatures maintained at 22/17°C or 18/13°C or 14/9°C (day/night) with a 12-h photoperiod and light intensity of 540 µM m^{-2} s^{-1}. These temperatures were selected to mimic temperatures commonly seen in the field in Western Australia during the first months of the winter growing season from May onwards to August, a period when root disease is particularly prevalent in subterranean clover forages (Barbetti, 1991); and as approximates the temperature ranges used in other studies of soilborne diseases of subterranean clover (e.g., Wong et al., 1984, 1986c; You et al., 2017a).

There were two levels of moisture, high moisture pots were watered to free draining with deionized water (DI) water daily [i.e., to 100% water holding capacity (WHC)], and water was added to low moisture pots by weight as required to maintain 50% WHC (e.g., every second day on high temperature pots) as described by You et al. (2017a).

Two types of soil, a sand-based mix representing light sand-based soil type (airing) and a Gingin red loam soil representing heavy soil type (compact), were used. The sand-based soil consisted of 2.5 m^3 fine composted pine bark, 1 m^3 coco peat, 5 m^3 brown washed river sand, 10 kg slow release fertilizer Osmoform® NXT 22 N + 2.2 P_2O_5 + 9.1 K_2O + 1.2 Mg + trace elements (Everris International B.V.), 10 kg Dolomite (CalMag®), 5 kg gypsum clay breaker, 5 kg extra fine limestone, 4 kg iron hepta sulphate, and 1 kg iron chelate). The Gingin red was a loam soil with a sand content of 85% (w/w) (McArthur, 1991) a soil with a texture that relates to extensive soil areas within and outside of Western Australia and this soil had no amendments. Each soil was pasteurised using aerated steam sterile on each of three consecutive days at 65°C for 90 min prior to use.

Nutrition levels and soil nutrient analyses

Two levels of nutrition were utilized, viz. high nutrition where seedlings were fertilised with a complete range of nutrients (Thrive, Yates®; N 25%, P5, K 8.8) required for seedling growth and development at the recommended rate weekly and low nutrition treatment where seedlings were watered with only DI water throughout the experiment. Twenty pots were used to make pooled soil samples collected from root zones around subterranean clover seedlings from each high or low treatment separately for sand-based and loam soils, and were air-dried at 25 to 30°C in a glasshouse, and sent to CSBP Plant and Soil Analysis Ltd., Western Australia for nutrient analyses. Characteristics including ammonium-N, nitrate-N, available-P, K and S, organic carbon, conductivity, pH ($CaCl_2$), pH (H_2O), DTPA Cu, Fe, Mn and Zn, exchangeable Al, Ca, Mg, K and Na, boron hot $CaCl_2$, total N, P and K were assessed using the protocols outlined by O'Rourke *et al*. (2012). For P levels, the Colwell-P the labile, easily plant available P pool was measured (Colwell 1963, 1965); while for Cu, Fe, Zn and Mn an extractable soil test using diethylenetriaminepentaacetic acid (DTPA) was utilized.

In comparison with the Gingin red loam, sand-based soil under high nutrient treatment contained higher nitrate Nitrogen (15.56 mg kg^{-1}), Phosphorus (Colwell 39.67 mg kg^{-1}), Potassium (Colwell, 79.72 mg kg^{-1}), Sulphur (28.8 mg kg^{-1}), Organic Carbon (4.22%), Conductivity (0.11 dS m^{-1}), Copper (0.71 mg kg^{-1}), Iron (17.27 mg kg^{-1}), Zinc (1.65 mg kg^{-1}), Potassium (89.33 meq $100g^{-1}$), Sodium (0.07 meq $100g^{-1}$), Boron (0.38 mg kg^{-1}), Nitrogen (Total, 0.09%), Phosphorus (Total, 79.95 mg kg^{-1}) and Potassium (Total, 89.33 mg kg^{-1}). Under low nutrition treatment, in comparison with the Gingin red loam, the sand-base soil contained the highest levels of Manganese (2.34 mg kg^{-1}), Calcium (6.321 meq $100g^{-1}$), and Exc. Magnesium (2.34 meq $100g^{-1}$). Exceptions to the above were where Gingin red loam under high nutrition treatment contained higher levels of ammonium Nitrogen (11.06 mg kg^{-1}) and under both low and high nutrition contained higher Aluminium (0.06 and 0.05 meq $100g^{-1}$, respectively) in comparison with the sand-based soil.

Inoculum production of p. irregulare and inoculation, confirmation of p. irregulare presence

A single isolate of *P. irregulare* was used, viz. WAC4953, from the Western Australian Culture Collection, Department of Agriculture and Food Western Australia. The isolate was originally from subterranean clover and had been used extensively in research on seedling damping-off and root rot in subterranean clover (e.g., You et al., 2005a) and different legumes (e.g., Li et al., 2015) and is representative of the prevailing *P. irregulare* populations on crop and forage legumes in Western Australia (Li et al., 2015). *P. irregulare* was cultured on potato dextrose agar (PDA) for 5 days at 25°C in the dark until mycelium had almost grown across the plate. Then, PDA plugs (0.5 by 0.5 cm) from the leading edge of a colony were cut and mixed with sterilized millet seed to produce inoculum. Millet seed was prepared by first soaking the seed in deionized water (40 g of millet seed soaked in

100 ml of water to make 150 ml of millet seed-water mixture/250 ml Erlenmeyer flask) overnight, draining the excess water, and autoclaving the moist millet seed at 121°C for 20 min, once on each of three consecutive days. The millet seed and five *P. irregulare*-colonized agar pieces were incubated at 25°C in the dark for 2 weeks. Flasks were shaken by hand once a day the first 2 days to ensure that colonised PDA plugs were evenly distributed among the seed.

One ha of each soil type was mixed thoroughly with *P. irregulare*-colonised millet seeds at a rate of 0.5% (w/w) immediately prior to sowing and used to fill pots. The control treatment for comparison was pots containing uninfested soil of each type, but without any uncolonised millet seeds added as uncolonised millet can readily 'bait-out' other non-target soil-borne pathogens present (Barbetti and Sivasithamparam, 1987). At the time of inoculation, quality of the inoculum was checked by plating approximately 15 colonized millet seeds onto corn meal agar (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) plates to confirm that *P. irregulare* was the sole organism present on the seed. In each experiment, 200 g of inoculated soil was placed in each pot.

In all experiments Koch's postulates were successfully completed to confirm that the disease symptoms observed were in fact caused by the *P. irregulare*. Root segments (8-10), 2 cm in length, were dissected from diseased plants and floated in Petri dishes containing sterile deionised water for 2-3 days at 20ºC. Five roots from each treatment were examined microscopically every 12 h using a light microscope and the presence of *P. irregulare* zoosporangia confirmed.

Disease and plant weight assessments

Germinated plants in each pot were counted to calculate emergence percentage before harvesting. Then, plants were harvested at 5 weeks after sowing, washed in running tap water to remove soil from roots and scored for their level of root disease. Plants were then floated in shallow trays of deionised water and both tap and lateral roots were visually scored independently using a modified scoring system described and used earlier by Wong et al. (1984). This assessment scale contained six disease severity categories: score 0 = root healthy, no discolouration; 1= < 25% of root brown, no significant lesions; 2 = 25- < 50% of root brown, lesions towards base of tap root; 3 = 50- < 75% root brown, lesions mid tap root; 4 = ≥ 75% root affected, significant lesions towards crown; 5 = plant dead and/or root system completely rotted off. The number of plants in each disease severity category was recorded. Then, all disease rating scores were transferred to a tap (TDI) or lateral (LDI) "Percent Disease Index" based on (McKinney, 1923) where:

$$\%\mathrm{DI} = \frac{(a\times 0)+(b\times 1)+(c\times 2)+(d\times 3)+(e\times 4)+(f\times 5)}{(a+b+c+d+e+f)\times g}\times 100$$

where a, b, c, d, e and f represent the number of plants with tap or lateral disease scores of 0, 1, 2, 3, 4 and 5 for root ratings and g represents the highest rating of tap or lateral root disease.

Shoots and roots from each pot were separated and dried at 60°C in an oven in separate paper bags for 3 days then dry shoot and root weights were recorded and calculated as mg plant^{-1}.

Experimental design, modelling approach and data analyses

There were four replicate pots for each treatment, with treatments in a full factorial arrangement, and all pots were maintained in their respective temperature-controlled environmental rooms throughout. All inoculated treatments were repeated using non-inoculated soils as control comparisons. This experiment was arranged in a randomized complete block design and the whole experiment for inoculated and uninoculated soils was fully repeated once under the same conditions. Data from the original and the repeat experiments were not significantly different

(*P*>0.05) using a pairwise *t*-test. Therefore, data from the original and repeat experiments were combined and re-analyzed together.

Experimental data were analysed using classical linear models (ANOVA) and generalised linear models (GLMs) to investigate the relationships between explanatory variables and the effects of Pythium damping-off and root disease on the three subterranean clover varieties. As these indicated complex and difficult to interpret interaction effects between explanatory variables, we also analysed the data using boosted regression trees (BRTs), a complementary method that is well-suited to data with complex interactions, and can be summarised in ways that give powerful biological insight (Elith et al., 2008, James et al., 2013). Simple regression trees and heat maps were also employed to help visualise the complex interactions within the data. Separate analyses and visualisations were conducted for each of the five measured dependent variables, dry shoot and root weights, emergence, and tap and lateral root disease indices for inoculated plants. For dry shoot and root weights, if seeds germinated and visible roots were present prior to drying, but weights were below the instrument detection limit, then a weight of 50% of the minimum weight was assigned. Differences between inoculated and control plants were also calculated for dry shoot and root weights and emergence, based on the mean of the replicates within each given treatment. Calculating differences between treatment means meant there was no replication within treatment, and so difference data were only analysed using BRTs as fitting of high order interactions within linear models, or GLMs would not have been possible. All analyses and data manipulations were conducted using the statistical package R (R Development Core Team, 2014), and its packages 'dismo' for boosted regression trees (Hijmans et al., 2016), 'plotly' for heat maps (Sievert et al., 2016) and 'rpart' and 'rpart.plot' for simple regression trees (Therneau et al., 2015, Milborrow, 2015).

Linear modelling was conducted on data from inoculated plants only. Linear models using boxcox power transformation were fitted for dependent variables dry shoot and root weights, while GLMs with binomial/quasibinomial error distribution and logit link function were performed for emergence, TDI and LDI. To accommodate zeros in the dataset, one was added to all dry shoot and root weight values prior to transformation. Initial models were fitted including all possible interactions, and simplified based on backwards selection using F-test (linear models) or Chi-squared tests (GLMs) starting with the highest interaction term (Crawley, 2013). This means that highest order interaction terms were removed from the model unless the test showed the existing model was significantly better than the model with the term removed (P-value <0.05). Diagnostic plots were examined to check model assumptions including homoscedasticity and normality of residuals.

BRTs were constructed for the inoculated plants data set and also on the calculated differences between inoculated and control plants, following the approach recommended by Elith and Leathwick (2008). For all BRTs, model parameters were set as: family 'Gaussian', tree complexity 5, learning rate 0.01, and bag fraction 0.5. These parameters allowed for a minimum of 1000 trees and maximised the model performance (lowest root mean squared error, RMSE). No terms were dropped from models. For dependent variables dry shoot and root weights, the same power transformation determined from linear modelling was used on the data prior to modelling.

For inoculated plants, BRT models were developed on the full dataset, as well as by cross-validation using training and test subsets of the data. For the latter, models were constructed using 75% of data and tested on the remaining 25%. Data were divided by replication number to provide balanced subsets (all possible explanatory variable combinations) and cross-validation was performed four times, separating a different replicate number out for testing each time. Root mean squared prediction error (RMSE) from the four cross-validations were then averaged, providing a more

realistic measure of model performance than obtained from models fitted to the full data set, which predict values for the same data used to create models.

To help visualise any complex interactions between explanatory variables, regression trees were also constructed. As the purpose here was purely visualisation, trees were grown and presented without pruning despite potential overfitting. Heat maps were created to further illustrate and examine relationships, as well as compare model predictions to actual data, using R package 'plotly' (Sievert et al., 2016).

3.3.2 Environmental interactions – *Phytophthora*

Subterranean clover varieties and other materials
Varieties Meteora, Riverina, Seaton Park and Woogenellup were used in this experiment as each of these have different levels of resistance to this pathogen. Seaton Park is known to have greatest resistance to the majority of known races compared with the other varieties. Woogenellup is susceptible to nearly all races of *P. clandestina* (You *et al.*, 2005b). Woogenellup is also utilized as the universal susceptible control comparison for all soilborne root disease studies on subterranean clover (Barbetti *et al*., 2006b, 2007). Subterranean clover seeds were surface sterilised in 70% ethanol for 30 seconds to remove any seed pathogen contamination; then scarified lightly with sandpaper to break dormancy and increase germination and then sown at 5 seeds per pot at a depth of 10 mm. Pots were 9 cm × 9 cm square pots × 10 cm depth.

Temperature regimes, moisture levels, soil types and treatments, nutrition levels and soil nutrient analyses were the same as with the environment x Pythium study above.

Isolation of P. clandestina
Isolates of *P. clandestina* were obtained from infested subterranean clover soils sampled from a region on the south coast of Western Australia where Phytophthora root disease and damping-off are prevalent. This was achieved using subterranean clover Woogenellup as the 'bait species'. In brief, Woogenellup plants were grown in infested soil samples in a controlled environment room at 18ºC/13ºC (day/night), with a 12/12 hour photoperiod; then pots flooded for 1 h at 1 and 3 weeks and harvested 4 weeks after sowing. Harvested plant roots were thoroughly washed under running tap water to remove soil. Whole root systems were floated in Petri dishes containing sterile distilled water and maintained in the dark in an incubator at 20ºC. Using a light microscope, individual *P. clandestina* zoosporangia were collected (at 24-48 h), using fine-tip tweezers, and placed directly onto Petri dishes containing a modified metalaxyl-benomyl-vancomycin agar (MBV) agar (Pfender *et al*. 1984). *P. clandestina* cultures were then sub-cultured onto fresh lima bean agar (LBA) and grown for 1 week in preparation for inoculum production. One isolate of *P. clandestina* identified as "race 173" was selected for these studies by testing isolates across the seven standard subterranean clover host differentials (You *et al*., 2005b), as it is the prevailing race on subterranean clover in south west of Western Australia (You *et al.,* 2005b).

Inoculum production of P. clandestina
Inoculum was prepared using a modified procedure from Barbetti (1989). In brief two-week-old *P. clandestina* colonies growing on LBA were cut into plugs 2 mm^2 and approximately 10 plugs were used to inoculate each 250 ml Erlenmeyer flask containing sterilized millet seeds (*Panicum miliaceum*). Millet seeds were prepared by soaking 100 g of millet seeds in 100 ml deionized water overnight in each 250 ml Erlenmeyer flask and then water drained and autoclaved at 120°C for 20 min three times on each of three consecutive days. Flasks with inoculated millet seeds were hand shaken vigorously to homogenize inoculum with millet seeds every second day to ensure equal colonization. Inoculated millet seeds were incubated at 22°C for three weeks. Colonized millet seeds

then were used for inoculating soils. At the time of inoculation, millet seed inoculum was also re-plated onto LBA to confirm that *P. clandestina* was present.

P. clandestina inoculation and disease assessments
One ha of each soil type was mixed thoroughly with *P. clandestina*-colonised millet seeds at a rate of 0.5% (w/w) and used to fill pots. The control treatment for comparison was pots containing uninfested soil of each type, but without any uncolonised millet seeds added as uncolonised millet can readily 'bait-out' other non-target soil-borne pathogens present (Barbetti & Sivasithamparam, 1987). Both inoculated and non-inoculated pots were flooded, but separately, for 1 h immediately after sowing, with flooding repeated two weeks later, and yet again flooded for 2 h at 4 weeks after sowing. Disease, nodulation and plant weight assessments were the same as for the Pythium x environment study.

Confirmation of P. clandestina presence
In all experiments Koch's postulates were successfully completed to confirm that the disease symptoms observed were in fact caused by the *P. clandestina*. Root segments (8-10), 2 cm in length, were dissected from diseased plants and floated in Petri dishes containing sterile deionised water for 2-3 days at 20ºC. Roots were examined microscopically every 12 h using a light microscope and the presence of *P. clandestina* zoosporangia confirmed.

Experimental design and analyses
There were four replicate pots for each treatment, with treatments in a factorial arrangement, and all pots were maintained in their respective temperature-controlled environmental rooms throughout. All inoculated treatments were repeated using non-inoculated soils as control comparisons. This experiment was arranged in a randomized block design and the whole experiment for inoculated and uninoculated soils was repeated once under the same conditions.

Analysis of variance (ANOVA) was conducted using GenStat® (14th edition, Lawes Agricultural Trust). Normality of data and homogeneity of variances from each experiment were tested before conducting analyses. Data from the original and the repeat experiments were not significantly different (P>0.05) using a t-test. Therefore, data from the original and repeat experiments were combined and re-analyzed together. Data on emergence, disease index of tap roots, disease index of lateral roots, root dry weight (RDW) and shoot dry weight (SDW) of seedlings from each experiment had a normal distribution and similar variance. Therefore, the effect of different treatment (viz. temperature, soil type, moisture and nutrition, varieties and pathogen inoculation) on emergence, tap root disease, lateral root disease, RDW and SDW of seedlings were determined by analyses of variance, and subsequent multiple comparisons between treatments were made using Fisher's protected least significant differences (l.s.d) at P=0.05. Standard errors (SE) of means were also computed. GenStat® was also used to test the significance of correlation co-efficients between the parameters assessed and the different treatments utilized.

3.3.3 Environmental interactions – *Rhizoctonia*

Rhizoctonia solani isolate
A single isolate of *R. solani* AG8 (ZG6; isolate WAC9086) was chosen for the current study as You *et al*. (2008) had shown that this AG8 (ZG6) group caused the greatest death of legume seedlings, across different genera (*Hedysarum, Medicago, Ornithopus and Trifolium*) that included eight different forage legume species (*H. coronarium, M. polymorpha, O. compressus, O. sativus, T. dasyurum, T. michelianum, T. purpureum* and *T. subterraneum*), in comparison with ZG1-5 and ZG11, ZG1-4 and especially ZG4. This isolate had been maintained as a lyophilised culture for approximately 10 years.
Subterranean clover varieties and other materials

Varieties Riverina, Seaton Park and Woogenellup were used in this experiment as they have varying combinations of resistances and susceptibilities to different soilborne pathogens or their races as affect subterranean clover (Nichols *et al.,* 2014). For example, against *P. clandestina* races 000, 001, 173 and against *P. irregulare*, *R. solani* and *Fusarium avenaceum*, listed pathogen resistance/susceptibility ratings for Riverina are HR, R, R, MR, S and R; for Seaton Park are R, R, MR, HS, MS and MS; and for Woogenellup are HS, S, HS, MS, MS and S (where HR = highly resistant, H = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, and HS = highly susceptible) (Nichols *et al.,* 2014). Further, Woogenellup is used as the universal susceptible control comparison for all soilborne root disease studies on subterranean clover (Barbetti *et al*., 2006b, 2007). Subterranean clover seeds were surface sterilised in 70% ethanol for 30 seconds to remove any seed pathogen contamination; then scarified lightly with sandpaper to break dormancy and increase germination and then sown at 5 seeds per pot at a depth of 10 mm. Pots were 9 cm × 9 cm square pots × 10 cm depth.

Temperature regimes, moisture levels, disease, nodulation and plant weight assessments and soil nutritional analyses were the same as for the Phytophthora x environment study.

Inoculum production of R. solani

Inoculum was prepared using a modified procedure from Barbetti (1989). In brief, two-week-old *R. solani* colonies growing on potato dextrose agar (PDA) were cut into plugs 2 mm^2 and approximately 10 plugs used to inoculate each flask containing sterilised millet seeds. Millet seeds were prepared by using 250 mL Erlenmeyer flasks containing 100 g of millet seeds and 100 mL of deionised water that were then autoclaved at 120°C for 20 min three times on each of three consecutive days. Flasks were hand shaken vigorously to homogenise inoculum with millet seeds. Inoculated millet seeds were then incubated at 22°C for two weeks, with flasks shaken every second day to ensure equal colonisation. Colonised millet seeds then were used for inoculating soils. At the time of inoculation, millet seed inoculum was also re-plated onto PDA to confirm that *R. solani* was present as expected.

R. solani inoculation

One ha of each soil type contained *R. solani* colonised millet seeds at a rate of three colonised seeds per pot evenly spaced on the surface of the soil then covered with a further 2 cm depth layer of pasteurised soil of either soil type as relevant. The inoculated pots were left in the growth room for 2 weeks before sowing to allow the pathogen to establish in the soil as has been used in earlier studies (e.g., You *et al.,* 2008). The control treatment for comparison consisted of pots containing uninfested soil of each type (with the additional 2 cm depth layer of pasteurised soil of either soil type as relevant), but without any uncolonised millet seeds added, as not only does uncolonised millet readily 'bait-out' other non-target soil-borne pathogens present at a low level even after soils pasteurisation (Barbetti and Sivasithamparam, 1987), but millet colonised by *R. solani* no longer constitutes millet seed *per se.*

Disease and plant weight assessments

Germinated plants in each pot were counted to calculate emergence percentage before harvesting and any seedlings that were dying before harvest were sampled at near-death and assessed. Plants were harvested at 5 weeks after sowing, washed in running tap water to remove soil from roots and scored for their level of root disease. Plants were then floated in shallow trays of deionised water and both tap and lateral roots were visually scored independently using a modified scoring system described and used earlier by Wong *et al*. (1984). This assessment scale contained six disease severity categories: score 0 = root healthy, no discolouration; 1= < 25% of root brown, no significant lesions; 2 = 25- < 50% of root brown, lesions towards base of tap root; 3 = 50- < 75% root brown, lesions mid tap root; 4 = ≥ 75% root affected, significant lesions towards crown; 5 = plant dead and/or root system completely rotted off. The number of plants in each disease severity category

was recorded. Then, all disease rating scores were transferred to a tap or lateral "Percent Disease Index (PDI)" based on (McKinney, 1923) where tap or lateral root PDI = (sum of all numerical grades) × 100 ÷ (total number of plants scored × maximum rating score).

Shoots and roots from each pot were separated and dried at 60°C in an oven in separate paper bags for 3 days then dry shoot and root weights were recorded and calculated as mg plant^{-1}.

Confirmation of R. solani presence
In all experiments Koch's postulates were successfully completed to confirm that the disease symptoms observed were in fact caused by the *R. solani*. Root segments (8-10 per treatment), 2 cm in length, were dissected from diseased plants, rinsed well under running water and plated to water agar containing 15 ppm of aureomycin hydrochloride (WA +A) and also floated in Petri dishes containing sterile deionised water for 2-3 days at 20ºC. Roots in water were examined microscopically every 12 h using a light microscope and the presence of *R. solani* hyphae confirmed in pathogen added treatments and its absence confirmed in control comparison treatments. Isolations on to WA + A also confirmed presence of *R. solani* where the pathogen inoculum had been added.

Experimental design and statistical analyses
There were the same as for the Phytophthora x environment study above.

3.3.4 Environmental interactions – *Aphanomyces*

Isolation of A. trifolii, inoculum production, inoculation and re-isolation
Isolations of *A. trifolii* isolates for this study were the same isolates as used in the study of You et al. (2016). Isolation details are as provided in You et al. (2016) and are as follows: First, 1 kg of topsoil was collected in 2013 from 25 field sites in the high rainfall parts of southwestern Western Australia, sites where *A. trifolii* had been determined in an earlier study to be present (Ma et al. 2008). Each topsoil sample was air-dried in a glasshouse at 25-30ºC for 7 d and stored until required. *A. trifolii* isolates were then baited-out from the stored soil samples, using subterranean clover variety Woogenellup as the bait species. Woogenellup seedlings were grown in soil samples maintained in a controlled environment room with an air temperature of 18ºC/13ºC (day/night), with a 12/12 hour photoperiod and a light intensity of 485 µM m^{-2}s^{-1}. Plants were flooded for 1 h at 1 and 3 weeks and harvested 4 weeks after sowing. Harvested plant roots were thoroughly washed under running tap water to remove soil. Whole root systems were floated in Petri dishes containing sterile distilled water and maintained in the dark in an incubator at 20ºC. With the aid of a light microscope, individual *A. trifolii* zoosporangia were collected (at 24-48 h), using fine-tip tweezers, and placed directly onto Petri dishes containing a modified metalaxyl, benomyl, and vancomycin agar (MBV) media [24]. *A. trifolii* cultures were then sub-cultured onto fresh cornmeal agar (CMA) and grown for 1 week in preparation for inoculum production as described below. As *A. trifolii* is currently impossible to maintain alive for more than a maximum of 8-10 weeks in agar or other culture (You et al. 2016), isolates obtained were utilized without delay. All isolates were microscopically identified and sequenced. Isolates collected in 2007 were identified as *A. trifolii* by O'Rourke et al. (2010). Sequences of isolates collected were BLAST searched in Genbank and showed 99 to 100 % similarity to *A. trifolii* [O'Rourke et al. 2010, You et al. 20-16).

Inoculum was prepared using a modified procedure from Barbetti (1989) and as used in You et al. (2016). Briefly, a mixture of the 20 freshly collected *A. trifolii* isolates were mixed at equal volume proportions. Moist sterile millet seeds (*Panicum miliaceum*) were soaked in deionized water over night then water drained and autoclaved on 3 consecutive days at 121ºC for 20 min. Two-week-old *A. trifolii* colonies growing on corn meal agar (CMA) were cut into plugs 2 mm^2 and approximately 20 plugs were added to each 250 ml flask containing 200 g of the prepared sterile moist millet seed.

Flasks were vigorously shaken every 2 days to homogenize inoculum. Inoculum was incubated in the dark at 20^{o}C for 3 weeks prior to use. At the time of inoculation, millet seed inoculum was also replated onto CMA to confirm that *A. trifolii* was present.

One ha of each soil type was mixed thoroughly with *A. trifolii*-colonised millet seeds at a rate of 0.5% (w/w) immediately prior to sowing and used to fill pots. The control treatment for comparison was pots containing uninfested soil of each type. No uncolonised millet seed was added to control treatments as uncolonised millet easily 'baits-out' any other soil-borne pathogens that may be present (Barbetti and Sivasithamparam 1987). At the time of inoculation, quality of the inoculum was checked by plating approximately 15 colonized millet seeds onto directly onto Petri dishes containing a modified metalaxyl, benomyl, and vancomycin agar (MBV) media (Pfender et al. 1984) to confirm that *A. trifolii* was the sole organism present on the seed. In each experiment, 200 g of inoculated soil was placed in each pot.

Koch's postulates were successfully completed for all experiments and confirmed that *A. trifolii* was the cause of the disease symptoms that occurred. Root segments (8-10), 2 cm in length, were dissected from diseased plants and floated in Petri dishes containing sterile deionised water for 2-3 days at 20ºC. Roots were examined microscopically every 12 h using a light microscope and the presence of *A. trifolii* zoosporangia confirmed.

Subterranean clover varieties
Varieties Riverina, Seaton Park and Woogenellup were used. While Riverina does show resistance to one or more specific individual isolates of *A. trifolii*, it is susceptible against a mixture of isolates that is more representative of the physiological variation existing in the pathogen population (You et al. 2016). In contrast, both Woogenellup and Seaton Park varieties were highly susceptible to Aphanomyces damping-off and root disease in the same experiments (You et al. 2016). Further, Woogenellup is utilized as the universal susceptible control comparison for all soilborne root disease studies on subterranean clover (Barbetti et al. 2006, 2007). Subterranean clover seeds were surface sterilised in 70% ethanol for 30 sec to remove any seed pathogen contamination, then scarified lightly with sandpaper to break dormancy and increase germination and then sown at 5 seeds per pot at a depth of 10 mm. Pots were 9 cm × 9 cm square pots × 10 cm depth.

Temperature regimes, moisture levels, soil types and treatments, nutrition levels and soil nutrient analyses, disease and plant weight assessments, experimental design, modelling approach and data analyses
These were the same as for the Pythium x environment study described above.

3.4DNA testing for soilborne pasture diseases

3.4.1 Development of DNA tests for Aphanomyces

Disease surveys and standard soil-baiting methodologies were used to successful isolation of new cultures of the root pathogen *A. trifolii* from infested soils collected across the southwest of Western Australia. This pathogen remains one of the most difficult soilborne pathogens worldwide to isolate and it took 18 months (i.e., research on this began well before the commencement of this project in anticipation of the challenge and importance of completing this task at an early stage in this project). This is only the second time worldwide that this pathogen has been successfully isolated and cultured. Six different isolates of *A. trifolii* were sent to the South Australian Research and Development Institute (SARDI) for utilization in developing the new DNA assay testing procedure for this pathogen. This would not only be the first ever development of a commercial DNA soil test to assess the presence and prevalence of this pathogen, but, more importantly would allow for the first

time the application of and assessment by DNA tests of the full range of soilborne pathogens in pastures, something available for crop soilborne pathogens but not ever before available for soilborne diseases of pastures.

3.5 Identification of effective host resistance to *Aphanomyces*

Forty-six subterranean clover varieties and twenty *A. trifolii* isolates mixed at equal volume and six replications were used for screening to identify host resistance against *A. trifolii*. Seeds were sown into potting mix contained in 6 x 6cm pots in a glasshouse with temperature ranging from 14 to 22°C. *A. trifolii* were grown on sterilized millet seeds and the fully colonized millet seeds were inoculated into the potting mix as a layer 4cm beneath the soil surface at a rate of 0.5% dry-soil weight. All other methodologies are the same as detailed above for the Aphanomyces x environment study.

3.6 Australia-wide field studies 2015

The following total of eight national famer field trials were undertaken in 2015 across southern Australia (2 farmer sites each in WA, SA, Vic and NSW).

i. *Fungicide seed treatments* (recommended rate, Woogenellup)
   - Metalaxyl, Phos-Jet, Thiram, Rancona Dimension x High and Low rate, Nil (10 reps; total = 60 x 1 m rows).

ii. *Complete fertilizer*, Nil (Woogenellup, soil tests before and after)
   - (10 reps; total = 20 x 1 m rows). There were interesting indications both from earlier studies (e.g., *O'Rourke, T.A., Scanlon, T.T., Ryan, M.H., Sivasithamparam, K. and Barbetti, M.J. 2012. Amelioration of root disease of subterranean clover (Trifolium subterraneum) by mineral nutrients. Crop and Pasture Science 63: 672–682; Barbetti et al. unpubl*) and from recent other experimental studies and farmer observations that application of 'adequate fertilizer regime' can influence levels of soilborne disease, sometimes adversely and sometimes beneficially. Hence, inclusion of a complete fertilizer treatment in both the 2015/2016 field trials.

iii. *Chemical sprays* (recommended rate, Woogenellup)
   - Metalaxyl, Phos-Jet, Nil (10 reps; total = 30 x 1 m rows).

iv. *Cultivation* (farmer strips, not resown)
   - Cultivated, non-cultivated (4 reps, plots cultivar width; paired comparisons across 10m cultivated/non-cultivated strips).

v. *Simulated grazing* (farmer pastures; mowing treatments; no stock allowed on trial area)
   - Simulated continuous grazing, simulated crash grazing, Nil (4 reps, plots mower width; paired comparisons across comparisons across 5m cultivated/non-cultivated strips)

vi. Varieties
   - Woogenellup, Seaton Park, Riverina, Meteora (10 reps; total 40 x 1 rows). This also aimed provide a benchmark for the glasshouse data collected on these same four varieties and provide the linkage between glasshouse and field disease levels-disease impacts.

All treatments were hand sown in 1m rows.

3.7 Australia-wide field studies 2016

Based on outcomes from the 2015 field trials, the main trial treatment targets were the focus for 2016 national famer field trials undertaken in 2016 across southern Australia (2 farmer sites each in WA, SA, Vic and NSW; total of 8 trials), were as follows.

Fungicide seed treatments: Seed treatment (10 reps) – mixed Rancona Dimension 160ml/100kg, Thiram 800 (100 g a.i./100 kg seed), Metalaxyl 350 (rate 500-1000 mL a.i./100 kg) + Phosphorous acid/Phosphite 200g/L (rate 10g ai/L); plus oestrogen (formononetin 12mg/100 seeds), Biochanin A (4mg/100 seeds) and Salicylic acid (5mM)

Fungicide sprays: Metalaxyl and Phos-jet separately when plants 10 weeks old (10 reps)
Cultivation: single 20m strip in farm field on last year's cultivated and non-cultivated field
Fertilizer: complete fertilizer, nil (10 reps)
Varieties (12): Woogenellup, Seaton Park, Riverina, Meteora, Losa, Dalkeith, Trikkala, Mt. Barker, Antas, Mounty, Naofil elite null and Novostar elite null (5 reps).

As with the 2015 field trials, all treatments were hand sown in 1m rows.

3.8 Modelling field environmental and other data against soilborne disease severity across southern Australia

Modelling of available field environmental and other data against soilborne disease severity across southern Australia was undertaken by Dr Michael Renton, UWA, using data available and/or collected from across southern Australia, including slope, pH, soil type, an estimate of water logging, and latitude and longitude. In addition, the total monthly rainfall and the mean monthly maximum temperature for each month for the year of sample collection were obtained from the Bureau of Meteorology website http://www.bom.gov.au/climate/data/index.shtml. This data was taken from the Bureau of Meteorology station nearest to the GPS co-ordinates of each field location site. Where data for a certain month was missing from a station for that year, the data from the next nearest station that had the data for that month was used instead. All data for all sites was collated into an excel spreadsheet, which was then cleaned, explored and analysed using the R statistical software package (R Cote Team 2017). The potential explanatory variables included average annual rainfall; soil type; pH; slope; temperature for January, February, March, June, and July; rainfall for January, February, March, June, and July; latitude; and longitude. Soil variables were also recoded into alternative binary classes for sand, loam, gravel and clay, which simply recorded whether or not that soil component had been recorded in the original soil description. For example, a 'sandy gravel' was recorded as 'true' for sand and gravel, and 'false' for loam and clay.

The R 'leaps' package (Lumley and Miller 2009) was used to conduct full subset multiple regression analysis on each of the three observed variables, total infection, lateral infection, and nodulation, against all of the potential explanatory variables. This means that all possible models explaining a given observed variable using any combination of any number of the potential explanatory variables were fitted. For each possible number of explanatory variables, the best two models (based on adjusted R-squared values) were recorded. Plots were constructed to show all these models ranked by adjusted R-squared values, and whether each potential explanatory variable was present in that model or not. A best model was selected, being the simplest model (model with least parameters) with an optimal or near-optimal adjusted R-squared value. Other possible variables of interest were identified from the model ranking plots.

This full subset multiple regression analysis was repeated twice for each of the three observed variables, total infection, lateral infection, and nodulation; once with the original soil classification as a categorical explanatory variable and no monthly rainfall/temperature variables, and once using the recoded binary soil classifications and monthly rainfall/temperature variables for months January to July.

4 Results

4.1 Glasshouse studies

4.1.1 Causes and losses from soilborne root disease pathogens

It is now well established from historical and, in particular current studies in this MLA project, that the main pathogens causing damping-of and root diseases of subterranean clover are: *Phytophthora clandestina, Pythium* spp., especially *P. irregulare, Rhizoctonia solani* and *Aphanomyces trifolii*. Studies have shown that root rot pathogens generally exist in the soil as complex of two to four of the above mentioned pathogens.

The main losses now quantified for subterranean clover pastures in studies undertaken in this MLA project are as follows:

Pre-damping off and corresponding low germination rates (%) across the autumn - winter period caused by *Phytophthora* ranged from 0 to 30%; by *Pythium* ranged from 0 to 70%; by *Rhizoctonia* ranged from 0 to 60%, and by *Aphanomyces* ranged from 0 to 55% losses. The extent of these losses was clearly cultivar-dependent, higher losses for more susceptible varieties and lower but still extensive losses for more resistant varieties.

Root dry weight losses (i.e., size reductions in root systems) due to *Phytophthora* ranged from 0 to 90%; by *Pythium* by up to 65%, by *Rhizoctonia* ranged from 15 to 100%, and by *Aphanomyces* by up to 45%.

Shoot dry weight losses (i.e., size reductions in shoot systems) due to *Phytophthora* were up to 85%, by *Pythium* up to 70%, *Rhizoctonia* up to 100%, and *Aphanomyces* up to 10%.

4.1.2 Field definition of the relationships between disease expression and association with soil-borne pathogens and environmental factors

Root diseases are generally overlooked by farmers, consultants and even pathologists. In particular, the vast majority of farmers only belatedly notice that their pasture is declining (less germination, stunted /small plants and/or simply no longer productive in the way expected) and that the subterranean clover content has and continues to gradually disappear year by year. Historically, there has been a strong tendency that when there are periods of drought or flood, low temperatures during parts of the growing season, poor soil quality and nutrition, low moisture, to readily implicate such ‘factors’ as the cause *per se* of subterranean clover decline. Yet, such adverse conditions that stress plants are well known to make plants more susceptible to one or more soilborne pathogens, with overall disease incidence, severity and impact greatly increasing in company with plant stress from adverse environmental factors. Studies in this MLA project have demonstrated the important role of ‘environmental factors’ in determining the severity and impact of soilborne root diseases on subterranean clover. A brief summary of some of the main results relating to environmental ‘drivers’ for individual pathogens is as follows:

Pythium root rot:

- Low temperature, heavy soil, low soil moisture content all caused the most severe *Pythium* pre-damping off (i.e., lowest germination rate);
- Low temperature, heavy soil, low moisture and low nutrition caused the most severe tap and lateral root disease;
- Low temperature, heavy soil, low soil moisture, poor nutrition caused the most severe root and shoot loses in the presence of this pathogen.

Phytophthora root rot:

- Low temperature, heavy soil, low soil moisture caused the most severe *Phytophthora* pre-damping off (i.e., lowest germination rate);
- Low temperature, heavy soil and high nutrition caused the most severe tap and lateral root disease;
- Low temperature, heavy soil, low nutrition, and low soil moisture caused the most severe root weight loss in the presence of this pathogen;
- Low temperature, heavy soil and low nutrition caused the most severe shoot weight loss in the presence of this pathogen.

Aphanomyces root rot:

- Intermediate temperatures, light soil (well 'aired'), and low soil moisture causes the most severe pre-damping off (i.e., lowest germination rate);
- Low and medium temperature, light soil, high soil moisture and high nutrition caused the most severe tap and lateral root disease;
- Low temperature, lighter soils, and high nutrition caused the most severe root and shoot dry weight losses from this pathogen.

Rhizoctonia root rot:

- Low and medium temperature, low moisture and light soil caused the most severe pre-damping off (i.e., lowest germination rate);
- Low moisture, high nutrition and light soil caused the most severe tap and lateral root diseases;
- Low and medium temperature, low moisture and low nutrition caused the most severe root and shoot dry weight losses from this pathogen.

4.1.3 Fungicide seed dressings – *Pythium irregulare*

Emergence rate. There were significant differences between fungicide seed treatment; inoculation and varieties on emergence rate ($P<0.001$, $LSD_{0.05} = 6.669$; $P<0.001$, $LSD_{0.05} = 2.983$; and $P<0.001$, $LSD_{0.05} = 4.218$, respectively). There were significant interactions between fungicide seed treatment and inoculation; between fungicide seed treatment and varieties; and between inoculation and varieties ($P<0.001$, $LSD_{0.05} = 9.432$; $P<0.001$, $LSD_{0.05} = 13.339$; and $P<0.001$, $LSD_{0.05} = 5.965$, respectively) (Table 2). Thiram showed the best protection from pre-emergence damping-off for all four varieties tested in comparison with other tested fungicides and the 'nil disease' control. Rovral gave high protection for varieties Meteora, Seaton Park and Woogenellup but not Riverina. Syn-A16874F and Rancona Dimension gave good protection for varieties Riverina, Seaton Park and Woogenellup but not for Meteora. Phosphorous acid gave the lowest protection for all the varieties tested against *P. irregulare* pre-emergence damping-off followed by metalaxyl which gave second lowest protection (Table 3).

Root Disease Severity (Index). There were significant differences between fungicide seed treatments and inoculation on root disease index ($P<0.001$, $LSD_{0.05} = 3.663$; $P<0.001$, $LSD_{0.05} = 1.638$,

respectively). There were significant interaction between fungicide seed treatments and inoculation and between fungicide seed treatments and varieties (P<0.001, $LSD_{0.05}$ = 5.18; P=0.009, $LSD_{0.05}$ =7.326, respectively). There was a three way interaction between fungicide seed treatment, inoculation and varieties (P=0.005, $LSD_{0.05}$ =10.36) (Table 2). Metalaxyl was the best seed treatment among nine tested fungicides against *P. irregulare* root rot disease across all four varieties tested. Phosphorous acid, fluquinconazole (Jockey) and Rovral were the second best group of fungicides, while propamocarb (Previcur) had the least effect on reducing levels of root rot disease.

Root Dry Weight and Shoot Dry Weight. There were significant differences between fungicide seed treatment, inoculation and varieties on root dry weight (P<0.001, $LSD_{0.05}$ = 2.573; P<0.001, $LSD_{0.05}$ = 1.151; and P<0.001, $LSD_{0.05}$ = 1.627, respectively). There were significant interactions between fungicide seed treatment and inoculation; between fungicide seed treatment and varieties; and between inoculation and varieties (P<0.001, $LSD_{0.05}$ = 3.639; P<0.001, $LSD_{0.05}$ = 5.146; and P<0.001, $LSD_{0.05}$ = 2.302, respectively). There was a three way interaction between fungicide seed treatment, inoculation and varieties (P=0.012, $LSD_{0.05}$ =7.278) (Table 2). There were significant differences between inoculation, and varieties in relation to shoot dry weight (P<0.001, $LSD_{0.05}$ = 1.741; and P<0.001, $LSD_{0.05}$ = 2.462, respectively). There were significant interactions between fungicide seed treatment and inoculation, and between fungicide seed treatment and varieties (P<0.001, $LSD_{0.05}$ = 5.506; and P<0.001, $LSD_{0.05}$ = 7.786, respectively). There was also a three way interaction between fungicide seed treatment, inoculation and varieties (P=0.027, $LSD_{0.05}$ =11.011) (Table 2). Phosphorous acid and metalaxyl were the best at maximising root and shoot dry weights while Rancona Dimension, Syn-A16874F and Thiram were least effective in terms of root and shoot dry weights.

Nodulation Index. There were significant differences between varieties in relation to nodulation (P = 0.043, $LSD_{0.05}$ = 1.866). There were significant interactions between fungicide seed treatment and inoculation and between inoculation and varieties (P<0.001, $LSD_{0.05}$ = 4.173; and P=0.006, $LSD_{0.05}$ = 2.639, respectively) (Table 2). Seaton Park had greatest ability to form nodules. Metalaxyl and Thiram consistently increased root nodulation on all tested varieties inoculated with *P. irregulare*, while Syn-A16874F also increased root nodulations on most of the tested varieties except Woogenellup. Non-inoculated Meteora with phosphorous acid treated seeds showed highest ability to form nodules, while inoculated Meteora with phosphorous acid treated seeds showed a total inability to form nodules. Inoculated Meteora and Seaton Park showed decreased nodulation while inoculated Riverina and Woogenellup showed increased nodulation (Table 3).

It is clear that there is outstanding potential to utilise chemical seed treatments to ensure seedling emergence, survival and along with significantly enhanced productivity from reducing tap and lateral root disease. Further, there are some interesting effects of seed treatments on nodulation that warrant further investigation.

Table 2. Statistical main effects and interactions (P-value and LSD value) in the presence of *Pythium irregulare*

	Emergence %		*Root Disease Index*		*Root Dry Weight (mg)*		*Shoot Dry Weight (mg)*		*Nodulation Index*	
Main effects	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*	*P-value*	*LSD=0.05*
Fungicide seed treatment	<0.001	6.669	<0.001	3.663	<0.001	2.573	NS	*	NS	*
Inoculation	<0.001	2.983	<0.001	1.638	<0.001	1.151	<0.001	1.741	NS	*
Varieties	<0.001	4.218	NS	*	<0.001	1.627	<0.001	2.462	0.043	1.866
Fungicide seed treatment*inoculation	<0.001	9.432	<0.001	5.18	<0.001	3.639	<0.001	5.506	<0.001	4.173
Fungicide seed treatment*varieties	<0.001	13.339	0.009	7.326	<0.001	5.146	<0.001	7.786	NS	*
Inoculation*varieties	<0.001	5.965	NS	*	<0.001	2.302	NS	*	0.006	2.639
Fungicide seed treatment*inoculation*varieties	NS	*	0.005	10.36	0.012	7.278	0.027	11.011	NS	*

Table 3. Effect of fungicide seed treatments on emergence rate (%), root rot disease index, root dry weight (mg), shoot dry weight (mg) and nodulation index in the presence of *Pythium irregulare*

	Varieties	*Meteora*		*Riverina*		*Seaton Park*		*Woogenellup*		
	Inoculum	*Nil*	*Pythium irregulare*	*Nil*	*Pythium irregulare*	*Nil*	*Pythium irregulare*	*Nil*	*Pythium irregulare*	*Mean*
Emergence Rate （%）										
	Dividend	75.00	45.00	80.00	72.50	90.00	85.00	72.50	85.00	*75.63*
	Fluquinconazole (Jockey)	65.00	22.50	80.00	75.00	90.00	100.00	75.00	87.50	*74.38*
	Metalaxyl	65.00	37.50	72.50	82.50	75.00	85.00	67.50	72.50	*69.69*
	Nil	80.00	52.50	75.00	47.50	87.50	82.50	80.00	70.00	*71.88*
	Phosphorous acid (Phos-Inject 200)	57.50	42.50	60.00	30.00	45.00	10.00	60.00	35.00	*42.50*
	Propamocarb (Previcur)	75.00	72.50	82.50	72.50	87.50	92.50	77.50	67.50	*78.44*
	Rancona Dimension	72.50	60.00	85.00	95.00	95.00	90.00	92.50	90.00	*85.00*
	Rovral	80.00	90.00	90.00	67.50	95.00	95.00	80.00	87.50	*85.63*
	Syn-A16874F	80.00	72.50	77.50	82.50	95.00	97.50	80.00	95.00	*85.00*
	Thiram	80.00	82.50	92.50	90.00	95.00	97.50	80.00	87.50	*88.13*
Mean		73.00	57.75	79.50	71.50	85.50	83.50	76.50	77.75	*75.63*
Root Disease Index	Dividend	0.00	58.33	0.00	56.10	0.00	51.53	0.00	52.92	*27.36*
	Fluquinconazole (Jockey)	5.83	50.48	0.00	61.67	0.00	46.67	0.00	50.00	*26.83*
	Metalaxyl	0.00	26.39	0.00	21.46	0.00	35.42	0.00	33.33	*14.58*
	Nil	0.00	68.75	0.00	67.70	0.00	56.67	0.00	55.56	*31.09*
	Phosphorous acid (Phos-Inject 200)	0.00	54.17	0.00	45.11	0.00	61.31	0.00	49.72	*26.29*
	Propamocarb (Previcur)	0.00	52.15	0.00	73.82	0.00	61.94	0.00	62.15	*31.26*
	Rancona Dimension	0.00	60.42	0.00	56.39	0.00	67.72	0.00	61.87	*30.80*
	Rovral	0.00	52.50	0.00	47.01	0.00	61.46	0.00	53.75	*26.84*
	Syn-A16874F	0.00	66.04	0.00	62.29	0.00	55.62	0.00	41.67	*28.20*
	Thiram	0.00	66.88	0.00	62.29	0.00	52.05	0.00	54.24	*29.43*
Mean		0.58	55.61	0.00	55.38	0.00	55.04	0.00	51.52	*27.27*
Root Dry Weight (mg)	Dividend	28.14	27.54	31.14	17.28	22.79	17.95	27.78	19.68	*24.04*
	Fluquinconazole (Jockey)	35.69	24.36	21.9	14.12	13.22	16.27	30.17	31.36	*23.39*
	Metalaxyl	27.72	36.85	27.04	22.14	11.77	16.05	30.19	26.94	*24.84*
	Nil	28.17	21.35	26.5	15.18	19.28	13.34	33.04	24.29	*22.64*
	Phosphorous acid (Phos-Inject 200)	28.41	23.47	32.27	17.49	28.19	29.96	25.91	27.22	*26.62*
	Propamocarb (Previcur)	28.94	17.3	27.76	19.3	22.63	13.76	31.94	30.99	*24.08*

	Rancona Dimension	24.37	23.94	33.35	12.85	23.65	13.79	20.51	16.8	*21.16*
	Rovral	25.97	18.75	26.52	16.34	22.33	13.23	29.84	27.39	*22.55*
	Syn-A16874F	26.42	10.7	30.99	15.03	25.2	12.27	27.92	22.93	*21.43*
	Thiram	30.9	14.62	24.02	13.06	24.35	11.07	31.08	23.33	*21.55*
Mean		28.47	21.89	28.15	16.28	21.34	15.77	28.84	25.09	*23.23*
Shoot Dry Weight (mg)	Dividend	42.71	53.28	45.86	28.28	31.86	28.18	40.22	31.74	*37.77*
	Fluquinconazole (Jockey)	49.19	44.44	30.84	26.00	21.77	22.30	48.79	45.78	*36.14*
	Metalaxyl	47.16	51.68	45.68	38.05	20.70	23.27	44.20	45.71	*39.56*
	Nil	53.57	35.57	36.94	25.32	29.53	17.58	52.05	35.28	*35.73*
	Phosphorous acid (Phos-Inject 200)	43.47	35.44	43.67	24.11	33.05	45.80	46.63	33.45	*38.20*
	Propamocarb (Previcur)	48.27	30.99	41.15	30.74	35.98	17.51	47.99	43.74	*37.05*
	Rancona Dimension	42.44	33.57	41.02	28.18	34.88	21.96	38.09	29.52	*33.71*
	Rovral	45.43	32.80	42.46	27.67	32.00	18.67	46.13	40.06	*35.65*
	Syn-A16874F	42.66	24.19	46.79	30.00	33.41	16.76	42.18	35.68	*33.96*
	Thiram	48.49	27.63	39.81	24.85	35.10	16.87	41.02	36.98	*33.84*
Mean		46.34	36.96	41.42	28.32	30.83	22.89	44.73	37.79	*36.16*
Nodulation Index	Dividend	10.38	4.75	3.12	9.78	12.83	8.50	7.67	6.75	*7.97*
	Fluquinconazole (Jockey)	5.00	6.36	7.75	5.92	5.12	10.50	0.50	6.13	*5.91*
	Metalaxyl	3.75	10.00	3.75	9.08	4.67	6.00	9.58	14.58	*7.68*
	Nil	11.29	2.08	2.91	6.90	5.38	7.38	8.13	9.75	*6.73*
	Phosphorous acid (Phos-Inject 200)	16.25	0.00	5.95	0.03	12.92	0.00	0.00	3.35	*4.81*
	Propamocarb (Previcur)	5.00	4.75	7.71	8.00	13.63	4.12	2.50	7.29	*6.63*
	Rancona Dimension	10.25	1.67	11.25	6.50	13.13	8.30	2.25	8.88	*7.78*
	Rovral	7.92	3.87	1.96	3.50	8.50	10.50	1.75	6.87	*5.61*
	Syn-A16874F	2.62	5.63	3.75	14.79	2.00	6.38	0.00	0.00	*4.40*
	Thiram	1.87	6.62	2.50	4.21	2.50	11.13	0.50	2.75	*4.01*
Mean		*7.43*	*4.57*	*5.07*	*6.87*	*8.07*	*7.28*	*3.29*	*6.64*	*6.15*

4.1.4 Fungicide seed dressings – *Aphanomyces trifolii*

Emergence rate: It was clear from this study that *A. trifolii* generally does not cause serious damping-off in the way that *P. irregulare*, for example, does with up to 90% or more seedling losses in disease-prone situations in Western Australia. Reductions in emergence from *A. trifolii* across the different varieties from 0 to 11% were overall of the order of only about 7%, a level at which there would be only a small economic impact for the farmer. Despite this, there were significant differences between fungicide seed treatment; and varieties, in terms of emergence rate ($P=0.023$, $LSD_{0.05} = 5.992$; and $P<0.001$, $LSD_{0.05} = 3.79$, respectively). There were significant interactions between fungicide seed treatment and inoculation; between fungicide seed treatment and varieties; and between inoculation and varieties ($P=0.01$, $LSD_{0.05} = 8.474$; $P<0.001$, $LSD_{0.05} = 11.984$; and $P<0.001$, $LSD_{0.05} = 5.359$, respectively) (Table 2). Against *A. trifolii*, Rovral, Dividend and Jockey showed the best protection from pre-emergence damping-off in Meteora in comparison with other tested fungicides. Rancona Dimension, Dividend and Syn-A16874F gave high protection for Riverina and Rovral, while Syn-A16874F and Dividend showed the best protection for Seaton Park. Thiram and Dividend showed good protection for Woogenellup (Table 3).

Percent Disease Indices: In contrast to emergence, *A. trifolii* did have a very significant effect and resulted in serious levels of both tap and lateral root disease, resulting in an average disease index of 45% for tap root disease and of 51% for lateral root disease across the four varieties. However, these high levels of root disease did not impact shoot weight as the experiment was assessed at only 4 weeks after sowing and this was not sufficient time to observe the impact of root disease on shoot weight, which is would only have occurred with additional time for a pathogen such as *A. trifolii* that takes a longer time to seriously impede plant productivity.

Fungicide seed treatments had no effect on tap root rot but had significant effect on lateral root rot ($P=0.041$, $LSD_{0.05} = 7.881$). Inoculation, cultivar and their interaction were significant in terms of both tap root rot ($P<0.001$, $LSD_{0.05} = 3.424$; $P<0.001$, $LSD_{0.05} = 4.842$ and $P = 0.011$, $LSD_{0.05} = 6.848$, respectively) and also lateral root rot ($P<0.001$, $LSD_{0.05} = 3.525$; $P<0.001$, $LSD_{0.05} =4.985$ and $P = 0.018$, $LSD_{0.05} =7.049$, respectively) (Table 4). Overall, Metalaxyl was the best seed treatment among nine tested fungicides against *A. trifolii* lateral root rot disease across all four varieties tested. While varieties Meteora and Riverina showed lest lateral root rot treated by fluquinconazole, Seaton Park and Woogenellup showed lest lateral root rot when treated by Thiram or Rancona Dimension (Table 5).

Root Dry Weight and Shoot Dry Weight: There were no significant effects on root dry weight from fungicide seed treatment, inoculation or varieties but there were significant effect on shoot dry weight from fungicide seed treatment, different varieties and with the interaction of fungicide seed treatment with varieties ($P=0.038$, $LSD_{0.05} = 3.886$; $P<0.001$, $LSD_{0.05} = 2.458$; and $P=0.001$, $LSD_{0.05} = 7.772$, respectively) (Table 4). Overall, Thiram and Rovral were the best at maximising shoot dry weights while Dividend and Rancona Dimension, were the least effective in terms of protecting losses in terms of shoot dry weight occurring from *A. trifolii* (Table 5).

Overall, there is very promising potential for use of chemical seed treatments to ensure seedling emergence, survival and along with significantly enhanced productivity from reducing lateral root disease and maximising shoot production, not only in the presence of this important pathogen, *A. trifolii*, but also in the presence of the more serious damping-off pathogen *P. irregulare.*

Table 4. Statistical main effects and interactions for seed treatment (P-value and LSD value) against *Aphanomyces trifolii*

	Emergence %		*Tap Root Disease Index*		*Lateral Root Disease Index*		*Shoot Dry Weight (mg)*	
Main effects	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*
Fungicide seed treatment	0.023	5.992	NS	NS	0.041	7.881	0.038	3.886
Inoculation	NS	*	<0.001	3.424	<.001	3.525	NS	*
Varieties	<0.001	3.79	<0.001	4.842	<.001	4.985	<0.001	2.458
Fungicide seed treatment*inoculation	0.01	8.474	NS	*	NS	*	NS	*
Fungicide seed treatment*varieties	<0.001	11.984	NS	*	NS	*	0.001	7.772
Inoculation*varieties	<0.001	5.359	0.011	6.848	0.018	7.049	NS	*

Table 5. Effect of fungicide seed treatments on emergence rate (%), tap root rot disease index, lateral root rot disease index, shoot dry weight (mg) infected by *Aphanomyces trifolii*.

	Varieties	*Meteora*		*Riverina*		*Seaton Park*		*Woogenellup*		
	Inoculum	*Nil*	*Aphanomyces trifolii*	*Nil*	*Aphanomyces trifolii*	*Nil*	*Aphanomyces trifolii*	*Nil*	*Aphanomyces trifolii*	*Mean*
Emergence Rate (%)										
	Nil	92.5	85.0	90.0	85.0	92.5	82.5	67.5	72.5	*83.4*
	Dividend	85.0	90.0	75.0	85.0	85.0	92.5	72.5	87.5	*84.1*
	Fluquinconazole (Jockey)	87.5	90.0	67.5	77.5	92.5	75.0	85.0	82.5	*82.2*
	Metalaxyl	82.5	82.5	72.5	67.5	90.0	77.5	87.5	80.0	*80.0*
	Phosphorous acid (Phos-Inject 200)	77.5	80.0	65.0	70.0	92.5	77.5	85.0	82.5	*78.8*
	Propamocarb (Previcur)	82.5	72.5	72.5	65.0	77.5	62.5	82.5	75.0	*73.8*
	Rancona Dimension	85.0	80.0	82.5	90.0	70.0	87.5	75.0	85.0	*81.9*
	Rovral	90.0	90.0	67.5	75.0	90.0	95.0	80.0	82.5	*83.8*
	Syn-A16874F	80.0	70.0	72.5	85.0	85.0	92.5	77.5	82.5	*80.6*
	Thiram	95.0	82.5	72.5	55.0	82.5	67.5	80.0	90.0	*78.1*
Mean		*85.8*	*82.3*	*73.8*	*75.5*	*85.8*	*81.0*	*79.3*	*82.0*	*80.7*
Tap Root Disease Index	Nil	14.96	35.08	29.25	49.0	6.25	41.58	22.5	53.5	*31.52*
	Dividend	23.79	43.58	10.0	34.38	10.0	36.63	17.92	39.17	*26.93*

	Fluquinconazole (Jockey)	21.75	27.12	26.67	37.04	8.5	38.88	3.17	50.0	*26.64*
	Metalaxyl	12.12	41.0	8.21	38.83	15.25	33.96	5.25	39.88	*24.31*
	Phosphorous acid (Phos-Inject 200)	28	25.29	22.29	48.42	5.87	32.88	6.25	43.25	*26.53*
	Propamocarb (Previcur)	16.67	37.04	20.96	34.42	10.0	27.71	6.75	46.58	*25.02*
	Rancona Dimension	18.83	23.08	21.33	39.12	9.17	17.12	16.54	52.83	*24.75*
	Rovral	42.13	32.75	31.67	34.71	3.62	38.75	26.62	40.25	*31.31*
	Syn-A16874F	14.62	27.5	11.58	44.12	2.75	27.88	23	28.83	*22.54*
	Thiram	22.62	46.08	27.17	32.08	3.12	15	29.42	42.5	*27.25*
Mean		*21.55*	*33.85*	*20.91*	*39.21*	*7.45*	*31.04*	*15.74*	*43.68*	*26.68*
Lateral Root Disease Index	Nil	24.33	56.46	37.25	71.08	3.13	30.54	26.04	44.21	*36.63*
	Dividend	30.54	58.92	17.63	56.75	7.38	34.63	16.46	14.0	*29.54*
	Fluquinconazole (Jockey)	20.63	42.75	41.38	51.83	0	34.21	3.0	42.42	*29.53*
	Metalaxyl	11.88	47.13	8.88	63.67	0	21.46	1.25	60.67	*26.87*
	Phosphorous acid (Phos-Inject 200)	44.67	44.54	28.96	57.63	0	30.63	3.96	60.88	*33.91*
	Propamocarb (Previcur)	31.79	46.67	24.67	56.75	0	23.75	15.13	47.38	*30.77*
	Rancona Dimension	28.42	49.17	22.88	52	1.67	14.04	12.75	43.63	*28.07*
	Rovral	46.00	46.13	32.04	59.58	2.50	33.25	24.0	59.42	*37.87*
	Syn-A16874F	29.00	55.21	21.88	50.63	3.0	25.75	19.75	57.25	*32.81*
	Thiram	32.12	64.29	12.17	56.25	0	14.0	23.13	30.71	*29.08*
Mean		*29.94*	*51.13*	*24.77*	*57.62*	*1.77*	*26.23*	*14.55*	*46.06*	*31.51*
Shoot Dry Weight (mg)	Nil	45.88	46.27	28.9	30.81	34.83	38.55	47.22	42.57	*39.38*
	Dividend	45.7	42.64	33.59	28.56	38.22	32.99	37.65	39.6	*37.37*
	Fluquinconazole (Jockey)	38.27	42.33	39.13	40.05	29.32	26.62	38.6	40.72	*36.88*
	Metalaxyl	38.35	38.14	39.26	45.49	26.11	29.2	47.64	41.1	*38.16*
	Phosphorous acid (Phos-Inject 200)	41.53	40.39	46.00	33.89	30.97	32.54	40.07	42.42	*38.48*
	Propamocarb (Previcur)	36.7	44.39	48.36	40.68	33.11	28.1	43.28	41.41	*39.50*
	Rancona Dimension	39.73	47.11	35.33	28.85	35.32	31.14	43.75	35.61	*37.11*
	Rovral	42.45	51.04	44.26	41.73	34.26	30.54	50.21	46.51	*42.63*
	Syn-A16874F	43.17	48.33	32.38	36.47	34.47	31.57	48.3	43.95	*39.83*
	Thiram	47.00	46.4	41.6	51.35	35.04	34.08	46.31	34.69	*42.06*
		44.70	37.79	31.53	40.86	41.88	38.88	33.17	44.30	*39.14*
Mean		*44.70*	*37.79*	*31.53*	*40.86*	*41.88*	*38.88*	*33.17*	*44.30*	*39.14*

4.1.5 Fungicide seed dressings – *Phytophthora clandestina*

Fungicides overall significantly reduced the levels of tap and lateral root disease but had no significant effect on the level of nodulation nor upon the plant shoot or root size (Tables 6a,b). Further, there were significant interactions of fungicide with pathogen, fungicide with variety, pathogen with variety and of fungicide with pathogen and variety (Tables 6a, b). In relation to both tap and lateral root disease, Seaton Park was highly resistant to root disease caused by *P. clandestina* with reasonable resistance expressed in both Meteora and Riverina, but Woogenellup was highly susceptible. For both tap and lateral root disease, Metalaxyl gave the greatest reductions in root disease, significantly so for Woogenellup, Meteora and Riverina. Phosphorous acid significantly reduces lateral root disease in Meteora, Riverina and Woogenellup but only for Meteora in relation to tap root disease (Table 6b, Fig. 1 and 2).

Table 6a. *Phytophthora clandestina* root disease seed treatment: Statistical effect of main effects and their interactions on tap and root disease index [TDI% and LDI%), nodulation index (NI%) and dry root weight and dry shoot weight per plant (DRW(mg/p) and DSW (mg/p)]

	TDI%		*LDI%*		*NI%*		*DRW (mg/p)*		*DSW(mg/p)*	
Main factor	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Fungicide	<0.001	2.356	<0.001	2.564	0.187	0.3783	0.459	34.6	0.416	6.725
Pathogen	<0.001	1.054	<0.001	1.147	0.003	0.1692	0.82	15.48	<0.001	3.007
Variety	<0.001	1.49	<0.001	1.622	0.343	0.2393	0.172	21.89	<0.001	4.253
Interactions										
Fungicide*pathogen	0.001	3.332	<0.001	3.626	0.19	0.535	0.482	48.94	0.619	9.51
Fungicide*variety	0.002	4.712	<0.001	5.128	0.12	0.7566	0.422	69.21	0.336	13.45
Pathogen*variety	<0.001	2.107	<0.001	2.294	0.343	0.3384	0.156	30.95	<0.001	6.015
Fungicide*pathogen*variety	0.003	6.664	<0.001	7.253	0.12	1.07	0.338	97.87	0.174	19.021

Table 6b. Effect of fungicides on tap and root disease index [TDI% and LDI %), nodulation index (NI %) and dry root weight and dry shoot weight per plant DRW (mg/p) and DSW (mg/p)] in the presence of *Phytophthora clandestina.*

		TDI%		*LDI%*		*DRW (mg/p)*		*DRW (mg/p)*	*DSW(mg/p)*		*DSW (mg/p)*
Fungicide	*Variety*	*Nil*	*Phyto*	*Nil*	*Phyto*	*Nil*	*Phyto*	*Deduction%*	*Nil*	*Phyto*	*Deduction%*
Dividend	Meteora	0.00	19.00	0.00	17.67	30.60	23.30	23.86	71.96	55.10	23.43
	Riverina	0.00	27.00	0.00	28.58	37.80	15.20	59.79	77.18	39.05	49.40
	Seaton Park	0.00	0.50	0.00	0.00	22.60	25.50	0.00	52.26	45.19	13.53
	Woogenellup	0.00	64.67	0.00	73.37	18.30	9.20	49.73	54.58	15.33	71.91
fluquinconazole	Meteora	0.00	22.83	0.00	25.58	26.20	20.40	22.14	70.64	45.37	35.77
	Riverina	0.00	35.25	0.00	30.08	38.10	15.40	59.58	76.66	39.11	48.98
	Seaton Park	0.00	2.25	0.00	0.00	26.20	27.60	0.00	58.73	56.57	3.68
	Woogenellup	0.00	60.25	0.00	71.13	21.20	3.80	82.08	59.71	13.30	77.73
Metalaxyl	Meteora	0.00	15.92	0.00	11.30	28.70	18.20	36.59	70.27	46.16	34.31
	Riverina	0.00	15.17	0.00	14.75	37.30	18.30	50.94	80.35	46.17	42.54
	Seaton Park	0.00	6.83	0.00	1.88	22.70	21.60	4.85	54.26	42.08	22.45
	Woogenellup	0.00	53.33	0.00	32.62	19.80	23.00	0.00	55.74	44.81	19.61
Nil	Meteora	0.00	27.50	0.00	22.88	29.00	28.80	0.69	80.80	62.50	22.65
	Riverina	0.00	27.13	0.00	35.25	34.10	17.50	48.68	73.20	45.56	37.76
	Seaton Park	0.00	1.67	0.00	2.50	31.40	22.80	27.39	62.68	48.29	22.96

	Woogenellup	0.00	64.79	0.00	71.67	22.70	8.20	63.88	63.32	24.26	61.69
Phosphorous acid	Meteora	0.00	13.96	0.00	13.96	30.80	28.90	6.17	74.03	60.73	17.97
	Riverina	0.00	26.58	0.00	26.50	32.60	20.40	37.42	75.81	41.37	45.43
	Seaton Park	0.00	1.50	0.00	0.00	29.50	17.40	41.02	57.76	36.53	36.76
	Woogenellup	0.00	66.46	0.00	59.92	29.40	9.20	68.71	70.09	24.63	64.86
Propamocarb		2.50	26.25	0.00	25.21	40.30	16.40	59.31	73.53	43.77	40.47
	Riverina	0.00	26.67	0.00	33.12	20.10	18.00	10.45	62.49	45.32	27.48
	Seaton Park	0.00	4.29	0.00	2.12	23.70	341.30	0.00	46.84	50.29	0.00
	Woogenellup	0.00	62.50	0.00	73.67	29.20	3.40	88.36	75.87	17.84	76.49
Rancona Dimension	Meteora	0.00	20.88	0.00	18.96	37.00	22.60	38.92	90.52	54.05	40.29
	Riverina	0.00	17.25	0.00	24.00	24.70	25.40	0.00	70.35	58.28	17.16
	Seaton Park	0.00	1.50	0.00	0.00	24.60	22.90	6.91	55.41	41.36	25.36
	Woogenellup	0.00	59.46	0.00	65.50	21.90	5.10	76.71	63.27	19.51	69.16
Rovral	Meteora	0.00	27.33	0.00	29.46	23.20	23.60	0.00	71.40	53.89	24.52
	Riverina	0.00	22.88	0.00	26.92	22.00	26.50	0.00	59.10	58.88	0.37
	Seaton Park	0.00	3.13	0.00	4.62	21.00	22.00	0.00	54.30	43.86	19.23
	Woogenellup	0.00	68.29	0.00	76.38	18.00	9.40	47.78	60.62	21.15	65.11
	Meteora	0.00	28.63	0.00	40.38	27.40	18.20	33.58	71.10	40.27	43.36

Syn-A16874F	Riverina	0.00	26.83	0.00	36.54	35.50	12.80	63.94	77.27	39.66	48.67
	Seaton Park	0.00	6.63	0.00	0.00	27.50	21.70	21.09	55.14	41.96	23.90
	Woogenellup	0.00	58.25	0.00	68.42	22.00	4.40	80.00	64.34	16.08	75.01
	Meteora	0.00	31.33	0.00	31.88	27.40	13.50	50.73	68.62	43.94	35.97
Thiram	Riverina	0.00	37.08	0.00	39.58	25.80	11.70	54.65	63.83	31.11	51.26
	Seaton Park	0.00	1.88	0.00	0.00	30.70	29.20	4.89	58.87	57.02	3.14
	Woogenellup	0.00	60.54	0.00	70.92	33.50	9.90	70.45	66.15	16.91	74.44

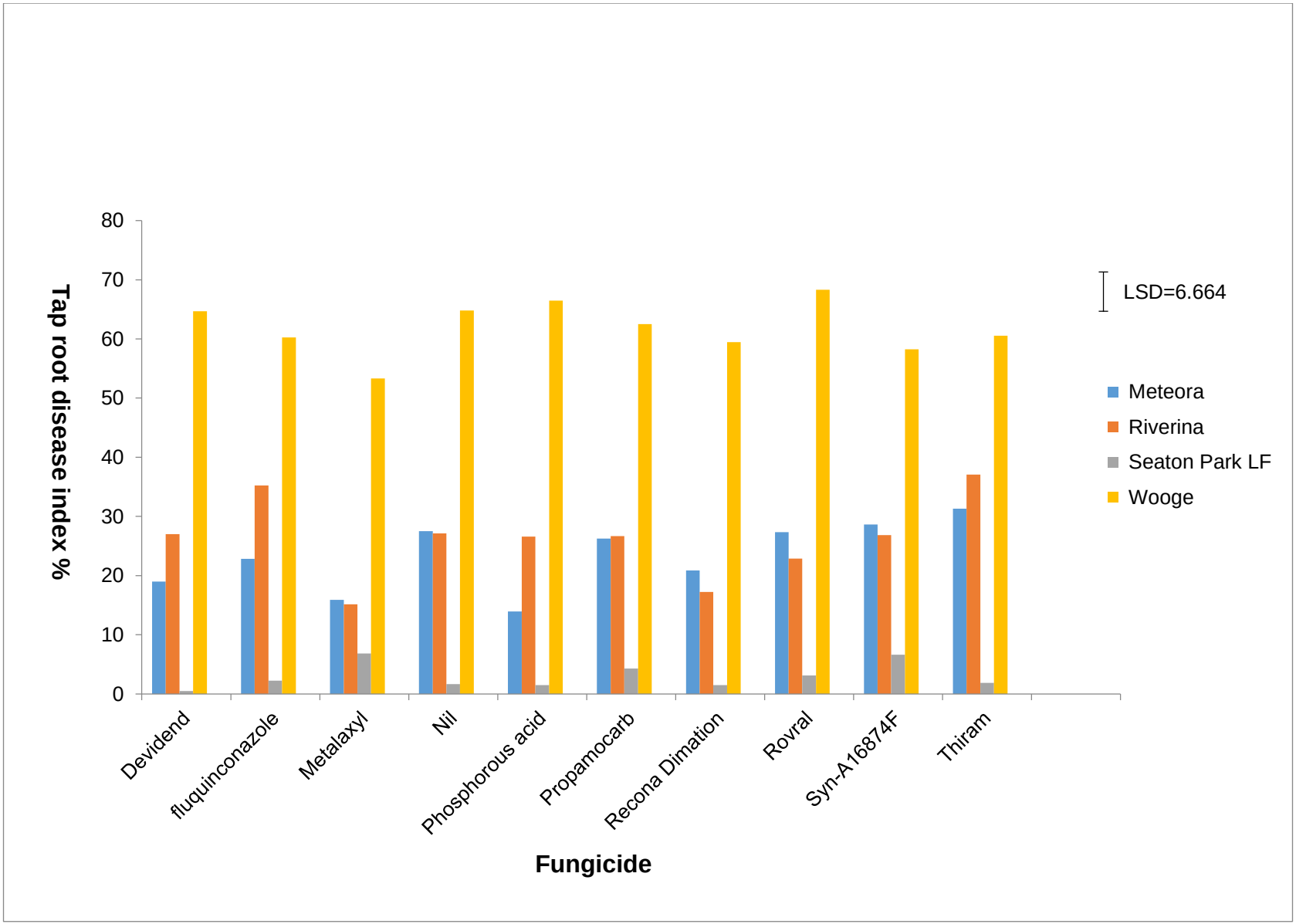


Fig. 1. Effects of fungicide seed treatment on *Phytophthora clandestina* tap root disease index (%) of varieties Meteora, Riverina, Seaton Park and Woogenellup

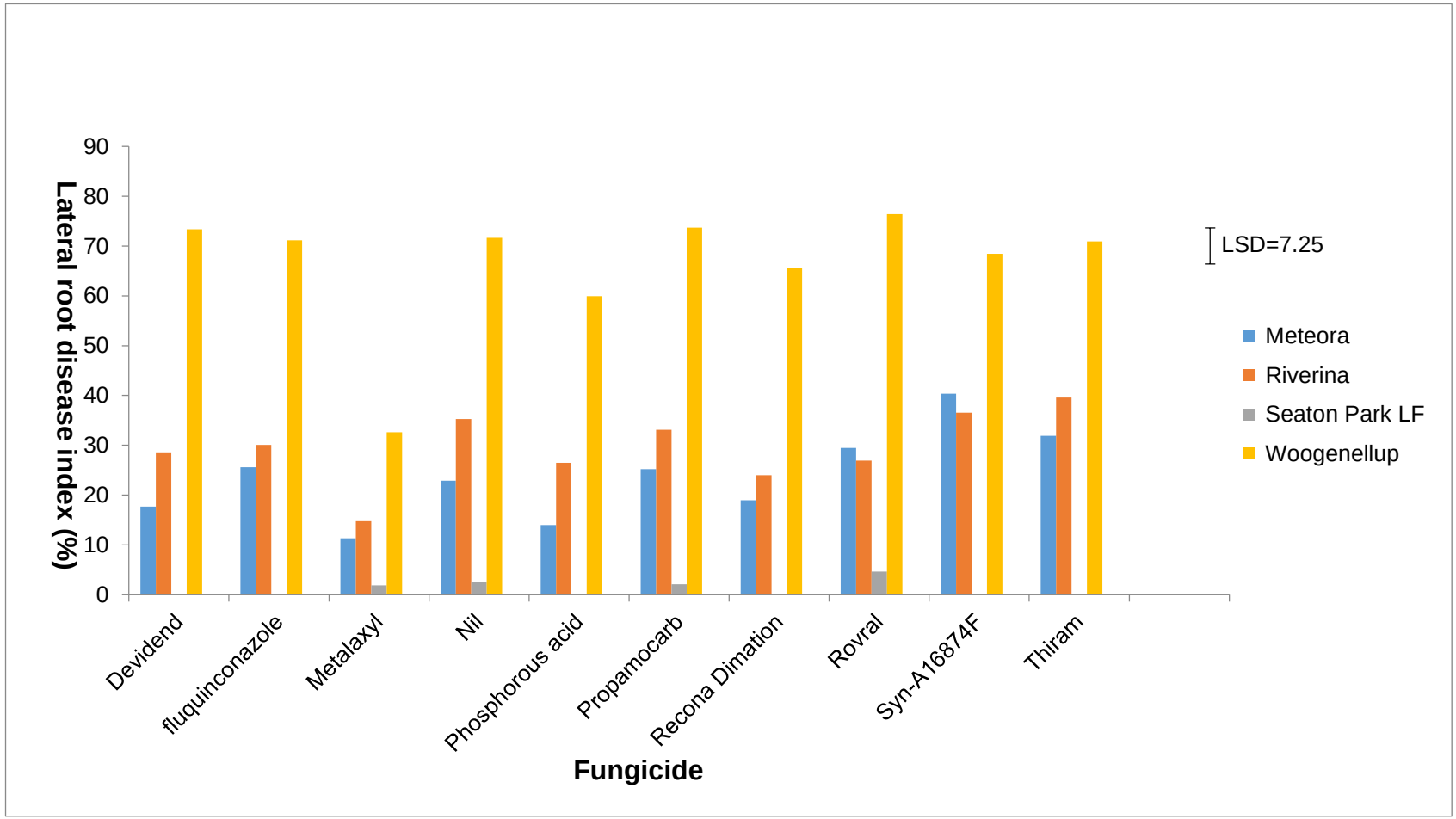


Fig. 2. Effects of fungicide seed treatment on *Phytophthora clandestina* lateral root disease index (%) of varieties Meteora, Riverina, Seaton Park and Woogenellup

4.1.6 Fungicide spray treatments

Pythium irregulare: Fungicide foliar treatments had no significant effect on tap root rot, lateral root rot level, nor on root dry weight, or shoot dry weight or level of nodulation. Disease caused by *P. irregulare* was always going to be the most challenging to manage with foliar sprays. However, the lack of effectiveness of these fungicide treatments applied as seedling sprays perhaps provides an answer for the sometimes lack of field responses to one or more of these foliar treatments historically, most apparent in situations where *P. irregulare* likely predominates. Despite this lack of overall significant effects of the fungicide spray treatments, there were some other significant differences such as those between inoculated with *P. irregulare versus* the uninoculated controls in relation to all the parameters measured *viz*. tap root rot, lateral root rot, germination rate, root dry weight, shoot dry weight and nodulation. Also, different varieties were significant different in terms of germination rate; root dry weight, and nodulation; with Seaton Park the best and Woogenellup second in terms of germination rate; with Riverina and Woogenellup better than Meteora and Seaton Park in terms of root dry weight; and with Riverina the best and Meteora second in terms of nodulation. In general, plants inoculated with *P. irregulare* showed significant root rot disease as expected, a lower germination rate as expected, lower dry root and shoot weights as expected and less nodulation caused by *P. irregulare* (data for this experiment not shown).

Aphanomyces trifolii: *A. trifolii* had no significant effect on the % emergence, but it did on tap and lateral root disease levels and on shoot and root dry weights. Fungicide foliar treatments had no significant effect on germination rate but had significant effects on both tap and lateral root disease levels; and also on root and shoot dry weight (Table 4). Different varieties showed significant differences in terms of germination rate, tap and lateral root disease levels, and also in terms of root and shoot dry weights (Table 6). Overall, Phos-Inject 200 (phosphorous acid) was the best in protecting against both tap and lateral root disease caused by *A. trifolii*, followed by Previcur (propamocarb); and effects were similar in terms of effects on dry root weights (Table 5). In terms of increasing shoot dry weight production, Rovral was best, followed by Phos-Inject 200 and Previcur; while Metalaxyl was the least effective spray fungicide for protecting tap and lateral roots from root disease caused by *A. trifolii* and for increasing root and shoot dry weight production (Table 7).

Phytophthora clandestina: Fungicide spray treatments were disappointing against *P. clandestina*, their being only a small overall significant effect of phosphorous acid on Meteora and metalaxyl on Woogenellup to reduce lateral root disease in those particular variety-chemical combinations, but there were no effects of chemicals on tap root disease (Table 8 Fig. 3).

Table 6. Statistical main effects and interactions for fungicidal spray treatments (P-value and LSD value) against *Aphanomyces trifolii*

	Emergence %		*Tap Root Disease Index*		*Lateral Root Disease Index*		*Shoot Dry Weight (mg)*		*Root Dry Weight (mg)*	
Main effects	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*	*P-value*	*LSD =0.05*
Fungicide spray treatment	NS	*	0.043	12.56	0.004	12.33	<.001	3.923	<.001	2.295
Varieties	0.003	8.76	0.013	11.24	<0.001	11.02	<0.001	3.509	<0.001	2.053
Fungicide spray treatment*inoculation	NS	*	NS	*	NS	*	NS	*	NS	*
Fungicide spray treatment*varieties	NS	*	NS	*	NS	*	NS	*	NS	*
Inoculation*varieties	NS	*	NS	*	NS	*	NS	*	0.047	2.903
Fungicide spray treatment*inoculation*varieties	NS	*	NS	*	NS	*	NS	*	NS	*

Table 7. Effect of fungicide spray treatments on emergence rate (%), tap and Lateral root rot disease indices, shoot and root dry weights (mg) infected by *Aphanomyces trifolii*.

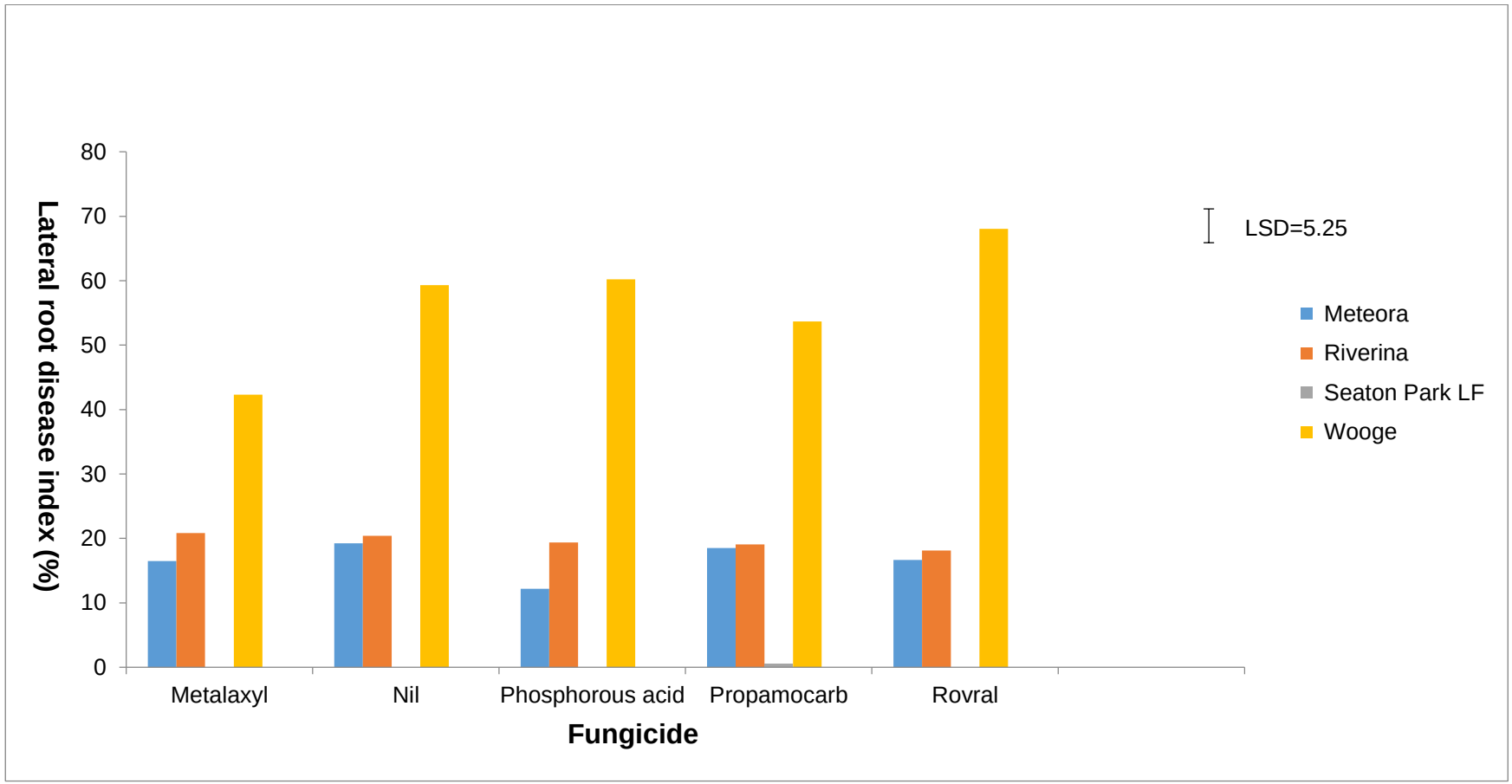
	Varieties	*Meteora*		*Riverina*		*Seaton Park*		*Woogenellup*		
	Inoculum	*Nil*	*Aphanomyces trifolii*	*Nil*	*Aphanomyces trifolii*	*Nil*	*Aphanomyces trifolii*	*Nil*	*Aphanomyces trifolii*	*Mean*
Emergence Rate （%）										
	Nil	85.0	70.0	75.0	72.5	82.5	77.5	80.0	77.5	*78.6*
	Metalaxyl	82.5	87.5	85.0	72.5	80.0	80.0	70.0	80.0	*78.6*
	Phosphorous acid (Phos-Inject 200)	80.0	82.5	85.0	67.5	100	90.0	80.0	90.0	*84.6*
	Propamocarb (Previcur)	87.5	80	70.0	67.5	92.5	95.0	87.5	85.0	*83.6*
	Rovral	82.5	87.5	85.0	72.5	80.0	80.0	70	80.0	*78.6*
Mean		83.5	81.5	77.0	70.5	91.0	86.5	76	82.5	*81.0*
Tap Root Disease Index	Nil	0	23.3	0	45.6	0	44.1	0	54.1	*41.8*
	Metalaxyl	0	31.5	0	40.0	0	20.5	0	39.5	*32.9*
	Phosphorous acid (Phos-Inject 200)	0	8.3	0	15.0	0	34.8	0	28.9	*21.8*
	Propamocarb (Previcur)	0	30.6	0	39.7	0	15.5	0	34.8	*30.2*
	Rovral	0	20.4	0	36.0	0	24.3	0	43.7	*31.1*
Mean		*0*	*22.8*	*0*	*35.3*	*0*	*27.8*	*0*	*40.2*	*31.5*
Lateral Root Disease Index	Nil	0	40.9	0	54.1	0	32.9	0	64.1	*48.0*

	Metalaxyl	0	41.2	0	54.4	0	18.8	0	32.7	*36.8*
	Phosphorous acid (Phos-Inject 200)	0	20.1	0	30.1	0	23.4	0	23.6	*24.3*
	Propamocarb (Previcur)	0	47.2	0	51.2	0	16.0	0	30.9	*36.3*
	Rovral	0	50.5	0	64.0	0	21.2	0	34.0	*42.4*
Mean		*0*	*40.0*	*0*	*50.8*	*0*	*22.5*	*0*	*37.1*	*37.6*
Shoot Dry Weight (mg)	Metalaxyl	22.8	25.4	16.3	26.9	17.7	18.6	21.3	23.8	*21.6*
	Nil	40.1	40.5	38.3	41.3	35.7	31.4	44.7	41.6	*39.2*
	Phosphorous acid (Phos-Inject 200)	40.4	47.1	34.2	39.0	38.0	24.0	44.5	38.5	*38.2*
	Propamocarb (Previcur)	39.9	43.7	37.8	37.9	33.5	29.1	40.9	34.2	*37.1*
	Rovral	47.1	45.4	42.9	32.1	37.7	34.9	52.5	42.4	*41.9*
Mean		*38.1*	*40.4*	*33.9*	*35.4*	*32.5*	*27.6*	*40.8*	*36.1*	*35.6*
Root Dry Weight (mg)	Nil	18.7	21.0	17.3	17.9	17.6	14.7	21.3	22.7	*18.9*
	Metalaxyl	8.4	12.7	7.4	13.1	9.5	9.8	12.1	13.4	*10.8*
	Phosphorous acid (Phos-Inject 200)	17.2	23.6	16.0	20.1	17.1	13.6	21.1	17.7	*18.3*
	Propamocarb (Previcur)	17.1	19.0	14.6	21.5	15.1	13.3	17.9	16.0	*16.8*
	Rovral	19.6	17.8	19.8	17.8	14.6	16.7	22.6	17.7	*18.3*
Mean		*16.2*	*18.8*	*15.0*	*18.1*	*14.8*	*13.6*	*19.0*	*17.5*	*16.6*

Table 8. Fungicide spray treatments *Phytophthora clandestina*. Statistical effect of main effects and their interactions on tap and root disease index [TDI% and LDI %), nodulation index (NI %) and dry root weight and dry shoot weight per plant (DRW (mg/p) and DSW (mg/p)]

	TDI%		*LDI%*		*NI%*		*Germination rate%*		*DRW (mg/P)*		*DSW (mg/P)*	
	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$
Main factor												
fungicide	0.883	2.154	0.028	1.856	0.082	2.776	0.922	6.8	0.303	6.25	0.326	12.71
pathogen	<.001	1.362	<.001	1.174	0.014	1.756	0.607	4.3	<.001	3.95	<.001	8.04
variety	<.001	1.926	<.001	1.66	<.001	2.483	<.001	6.08	<.001	5.59	<.001	11.37
Interaction												
fungicide*pathogen	0.883	3.046	0.028	2.624	0.279	3.926	0.462	9.61	0.499	8.83	0.685	17.98
fungicide*variety	0.239	4.307	<.001	3.711	0.433	5.552	0.875	13.6	0.272	12.49	0.441	25.43
pathogen*variety	<.001	2.724	<.001	2.347	<.001	3.511	0.51	8.6	<.001	7.9	<.001	16.08
fungicide*pathogen*variety	0.239	6.091	<.001	5.249	0.373	7.852	0.674	19.23	0.092	17.67	0.681	35.96

Fig. 3. Fungicide spray effect on *Phytophthora clandestina* lateral root disease index (%) of varieties Meteora, Riverina, Seaton Park and Woogenellup



4.2 Cultural control treatments

4.2.1 Cultivation – field and glasshouse

***Controlled environmental room test for field cores*:** Significances of treatments in the field core experiment are shown in Table 9 and treatment means in Table 10. Cultivation significantly (P<0.001) affected subterranean clover germination rate, tap and lateral root disease severity (expressed as % disease indices), and dry root and shoot weights. While germination rate (%) was higher on non-cultivated soil, tap and lateral root diseases were significantly more severe in non-cultivated soil, subterranean clover root and shoot dry weights were significantly higher in cultivated soil. Germination rate (%) was significantly (P<0.005) affected by three way interactions between cultivation, cultivar and field site with most varieties grown on all five sites having a higher germination rate on non-cultivated soil. Tap root disease severity was also significantly (P<0.05) affected by three way interaction of cultivation with cultivar and field site. Generally, cultivation of soil suppressed tap root disease (i.e., lower disease index) across all tested varieties on soils from all sites with the exception of Woogenellup from three sites (sites A, B and D), Cultivar Seaton Park from sites B and M showed significantly lower tap root disease in cultivated soil than non-cultivated soil. Varieties Meteora and Riverina also showed significantly lower tap root disease in cultivated soil across the five sites.

***Field trials*:** In terms of the overall effects of treatments on subterranean clover productivity, the best treatment was cultivation plus fumigation at Coolup which reduced tap and lateral root disease indices and increased nodulation indices and root and shoot dry weights across the three tested varieties (Tables 11 and 12) while untreated at Wagerup was the worst. There was significantly more severe tap (P<0.001) and lateral (P<0.001) root disease at the Wagerup site compared with the Coolup site. As such, there were corresponding significant benefits in terms of reduced root disease (P<0.001) and increased nodulation (P<0.001) from cultivation expressed as increased plant shoot (P<0.001) and root (P<0.001) dry weights at the Coolup compared with the Wagerup site (Tables 11 and 12). There were no significant differences (P>0.05) between varieties in terms of severity of tap or lateral root disease nor for extent of nodulation. Cultivation plus fumigation at both sites had higher germination rate than other treatments, with non-treated at Wagerup the lowest germination with exception of Riverina. Germination rate was reduced by fumigation in comparison with the untreated treatment, suggesting that Riverina seed could have been particularly sensitive to any residual fumigation (Metham sodium) remaining. However, germination rates were increased by fumigation in comparison with the untreated treatment for the other tested varieties, while there were no significant differences in terms of germination for the same treatment on the same cultivar grown across both sites (Tables 11 and 12).

There were significant (all P <0.001) negative correlations between tap root disease and nodulation ($y = -0.5911x + 41.193$, $R^2 = 0.8496$); between lateral root disease and nodulation ($y = -0.7325x + 53.232$, $R^2 = 0.5846$); between tap root disease and root dry weight ($y = -0.7325x + 53.232$, $R^2 = 0.5846$); between tap root disease and shoot dry weight ($y = -0.0373x + 2.892$, $R^2 = 0.854$); between lateral root disease and root dry weight ($y = -0.1853x + 11.862$, $R^2 = 0.8277$); between lateral root disease and shoot dry weight ($y = -0.0481x + 3.7372$, $R^2 = 0.6374$); but a significant positive correlation between shoot and root dry weights ($y = 4.6678x - 2.0392$, $R^2 = 0.8543$).

Table 9. *Field cores:* Statistical main effects and interactions of simulated cultivation, subterranean clover cultivar and field site on germination percentage, disease indexes of tap and lateral roots, nodulation index and dry root and shoot weights.

	Germination rate (%)		*Tap root disease index (%)*		*Lateral root disease index (%)*		*Nodulation index (%)*		*Root dry weight (mg/p)*		*Shoot dry weight (mg/p)*	
	P-value	$LSD_{0.05}$	*P-value*	$LSD_{0.05}$	*P-value*	$LSD_{0.05}$	*P-value*	$LSD_{0.05}$	*P-value*	$LSD_{0.05}$	*P-value*	$LSD_{0.05}$
Main effects												
Cultivation	<.001	3.474	<.001	2.235	<.001	1.803	0.159	1.291	<.001	6.07	0.001	10.7
Cultivar	<.001	4.913	<.001	3.16	<.001	2.549	0.065	1.825	<.001	8.58	<.001	15.14
Site	<.001	5.493	<.001	3.533	<.001	2.85	<.001	2.041	<.001	9.59	<.001	16.92
Interaction												
Cultivation*Cultivar	0.345	6.948	0.221	4.469	0.885	3.605	0.217	2.581	0.039	12.14	0.359	21.4
Cultivation*site	0.259	7.768	0.141	4.997	0.568	4.031	<.001	2.886	<.001	13.57	<.001	23.93
Cultivar*Site	0.114	10.986	0.092	7.067	0.028	5.701	0.002	4.081	0.011	19.19	0.846	33.84
Cultivation*Cultivar*Site	0.002	15.537	0.02	9.994	0.78	8.062	0.329	5.772	0.12	27.14	0.141	47.86

Table 10. *Field cores:* Effects of simulated cultivation, field site (A,B,D,M,R) and subterranean clover cultivar and their interactions on subterranean clover germination rate (%), disease indexes of tap (TDI%) and lateral (LDI%).

Cultivation	*Site*	*Cultivar*	*Germination rate (%)*	*TDI (%)*	*LDI (%)*
	A	Meteora	39	11.67	16.83
Cultivated	A	Riverina	39	14.33	20.53
	A	Seaton Park	60	23.15	28.28
	A	Woogenellup	51	23.98	29.72
	A	Meteora	57	20.31	24.43
Non-cultivated	A	Riverina	55	23.88	26.19
	A	Seaton Park	67	26.8	31.44
	A	Woogenellup	61	20.6	31.04
	B	Meteora	72	10.47	9.3
Cultivated	B	Riverina	56	10.45	16.5
	B	Seaton Park	44	15.53	23.55
	B	Woogenellup	63	23.38	25.57
	B	Meteora	65	6.17	10.84
Non-cultivated	B	Riverina	47	9.93	12.57
	B	Seaton Park	81	29.87	30.55
	B	Woogenellup	69	17.92	23.91
	D	Meteora	52	16.56	23.73
Cultivated	D	Riverina	38	14.73	19.21
	D	Seaton Park	42	23.8	27.2
	D	Woogenellup	48	34.54	33.65
	D	Meteora	57	21.98	31.61
Non-cultivated	D	Riverina	54	17.88	22.01
	D	Seaton Park	71	23.9	29.84
	D	Woogenellup	53	29.04	36.45
	M	Meteora	55	14.45	18.53
Cultivated	M	Riverina	31	15.28	20.7
	M	Seaton Park	67	8.22	24.5
	M	Woogenellup	67	16.12	25.87
	M	Meteora	63	16.18	18.66
Non-cultivated	M	Riverina	56	20.13	26.07
	M	Seaton Park	72	26.23	30.92
	M	Woogenellup	71	27.1	32.19
	R	Meteora	34.52	12.69	21.83
Cultivated	R	Riverina	32	17.34	20.63
	R	Seaton Park	51	24.32	29.86
	R	Woogenellup	36.75	20.17	29.68
	R	Meteora	60	28.25	27.96
Non-cultivated	R	Riverina	50	18.47	27.07
	R	Seaton Park	61	22.65	32.47
	R	Woogenellup	59.86	25.87	35.27

Table11. *In-field trials*: Statistical accumulated analysis of variance on main effects and interactions of soil treatments on germination rate (%), tap root disease index (%), lateral root disease index (%), nodulation index (%), root dry weight (mg) and shoot dry weight (mg) of three subterranean clover varieties (Riverina, Seaton Park and Woogenellup) at two field sites Coolup and Wagerup, 90 and 100 km south of Perth, respectively.

	Germination rate (%)		*Tap Root Disease Index*		*Lateral Root Disease Index*		*Nodulation Index*		*Root Dry Weight (mg)*		*Shoot Dry Weight (mg)*	
Main effects	P-value	LSD=0.05	P-value	LSD=0.05	P-value	LSD=0.05	P-value	LSD=0.05	P-value	LSD=0.05	P-value	LSD=0.05
Varieties	<0.001	6.412	0.778	6.607	0.623	7.456	<0.001	5.03	0.023	0.2298	0.109	1.076
Treatment	0.024	6.58	<0.001	6.781	<0.001	7.652	0.014	5.162	0.017	0.2359	0.039	1.104
Varieties*Treatments	<0.001	11.37	0.008	11.72	0.056	13.22	0.353	8.92	0.014	0.4076	0.176	1.908
Site	0.209	5.252	<0.001	5.412	<0.001	6.108	<0.001	4.12	<0.001	0.1883	<0.001	0.8811
Site*Soil treatment	0.433	7.227	0.037	7.448	0.549	8.405	0.039	5.67	0.028	0.2591	0.013	1.212
Site*Cultivar	0.259	9.085	0.281	9.362	0.055	10.56	0.711	7.127	0.001	0.3256	0.188	1.524
Site*Cultivar*Soil treatment	0.228	12.653	0.947	13.04	0.047	14.72	0.577	9.926	0.159	0.4536	0.298	2.123

Table 12. *In-field trials*: Effect of subterranean clover cultivar, soil treatments and their interactions on germination rate (%), tap root disease index (%), lateral root disease index (%), nodulation index (%), root dry weight (mg) and shoot dry weight (mg) of three subterranean clover varieties (Riverina, Seaton Park and Woogenellup) at two field sites Coolup and Wagerup, 90 and 100 km south of Perth, respectively (figures from ANOVA and also confirmed by predictions from regression model).

Location	*Soil treatment*	*Cultivar*	*Germination rate %*	*Tap root disease index %*	*Lateral root disease index %*	*Nodulation index %*	*Root Dry Weight (mg/plant)*	*Shoot Dry Weight (mg/plant)*
Coolup	Cultivation	Riverina	8.75	19.33	34.36	25.04	2.34	6.78
		Seaton Park	41.50	22.60	42.68	34.58	2.23	9.97
		Woogenellup	49.25	17.20	43.56	29.58	2.25	10.03
		Mean	*33.17*	*19.71*	*40.2*	*29.73*	*2.27*	*8.93*
Coolup	Cultivation + Fumigation	Riverina	4.50	3.00	18.67	34.33	3.48	11.47
		Seaton Park	56.50	7.94	28.89	43.15	2.05	9.96
		Woogenellup	56.50	10.16	33.23	39.29	2.55	12.64
		Mean	*39.17*	*7.03*	*26.93*	*38.92*	*2.69*	*11.36*
Wagerup	Untreated	Riverina	18.67	57.51	53.14	4.32	0.93	1.66
		Seaton Park	36.67	51.91	53.04	10.58	0.91	2.24
		Woogenellup	26.00	74.43	69.94	5.31	0.48	1.54
		Mean	*27.11*	*61.28*	*58.71*	*6.74*	*0.77*	*1.81*
Wagerup	Cultivation	Riverina	4.33	52.96	68.52	6.30	0.93	1.75
		Seaton Park	51.67	44.10	55.21	19.00	1.28	2.4
		Woogenellup	49.00	36.96	50.99	13.84	1.03	2.48
		Mean	*35.00*	*44.67*	*58.24*	*13.05*	*1.08*	*2.21*
Wagerup	Cultivation + Fumigation	Riverina	3.00	48.89	46.67	3.33	1.08	1.89
		Seaton Park	48.33	44.29	39.04	14.47	1.06	2.26
		Woogenellup	58.33	40.10	37.37	20.69	0.98	2.41
		Mean	*36.55*	*44.43*	*41.03*	*12.83*	*1.04*	*2.19*

4.2.2 Cultural control treatments – species composition

Brief summary: The species composition study on the effects of the relative proportions of subterranean clover to annual ryegrass showed that the levels of tap and lateral root disease on subterranean clover and also their shoot dry weights were affected by the different sward compositions. In particular, tap and lateral root disease was most severe when subterranean clover percent composition was as low (e.g., 20% subterranean clover and 80% ryegrass). When subterranean clover composition was 100% (i.e., no ryegrass at all), shoot dry weight per plant for subterranean clover was the greatest and the level of tap and lateral root disease much less. This is the first study to demonstrate the reasons for why, as observed in the field, the level of root disease greatly increases and the rate of decline in deteriorating pastures rapidly accelerates as the subterranean clover content diminishes.

Overview of main findings: Overall, the subterranean clover/ryegrass compositions significantly (P<0.001) effected the levels of tap and lateral root disease on subterranean clover (Table 13) and also their shoot dry weight (Table 13). Tap and lateral root disease was most severe when subterranean clover composition was 20% (20% subterranean clover and 80% ryegrass) (Figs 4 & 5, Table 14). When subterranean clover composition was 100% (no ryegrass at all), shoot dry weight per plant for subterranean clover was the greatest (Fig. 6, Table 14). There was a significant (P<0.001) interaction between subterranean clover composition and pathogen (i.e., *Rhizoctonia vs Pythium*) in relation to tap and lateral root disease (Tables 13), where subterranean clover composition increases, tap and lateral root diseases decreases (Figs 7 & 8, Table 14). It is noteworthy that there was much faster decrease in both tap and lateral root disease with increasing % subterranean clover composition in relation to *P. irregulare* rather than *R. solani*. This helps to explain for the first time the field observations that root disease becomes more severe in conjunction with an accelerating overall decline in subterranean clover pastures as the % subterranean clover in the pasture decreases, particularly in areas where *P. irregulare* predominates. There was a significant (P<0.001) interaction between subterranean clover composition and variety in relation to nodulation, and dry root and shoot weights (Table 13); where nodulation index (%) was greatest when subterranean clover was 40% for varieties Riverina and Woogenellup and where it was 100% for varieties Meteora and Seaton Park(Figs 9, 10, 11, and 12). There was a significant (P<0.001) three way interaction between subterranean clover composition with pathogen and variety in relation to nodulation index (%) and root and shoot dry weights (Table 13). In the presence of *R. solani*, Seaton Park and Woogenellup were still able nodulate at a low level when subterranean clover composition was only 60% and 40%, respectively; but Meteora and Riverina were not able to nodulate at these same % subterranean clover compositions. However, in the presence of *P. irregulare*, all tested varieties were able to nodulate at 100% subterranean clover composition, with Meteora having the best nodulation, followed by Seaton Park, and with Riverina able to nodulate comparably highly at 40% subterranean clover composition followed by Woogenellup (Table 14, Fig 13). When root rot pathogens were absent (Nil comparison control treatment), nodulation indices for Meteora and Woogenellup showed a strong negative correlation with % subterranean clover composition (R^2 = 0.909; R^2 = 0.966, respectively; Figs 14 & 15); Seaton Park showed very high positive correlation with increasing percentage composition (R^2 = 0.958, Fig. 16); but Riverina showed no significant correlation (R^2 = 0.028, Fig. 17) between percentage composition and nodulation. This result suggests that Riverina is most robust variety in terms of ability to nodulate across different % subterranean clover compositions; in contrast to Meteora and Woogenellup that have greater nodulation at low percentage subterranean clover composition and Seaton Park that has greater nodulation at high percentage subterranean clover composition.

In relation to the impact of pasture composition on soil-borne *Pythium* and *Rhizoctonia* root rot diseases and quantity of pathogen in the soil (expressed as DNA weight) when using subterranean

clover composition at 20% and 100% were tested for *Pythium* and *Rhizoctonia* DNA (pg/g soil) weight by "Predicta B", it was found that DNA weight were significantly differed in pasture composition, pathogen, variety, and also their interactions (Table 15). Tap and lateral root disease indices and DNA weight were lower in 100% than 20% subterranean clover composition for all tested varieties (Fig. 18). Varieties Seaton Park and Riverina suffered lest *Pythium* root rot and contain lest *Pythium* DNA weight for 10% subterranean clover in soil in 100 % composition (Fig. 18). Both *Pythium* and *Rhizoctonia* DNA weights were positively correlated with tap and lateral root diseases. "Predicta B" can give about 70 to 80 % indication for *Pythium* root disease (Fig. 19, 20) and about 40% for *Rhizoctonia* (Fig. 21, 22). For 10% subterranean clover, the root rot disease indices were only about 10 to 15 % when DNA weight was around 500 pg /g soil for *Pythium* but were 60 to 80% disease indices when DNA weight was only about 100pg /g soil for *Rhizoctonia*. Therefore, when use "Predicta B" for soil testing, it is critical to bear in mind that **Rhizoctonia** root rot is effected by more factors than just it's DNA weight as indicated by "Predicta B" and also a relatively low amount of 100pg DNA weight would be sufficient to cause severe root rot.

Table 13. Statistical main effects and interactions of pathogen (*Pythium irregulare, Rhizoctonia*), subterranean clover variety and subterranean clover variety/ rye grass composition on germination rate (%), disease indices (%) of tap and lateral root rot, nodulation index (%) and dry weights (mg/p) of root and shoot

	Germination rate%		*Tap root disease index (%)*		*Lateral root disease index (%)*		*Nodulation index*		*Root dry weight (mg)*		*Shoot dry weight (mg)*	
	P-value	*LSD at P=0.05*	*P-value*	*LSD at P=0.05*	*P-value*	*LSD at P=0.05*	*P-value*	*LSD at P=0.05*	*P-value*	*LSD at P=0.05*	*P-value*	*LSD at P=0.05*
Main effects												
composition	0.887	7.63	<.001	4.58	<.001	4.929	0.122	1.954	0.165	6.32	0.001	8.96
pathogen	<.001	5.91	<.001	3.548	<.001	3.818	<.001	1.514	<.001	4.89	<.001	6.94
variety	0.412	6.83	0.078	4.097	0.03	4.409	0.014	1.748	<.001	5.65	<.001	8.01
Interactions												
composition*pathogen	0.073	13.22	<.001	7.934	<.001	8.538	0.115	3.385	0.014	10.94	<.001	15.52
composition*variety	0.25	15.26	0.29	9.161	0.158	9.858	<.001	3.909	<.001	12.63	<.001	17.92
pathogen*variety	0.356	11.82	0.021	7.096	0.045	7.636	0.082	3.028	<.001	9.78	0.013	13.88
composition*pathogen*variety	0.402	26.44	0.15	15.867	0.071	17.075	<.001	6.77	<.001	21.88	<.001	31.03

Table 14. Effects of pathogen (*Pythium irregulare, Rhizoctonia)*, subterranean clover variety and subterranean clover variety/rye grass and their interactions on pasture germination rate (PGR)(%), disease indexes (%) of pasture tap (TDI) and lateral (LDI) roots, pasture nodulation index (NI %), root dry weight (mg) per plant (RDW/p) and shoot dry weights (mg) per plant (SDW/p).

Pasture composition	*Pathogen*	*Variety*	*PGR (%)*	*TDI (%)*	*LDI (%)*	*RDW (mg/p)*	*SDW (mg)/p*	*NI (%)*
		Meteora	100	0	0	104.4	167.2	7.5
	Nil	Riverina	87.5	0	0	28.3	78.8	0.08
		Seaton Park	100	0	0	34.3	99.6	0
		Woogenellup	75	0	3.22	96.5	175	14.2
		Meteora	75	52.5	52.5	35.1	79.4	0
20%	*Pythium*	Riverina	62.5	45	52.5	41.8	62.8	5
		Seaton Park	50	62.5	72.5	26.5	32.3	5
		Woogenellup	50	75	65	19.3	30.4	2.5
		Meteora	12.5	97.5	97.5	1.4	5.5	0
	Rhizoctonia	Riverina	0	100	100	0	0	0
		Seaton Park	0	100	100	0	0	0
		Woogenellup	25	95	95	5.7	6	0
		Meteora	75	0	0	56.6	151.7	4.37
	Nil	Riverina	87.5	0	0	45	131.3	7.5
		Seaton Park	93.8	0	0	70.9	124.1	1.25
		Woogenellup	81.3	0	0	92.5	171.7	12.5
		Meteora	62.5	33.75	38.75	34.4	82.1	0
40%	*Pythium*	Riverina	68.8	31.25	31.25	28.9	61.8	18.8
		Seaton Park	93.8	18.75	23.75	38.7	59.9	7.5
		Woogenellup	68.8	30	42.5	27.2	41.5	12.5
		Meteora	0	100	100	0	0	0
	Rhizoctonia	Riverina	12.5	80	85	7.1	7.9	0
		Seaton Park	6.3	97.5	95	0.2	2.1	0
		Woogenellup	18.8	85	85	7.9	16.2	1.25
		Meteora	75	2.5	2.5	89.4	123.1	4.17
	Nil	Riverina	66.7	0	0	41.6	93.8	0
		Seaton Park	75	0	0	68.4	128.8	7.92
		Woogenellup	66.7	0	0	47.9	110.2	8.46
		Meteora	75	30.83	34.17	27.8	73.2	0
60%	*Pythium*	Riverina	70.8	22.92	27.92	53.3	96.3	11.3
		Seaton Park	75	13.33	21.67	61.3	93.1	9.17
		Woogenellup	70.8	42.92	43.75	21.7	42.8	10.4
		Meteora	25	88.75	86.25	13.4	19.3	0
	Rhizoctonia	Riverina	4.2	97.5	97.5	8.1	3.7	0
		Seaton Park	12.5	92.5	90.83	10.2	22	2.5
		Woogenellup	8.3	100	100	0.7	0.7	0
		Meteora	81.3	0	0	41	131	0
		Riverina	81.3	0	0	62.1	140.1	8.13

	Nil	Seaton Park	90.6	2.5	1.25	63.4	124.6	14.4
		Woogenellup	87.5	0	0	39.3	107.7	2.5
		Meteora	78.1	21.87	28.75	39.7	88.9	11.9
80%	*Pythium*	Riverina	65.6	27.5	32.5	33	72	0
		Seaton Park	68.8	15.83	23.33	47.5	70.6	11.3
		Woogenellup	62.5	23.75	30.21	41	82.6	2.5
		Meteora	31.3	80	67.5	33.2	51.8	0
	Rhizoctonia	Riverina	0	100	100	0	0	0
		Seaton Park	9.4	91.25	80	11.7	6.5	0
		Woogenellup	3.1	97.5	97.5	1	2.9	0
	Nil	Meteora	67.5	0	0	87	208.9	0
		Riverina	77.5	0	0	65.5	138.7	1.88
		Seaton Park	87.5	0	0	67.7	134.1	16.7
		Woogenellup	92.5	0	0	41.3	104.2	0.5
		Meteora	57.5	25.83	28.54	56.3	111.6	21.5
100%	*Pythium*	Riverina	65	13.5	17.92	35.3	53.9	0.83
		Seaton Park	72.5	6.96	13.88	60.8	76.5	12.9
		Woogenellup	82.5	19.62	31.29	37.4	68.8	1.75
		Meteora	25	82.08	64.17	17.3	32.1	0
	Rhizoctonia	Riverina	20	84.38	80	10.1	21.7	0
		Seaton Park	7.5	93.75	93.75	2.6	7.1	0
		Woogenellup	0	100	100	0	0	0

Table 15. Statistical main effects and interactions of pathogen, variety and subterranean clover composition (with rye grassed) on tap and lateral root disease indices (TD% and LD%) and pathogen DNA weight (pg/g soil) in 10% subterranean clover

	TD%		*LD%*		*DNA (pg/g soil) in 10% subterranean clover*	
Factor	*P0.05*	*LSD0.05*	*P0.05*	*LSD0.05*	*P0.05*	*LSD0.05*
Pasture composition	<0.001	7.45	<0.001	7.6	<0.001	26.21
Pathogen	<0.001	7.45	<0.001	7.6	<0.001	26.21
Variety	NS	*	NS	*	<0.001	37.06
Pasture composition*Pathogen	<0.001	10.54	0.002	10.75	<0.001	37.06
Pasture composition*Variety	NS	*	NS	*	<0.001	52.42
Pathogen*Variety	NS	*	NS	*	<0.001	52.42
Pasture composition*Pathogen*Variety	0.057	21.09	0.028	21.51	<0.001	74.13

Fig. 4. Tap root disease index (%) at diferent %subterranean clover composition

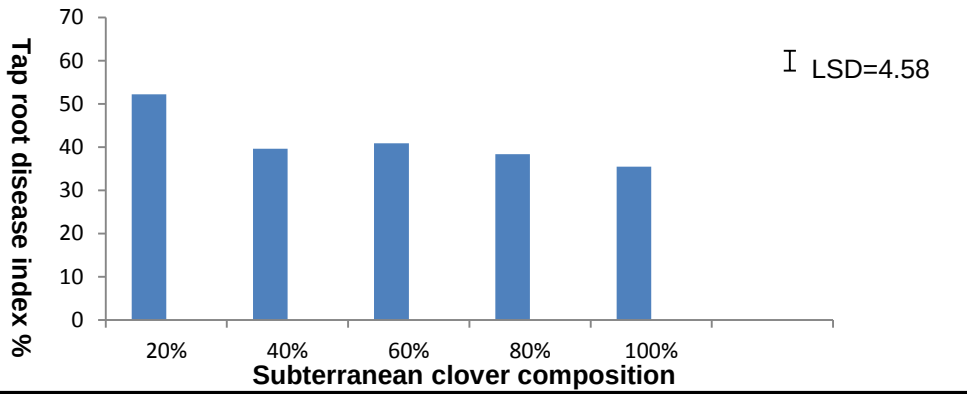


Fig. 5. Lateral root disease index (%) at different % subterranean clover composition

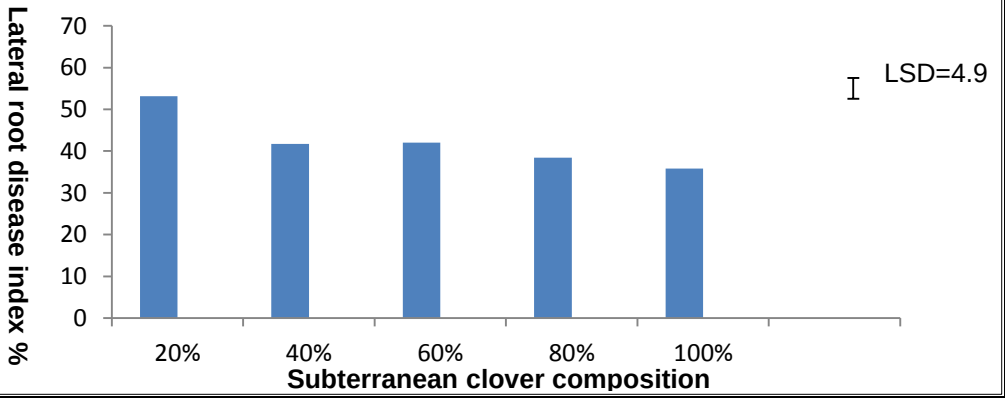


Fig. 6. Shoot dry weight (mg/plant) at different % subterranean clover composition

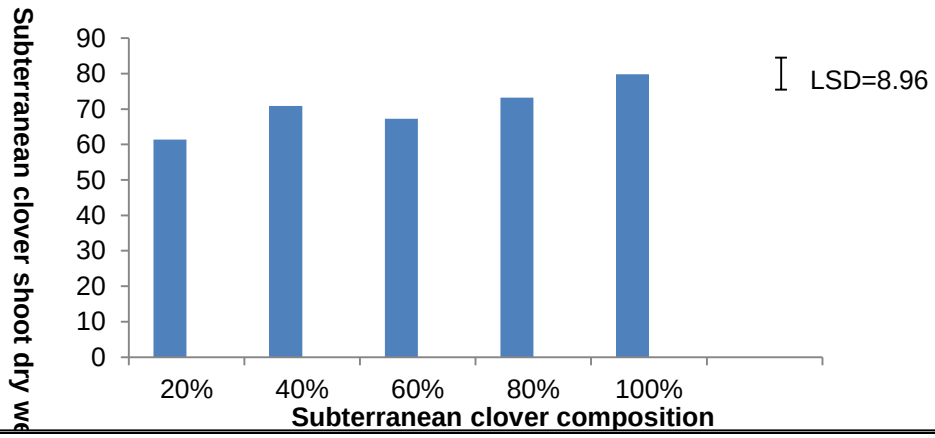


Fig. 7. Correlationbetween tap root disease index (%) and % subterranean clover composition

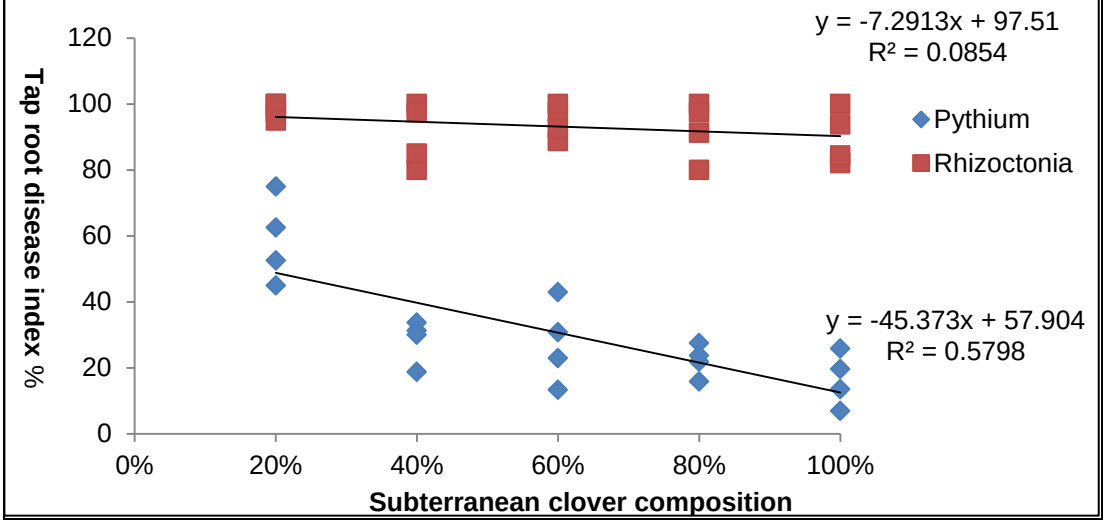


Fig. 8. Correlation between lateral root index (%) and % subterranean clover composition

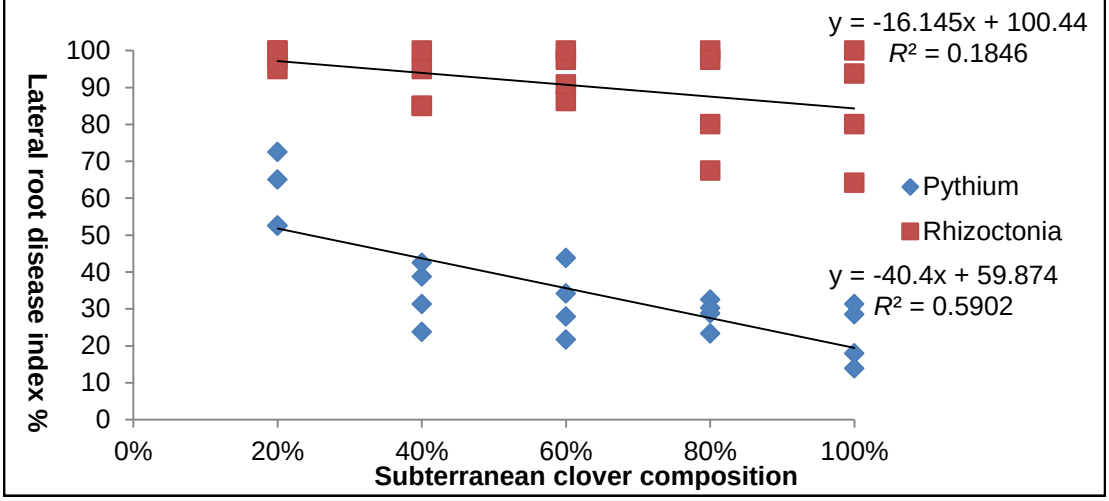


Fig. 9. Riverina nodulation index (%) at different % subterranean clover composition

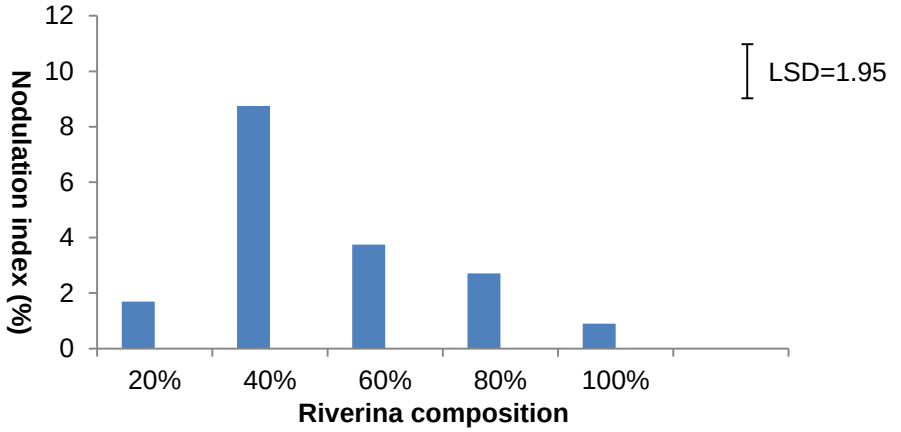


Fig. 10. Woogenellup nodulation index (%) at different % subterranean clover composition

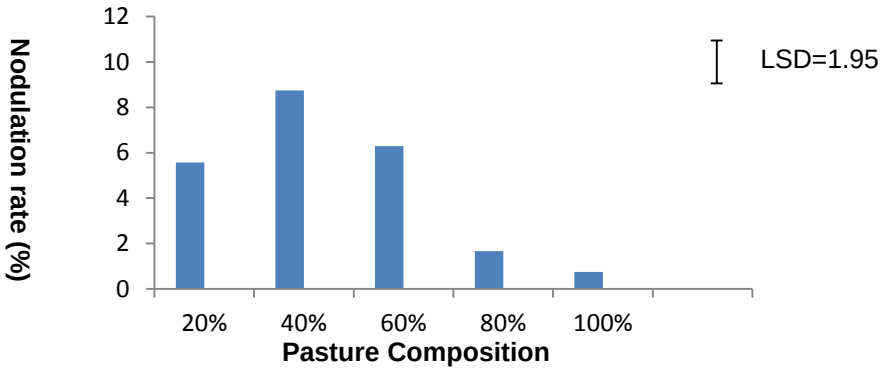


Fig.11. Meteora nodulation index (%) at different % subterranean clover composition

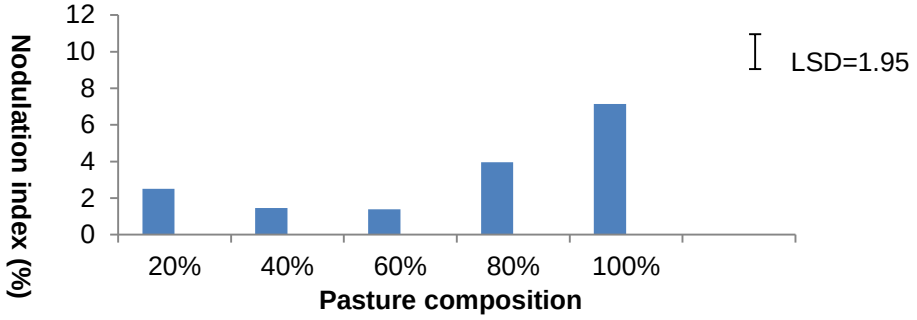


Fig. 12. Seaton Park nodulation index (%) at different % subterranean clover composition

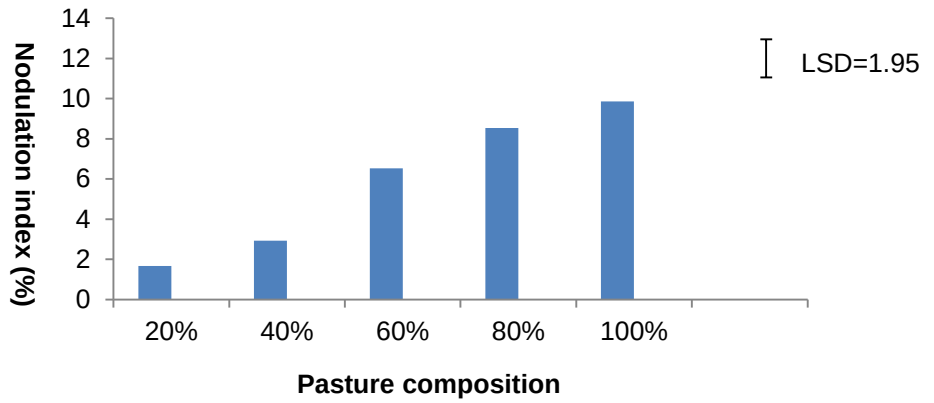


Fig. 13. Nodulation index (%) in presence of root rot pathogens for four subterranean varieties at diferrent % subterranean clover composition

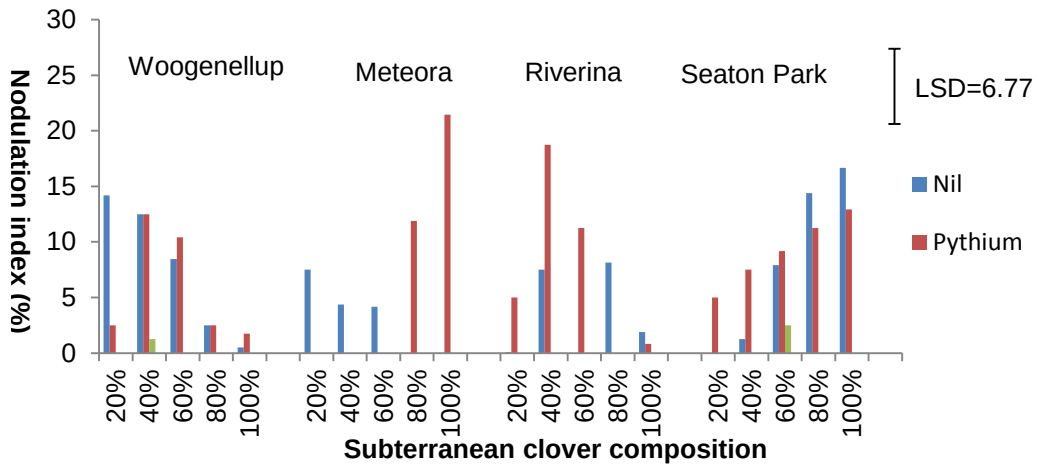


Fig. 14. Relationship between % Meteora composition & nodulation

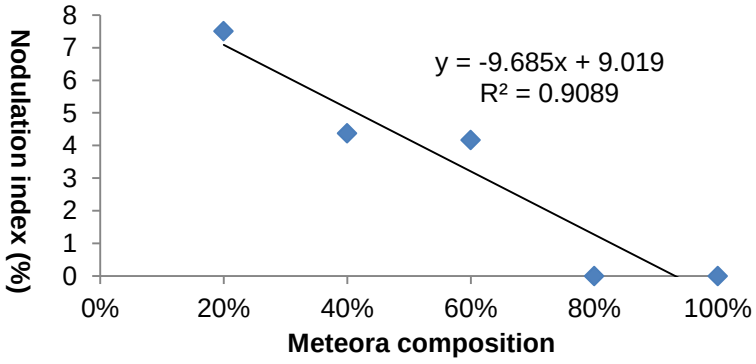


Fig. 15. Relationship between % Woogenellup composition & nodulation

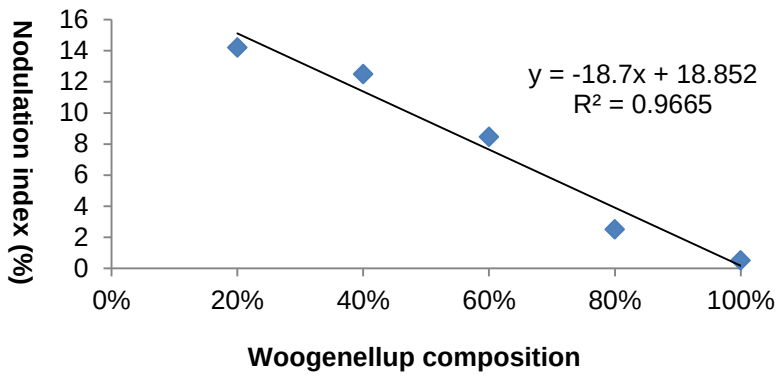


Fig. 16. Relationship between % Seaton Park composition & nodulation

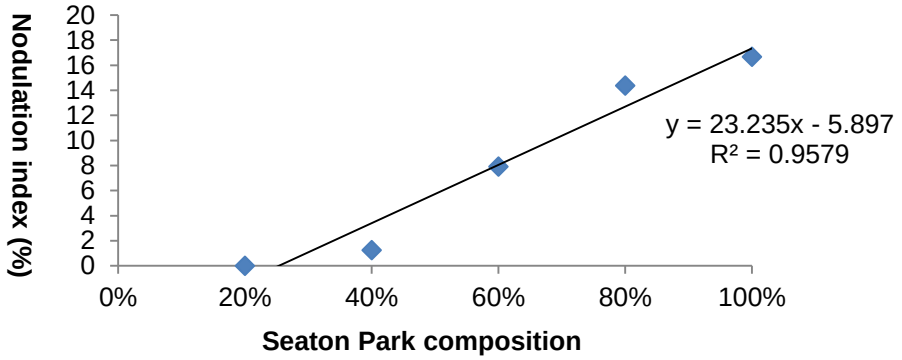


Fig. 17. Relationship between % Riverina composition & nodulation

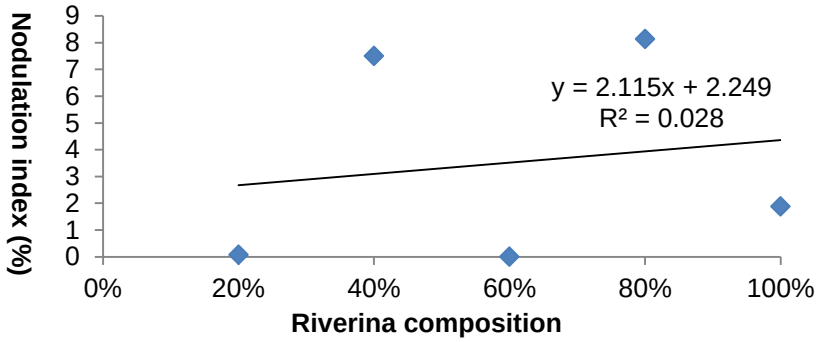


Fig. 18. Tap and lateral disease indices (%) and DNA weight (pg/g soil) at10% of subterranean clover in comparison with 20% and 100% composition

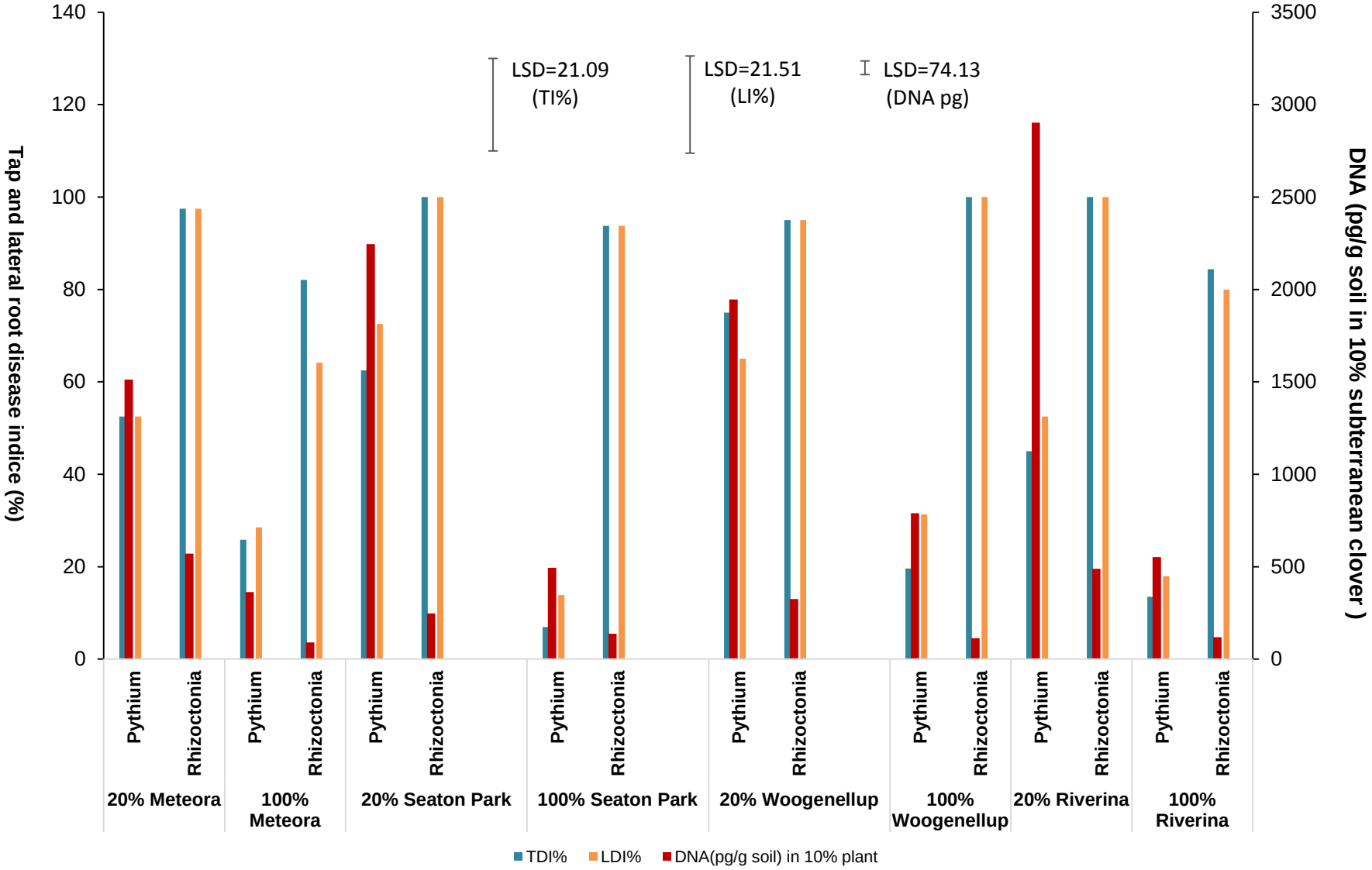


Fig. 19. Correlation between *Pythium* tap root disease index (%) and *Pythium* DNA weight (pg/g soil) per 10% plant

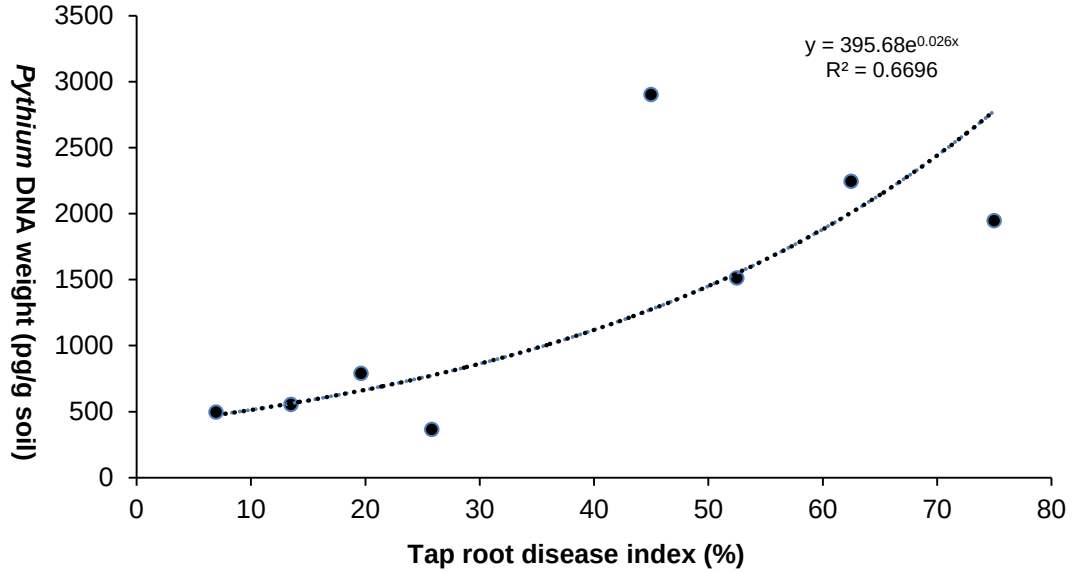


Fig. 20. Correlation between *Pythium* lateral root disease index (%) and *Pythium* DNA weight (pg/g soil) per 10% plant

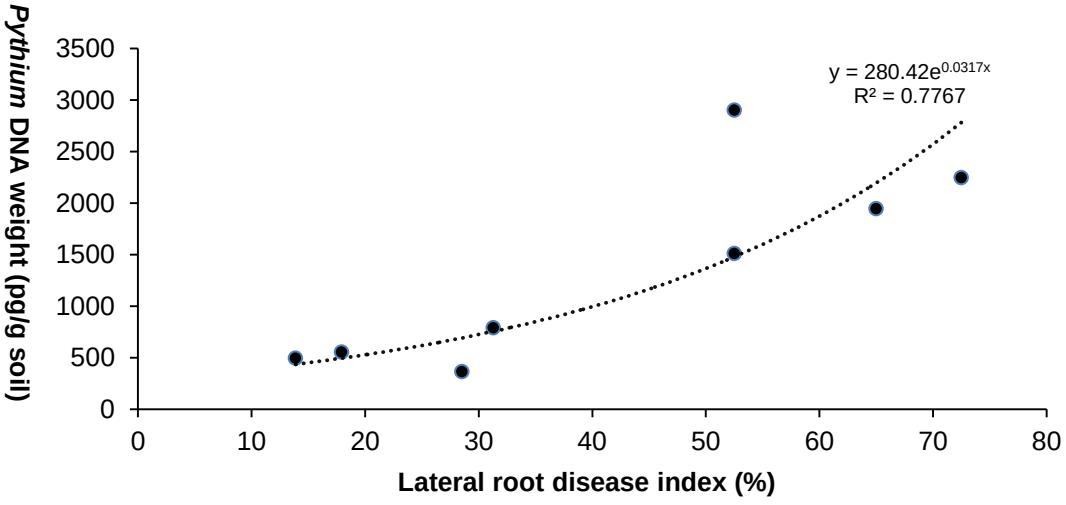


Fig. 21. Correlation between *Rhizoctonia* tap root disease index (%) and *Rhizoctonia* DNA weight (pg/g soil) at 10% subterranean clover composition

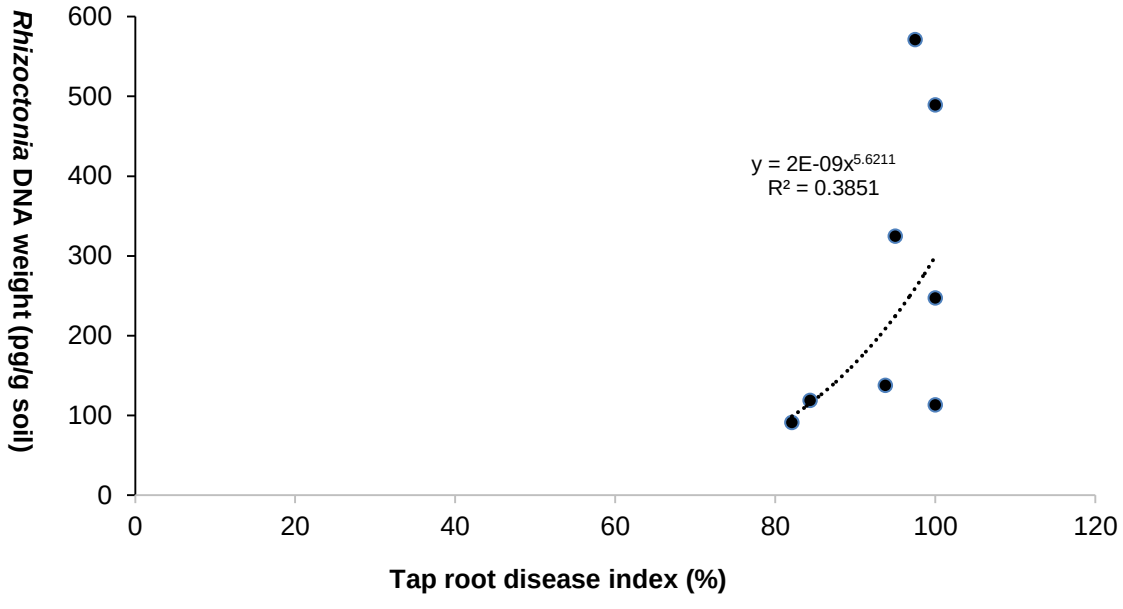
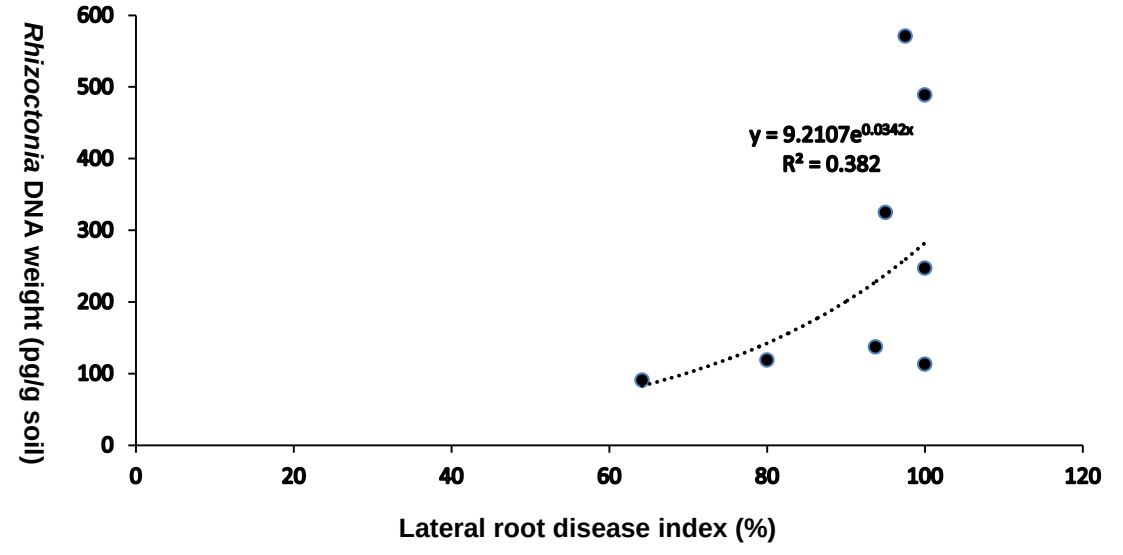


Fig. 22. Correlation between *Rhizoctonia* lateral root disease index (%) and *Rhizoctoni* DNA weight (pg/g soil) at 10% subterranean clover composition



4.2.3 Cultural control treatments – simulated grazing (glasshouse)

Brief summary simulated grazing study: The simulated grazing study showed that grazing, particularly continuous grazing, leads to more severe tap and lateral root disease, poorer nodulation and smaller plants in terms of both roots and shoots. Tap and lateral root disease were most severe, nodulation poorest and dry root and shoot weights lowest when subterranean clover was under intensive grazing in the presence of and affected by *P. irregulare*. Reducing grazing pressure offers

potential for significantly increasing subterranean clover pasture productivity during the critical autumn feed-gap period from decreased root disease and increased nodulation associated with less intensive grazing of root rot affected subterranean clover pastures.

Main findings simulated grazing study: Simulated grazing treatments had significant (P<0.001) effects on tap and lateral root disease indices (%), nodulation indices (%) and dry root and shoot weights (mg/p) (Table 16). Tap and lateral root disease indices were highest (i.e., most severe disease), and nodulation indices and dry root and shoot weights were the lowest (i.e., lest nodules and smallest root and shoot systems) when subterranean clover was under intensive grazing (the 'cut all' treatment) (Figs. 23, 24, 25, 26 and 27). Tap and lateral root disease indices, nodulation index and dry shoot weights were significantly affected by interactions of grazing with pathogen (P<0.001) (Table 16). Tap and lateral root disease indices were highest, and nodulation indices and dry shoot weights were lowest when subterranean clover was under intensive grazing in the presence of and affected by *Pythium irregulare* (Figs. 28, 29, 30 and 31). There was no loss in plant numbers from the simulated grazing treatments.

Table 16. Statistical main effects and interactions of simulated grazing, pathogen (Pythium irregulare) and subterranean clover variety on germination/survival percentage, disease indices for tap (TDI %) and lateral (LDI %) roots, nodulation indices (NI %) and dry weights for root (DRW mg/p) and shoot (DSW mg/p) per plant

	Germination rate (%)		TDI (%)		LDI (%)		NI (%)		DRW (mg/p)		DSW (mg/p)	
	P-value	LSD at P=0.05	P-value	LSD at P=0.05	P-value	LSD at P=0.05	P-value	LSD at P=0.05	P-value	LSD at P=0.05	P-value	LSD at P=0.05
Main factors												
Grazing	0.343	7.021	<.001	5.822	<.001	5.882	<.001	3.863	<.001	22.34	<.001	57.57
Pathogen	<.001	3.31	<.001	2.744	<.001	2.773	<.001	1.821	<.001	10.53	<.001	27.14
Variety	<.001	4.681	<.001	3.881	<.001	3.921	<.001	2.575	<.001	14.89	<.001	38.38
Interactions												
Grazing*pathogen	0.761	9.93	<.001	8.233	<.001	8.318	<.001	5.463	0.089	31.59	<.001	81.42
Grazing*variety	0.803	14.043	0.067	11.643	0.238	11.764	0.211	7.725	0.044	44.67	0.167	115.15
Pathogen*variety	0.05	6.62	0.179	5.489	0.316	5.546	<.001	3.642	0.09	21.06	0.536	54.28
Grazing*pathogen*variety	0.822	19.86	0.482	16.466	0.439	16.637	0.036	10.925	0.541	63.18	0.358	162.85

Fig. 23. Tap root disease index (%) affected by grazing

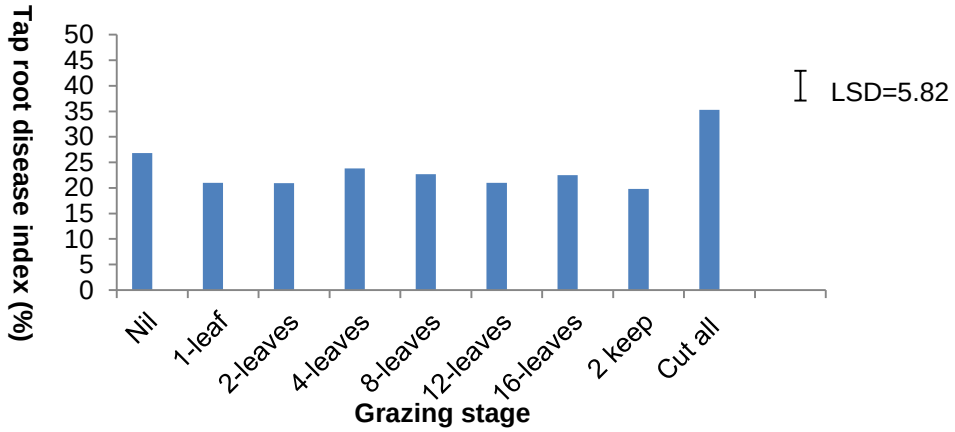


Fig. 24. Lateral root disease index (%) affected by grazing

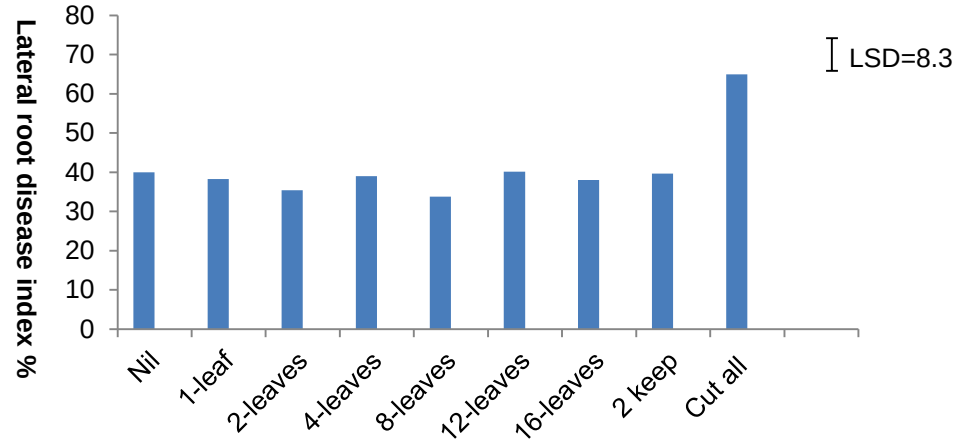


Fig. 25. Nodulation index (%) affected by grazing

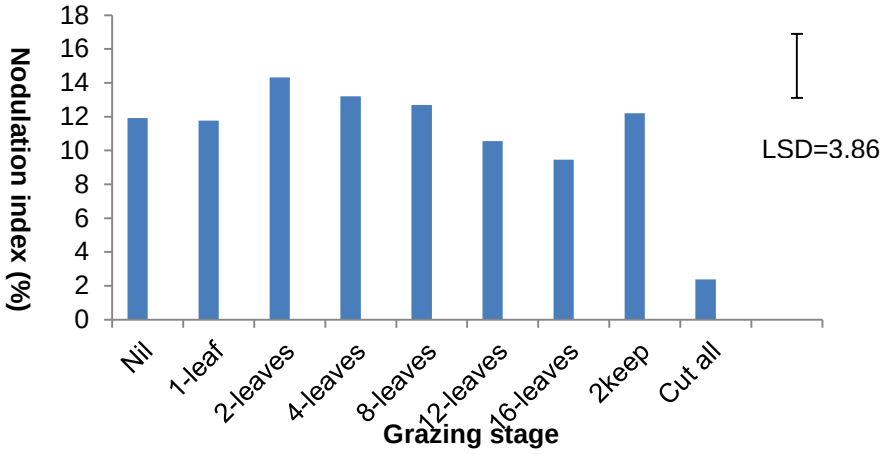


Fig. 26. Dry Root weight (mg/p) affected by grazing

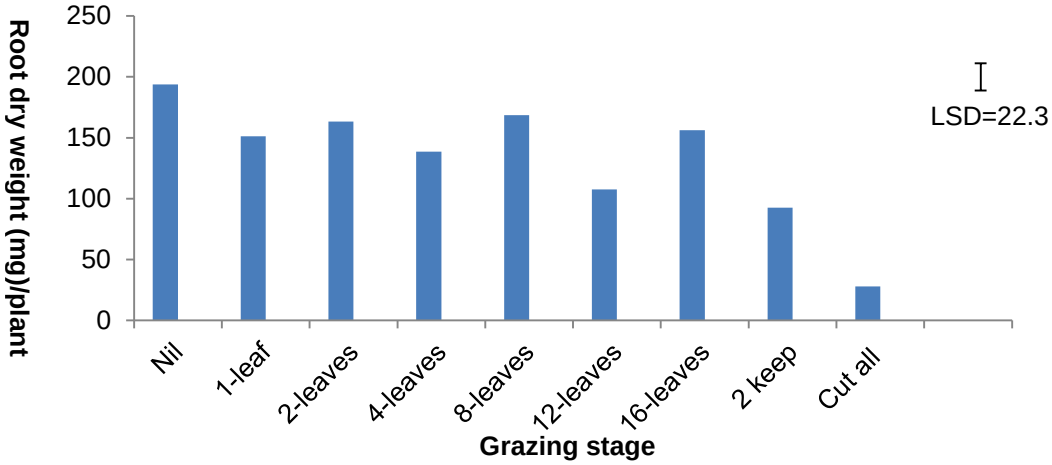


Fig. 27. Dry shoot weight (mg/p) affected by grazing

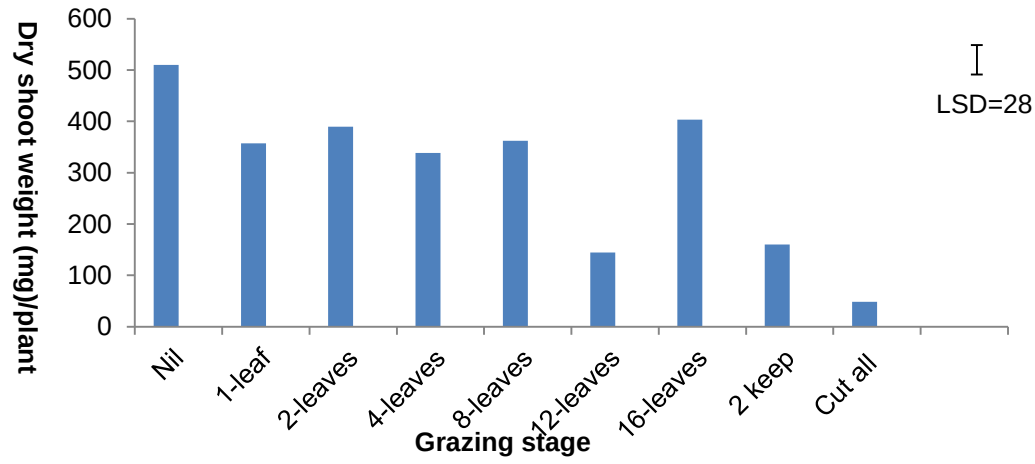


Fig. 28. Tap root disease index (%) affected by interaction of grazing with presence of *Pythium irregulare*

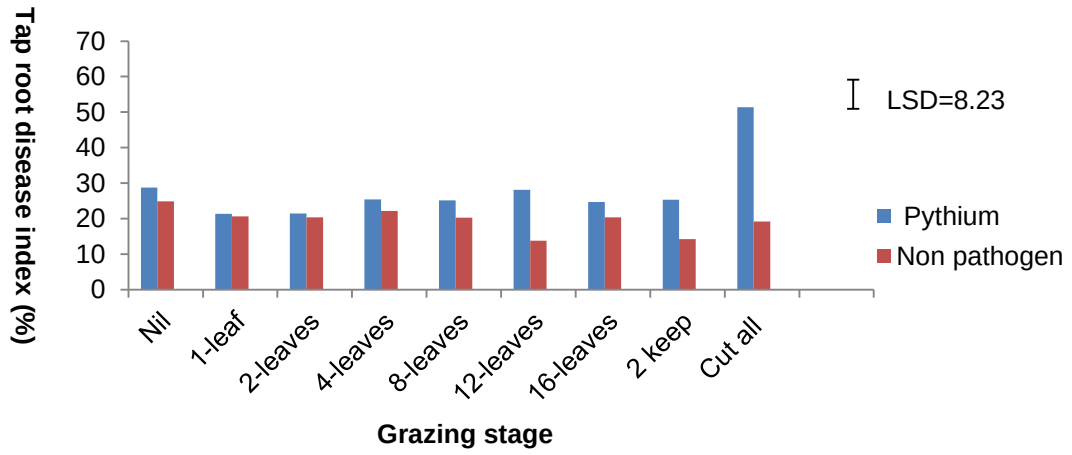


Fig. 29. Lateral root disease index (%) affected by interactions of grazing with presence of *Pythium irregulare*

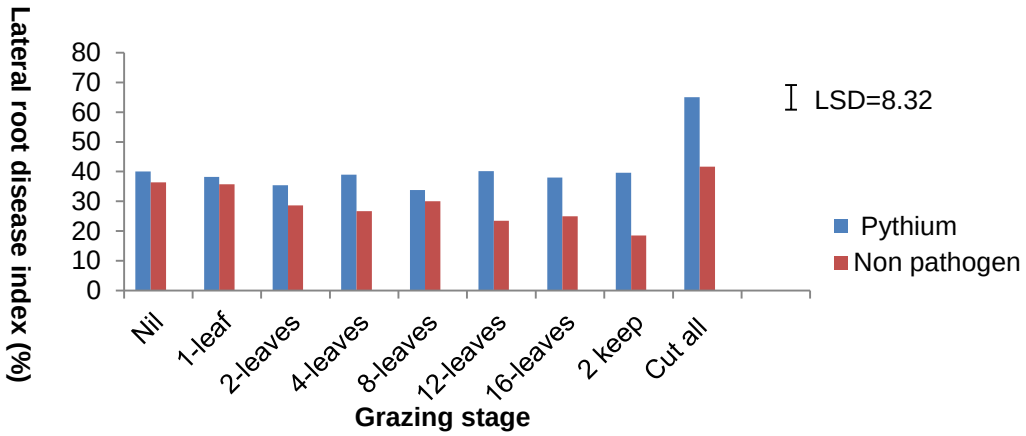


Fig. 30. Nodulation index (%) affected by interaction of grazing with presence of *Pythium irregulare*

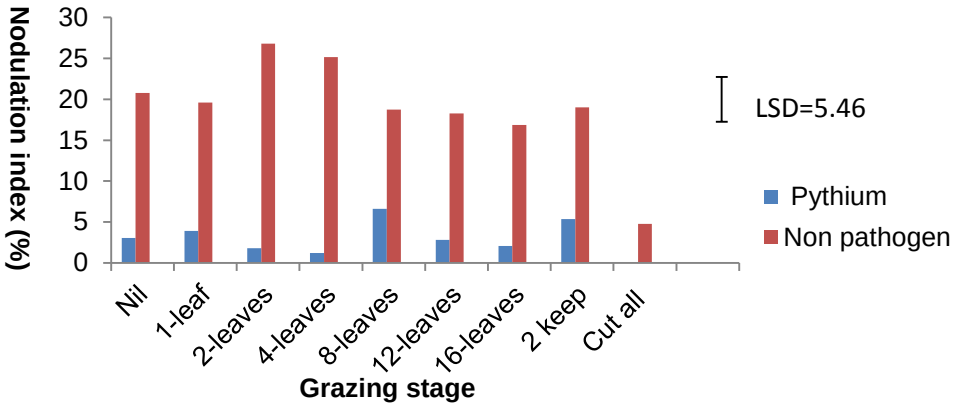
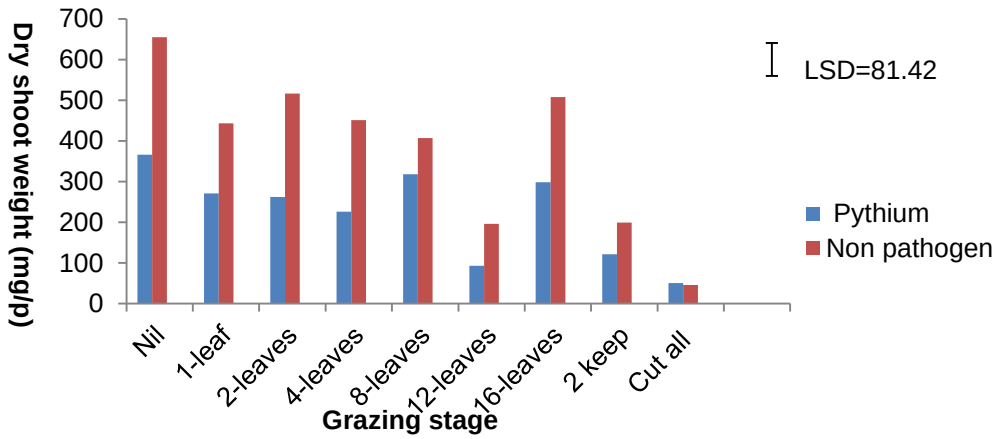


Fig. 31. Dry shoot weight (mg/p) affected by interaction of grazing with *Pythium irregulare*



4.2.4 Cultural control treatments – rhizobium

Brief summary influence of rhizobium study: This study on the influence of rhizobium on subterranean clover root disease showed that rhizobium application via seed or as a granule treatment significantly increased nodulation. However, rhizobium application did not increase either germination rate or shoot dry weight; and when applied as seed treatment it actually decreased both germination rate and shoot dry weight. The presence of the root rot pathogens *P. irregulare* and *R. solani* significantly reduced nodulation indices, but the extent of this depended upon the pathogen and the subterranean clover variety.

Main findings influence of rhizobium study: Application of rhizobium significantly affected subterranean clover germination rate (%) and nodulation rate (%) (P <0.001) and also significantly affected dry shoot weight (mg/p) (P<0.05) (Table 17). The interaction between pathogen with rhizobium significantly affected nodulation index (P<0.005) and dry shoot weight (P<0.001) (Table 17). A three way interaction of pathogen with rhizobium and with variety also significantly affected lateral root disease index and nodulation index (P<0.005) (Table 17). Rhizobium seed treatment decreased germination rate while granule application had similar germination rate as the nil rhizobium application treatment (Fig. 32). Nodulation rate were similar for using rhizobium granules as for rhizobium seed treatment while nodulation rate was much lower in the nil rhizobium treatment (Fig. 33). Dry shoot weight was lowest in the rhizobium seed treatment while rhizobium granule treatment was similar to the nil rhizobium treatment in terms of dry shoot weight (Fig. 34). No nodules formed (nodulation rate zero) when roots were affected by *P. irregulare* in the nil rhizobium treatment. Nodulation rates were similar for granule and seed treatments when pathogens *P. irregulare* or *R. solani* were present and both pathogens significantly reduced the nodulation index (Fig. 35). When there was no pathogen (i.e., nil pathogen), there were no significant differences in terms of dry shoot weights between the rhizobium granule and seed or when compared with the nil rhizobium treatment (Fig. 36). Lateral root disease indices were highest when Meteora was seed treated with rhizobium in the presence of root rot pathogen *P. irregulare* (Fig. 37). Overall, in general, the effect of rhizobium application increased numbers of nodules but without any major effects on root rot or upon dry root and shoot weight.

Table 17. Statistical main effects and interactions of rhizobium application, pathogen (*Pythium irregulare* or *Rhizoctonia solani*) and subterranean clover variety on germination percentage, disease indices of tap (TDI %) and lateral (LDI %) roots, nodulation index (NI %) and dry weights of root (DRW mg/p) and shoot (DSW mg/P) per plant

	Germination rate (%)		TDI (%)		LDI (%)		NI (%)		DRW (mg/p)		DSW (mg/p)	
	P-value	LSD at P=0.05	P-value	LSD at P=0.05	P-value	LSD at P=0.05	P-value	LSD at P=0.05	P-value	LSD at P=0.05	P-value	LSD at P=0.05
Main effect												
Pathogen	<.001	5.28	<.001	3.079	<.001	2.324	<.001	2.073	<.001	2.904	<.001	4.99
Rhizobium	<.001	5.28	0.223	3.079	0.205	2.324	<.001	2.073	0.13	2.904	0.045	4.99
Cultivar	<.001	6.1	0.038	3.555	0.006	2.683	<.001	2.394	<.001	3.354	<.001	5.76
Interaction												
Pathogen*rhizobium	0.123	9.14	0.775	5.333	0.34	4.025	0.002	3.591	0.095	5.03	<.001	8.64
Pathogen*cultivar	<.001	10.56	0.137	6.158	0.013	4.648	0.049	4.147	0.511	5.809	0.099	9.98
Rhizobium*cultivar	0.331	10.56	0.66	6.158	0.744	4.648	0.021	4.147	0.248	5.809	0.471	9.98
Pathogen*rhizobium*cultivar	0.509	18.29	0.021	10.666	0.001	8.05	0.004	7.182	0.684	10.061	0.141	17.28

Fig. 32. Germination rate (%) as affected by application of rhizobium

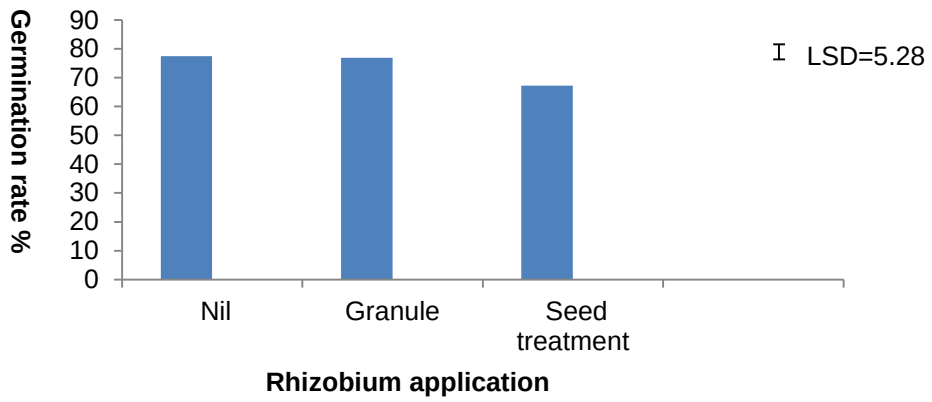


Fig. 33. Nodulation index (%) as affected by application of rhizobium

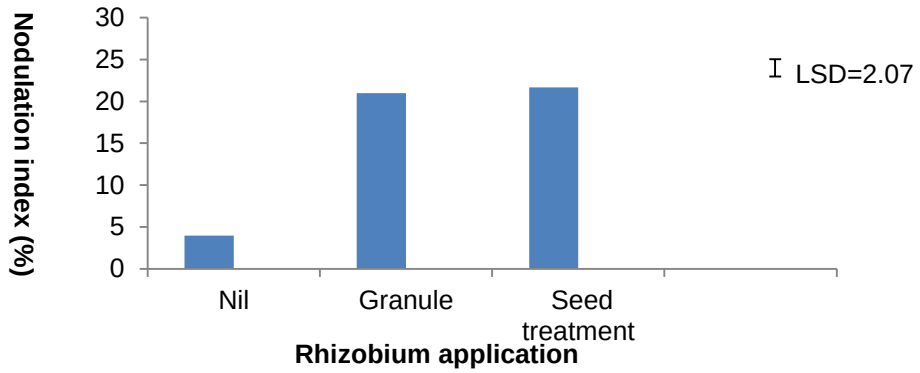


Fig. 34. Dry shoot weight (mg/p) as affected by application of rhizobium

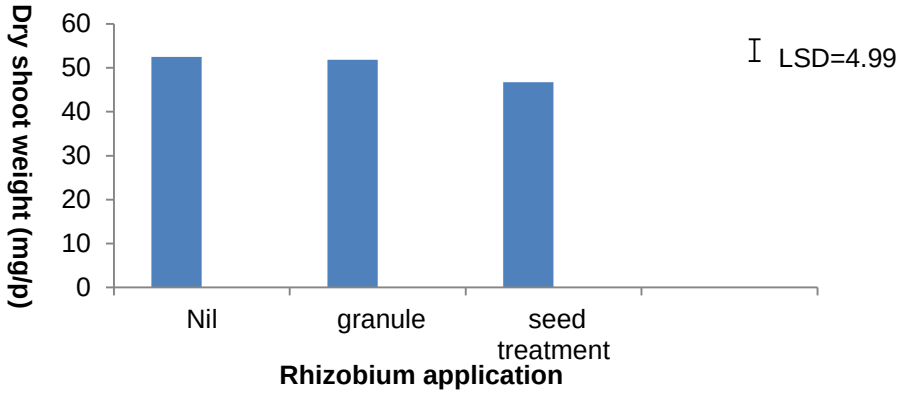


Fig. 35. Nodulation index (%) as affected by the interactions of rhizobium application and pathogen

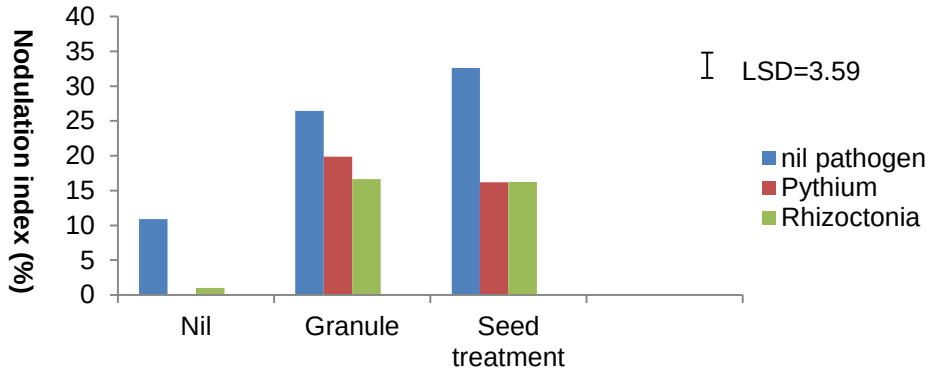


Fig. 36. Dry shoot weight (mg/p) as affected by the interactions of rhizobium application and pathogen

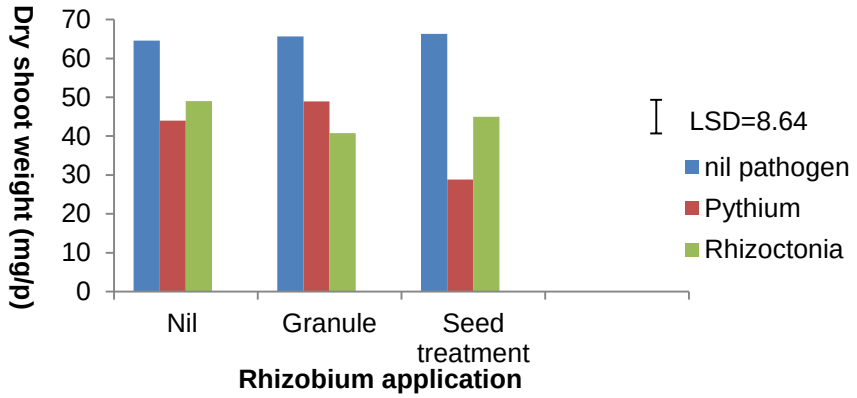
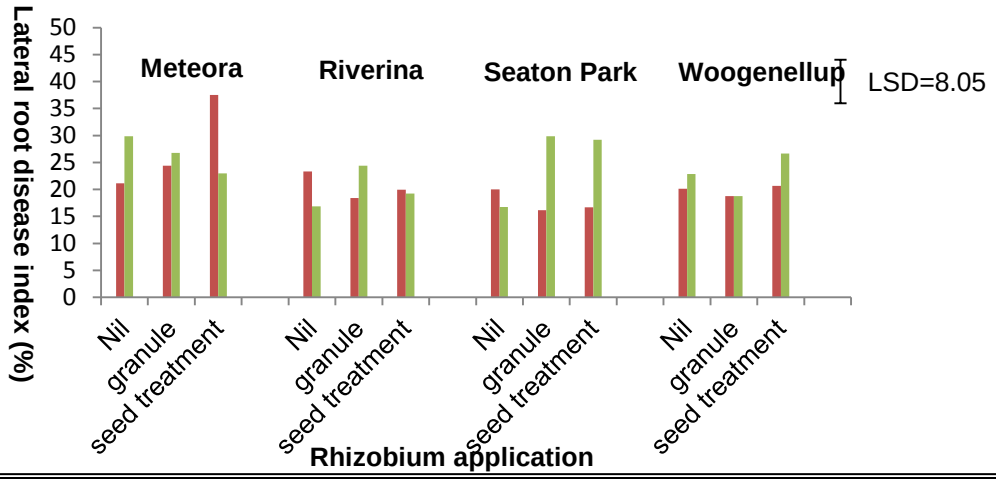


Fig. 37. Lateral root disease index (%) as affected by rhizobium application, pathogen and variety



4.3 Environmental interactions with soilborne diseases

4.3.1 Environmental interactions – *Pythium*

Overview and Outcomes from Modelling Approaches: Modelling found significant but complex relationships between explanatory variables and the presence of Pythium damping-off and root disease. Linear modelling identified high-level (4 or 5-way) significant interactions between explanatory variables for each response variable (dry shoot weight, dry root weight, emergence, tap root disease index, and lateral root disease index). Furthermore, all explanatory variables (temperature, soil, moisture, nutrition, variety) were found significant as part of some interaction within these models. The five-way interaction between all explanatory variables was significant when explaining both dry shoot and root weight, and a four way interaction between temperature, soil, moisture, and nutrition was significant when explaining both tap root disease index and lateral root disease index (Table 18).

The second approach to modelling using boosted regression trees provided support for the complex nature of the relationships found in linear models. All explanatory variables showed at least 5% relative effect on each of the five dependent variables for both diseased plants as for the differences between diseased and control plants (Table 19, Fig. 38).

A visual representation of the complex relationships identified in linear and boosted regression tree modelling is provided in Figs 39, 40, 41 and unpruned decision trees for inoculated plant data is provided in Figures 39, 40. All inoculated plant decision trees, aside from the tree for lateral root disease index, initially split with the factor soil. This was consistent with outputs from boosted regression trees which showed soil to have the highest relative effect (Table 19, and Fig. 38). For difference data, all models initially split with factors other than those found most influential in boosted regression trees (Fig. 41).

Heat maps illustrating the mean actual values across explanatory variable combinations for inoculated plant data and the differences between inoculated and control are shown in Figs 42, 43. For all models, soil type was important, with plants in sand-based soils having either higher weights, greater emergence, or lower disease indices. Lowest weights and proportion emergence, as well as higher disease indices were found for loam soil and low temperature. In sand based soils, the greatest differences between inoculated and control plants were seen in high moisture conditions, while in loam soils, the greatest differences were seen in high nutrition conditions, although these relationships were somewhat obscured by interactions with variety and temperature (Fig. 43). An overview of relationships between factors (moisture, temperature, nutrition, soil type, variety) and their interactions influencing emergence, tap root disease index, lateral root disease index, dry root weight, and dry shoot weight in the presence of *P. irregulare* is illustrated in Figs 44 A-E.

Explanatory Variables - Dry Shoot Weight and Dry Root Weight: Modelling revealed a complex relationship between dry shoot weights of inoculated plants with Pythium damping-off and root disease and environmental factors. Linear modelling found significant 5-way interactions between all explanatory variables for both dry shoot and root weights (Table 18). BRTs provided further support for the effect of all explanatory variables on differences in dry root weights (Table 19, Fig. 38). Relative effect of explanatory variables in BRT models was consistent for both dry shoot and root weights, with soil the most influential factor, followed by temperature, nutrition, variety and moisture.

Soil was also the initial splitting factor for both dry shoot and root weight decision trees (Fig. 40). In these models, plants with the highest dry shoot weight were grown under conditions of sand-based

soil, high or medium temperatures, while plants with the highest dry root weight were grown under conditions of sand-based soil, high moisture and high nutrition. The lowest dry shoot and root weights were varieties Riverina and Seaton Park, grown under the conditions of loam soil with high nutrition and low temperature.

Heat maps also provided a visual representation of the relationships showing a clear increase in dry shoot weight for plants grown under the conditions of sand-based soil and medium or high temperatures. A similar pattern was evident for dry root weight, but less clear. Lowest dry shoot and root weight were found from loam soils, particularly under low temperatures (Fig. 42).

Results of modelling the differences between mean emergence of inoculated plants and control plants showed that temperature had the strongest relative influence on dry root weight (Table 19) and neither decision tree split initially with soil (Fig. 41).

Explanatory Variables – Emergence: Linear modelling identified two significant interactions for explaining the relationship between emergence and explanatory variables, a 3-way interactions between temperature, soil and variety, and a 4-way interactions between temperature, moisture, nutrition and variety (Table 1). Relative effect of these factors from BRT modelling found soil had a 50 % effect on emergence, followed by variety, temperature, nutrition, and moisture (Table 19, Fig. 38).

Decision trees found highest emergence were for varieties Woogenellup and Seaton Park grown in sand-based soil, while the lowest emergence proportions were from plants grown in loam soil, high nutrition and high moisture (Figure 40). Heat maps also showed higher emergence proportions for plants grown in sand-based soil rather than loam soil (Fig. 42).

Results of modelling for the differences between mean emergence of inoculated plants and control plants showed a strong effect from soil type (Table 19, Fig. 41) with larger differences in loam soil (Fig. 43) but less clear for other factors.

***Explanatory Variables - Tap Root and Lateral Root Disease Index*:** Tap root disease index were found significantly affected by two 4-way interactions between temperature, soil, moisture and nutrition, and also interactions between soil, moisture, nutrition and variety. Lateral root disease index were found significantly affected by one 4-way and one 2-way interactions which were the interactions between temperature, soil, moisture and nutrition (same as for tap root disease index) and the interactions between soil and variety (Table 18). Boosted regression trees found similar relative effect on both tap and lateral root disease indices from soil, temperature and variety (Table 19).

In decision trees, the lowest disease indices were for Seaton Park and Woogenellup varieties grown in sand-based soil under high and medium temperatures and high moisture for tap root disease index. A similar pattern was found for lateral root disease index, but with a slightly different splitting pattern. Varieties Seaton Park and Woogenellup grown in sand-based soil under high moisture and medium temperature had the lowest disease indexes. The highest levels of root disease index were found from the conditions of loam soil, low moisture and high nutrition for tap roots and low moisture for lateral roots of variety Riverina (Fig. 41).

Heat maps showed higher disease indices for plants grown on loam soil; however, this pattern was more apparent in tap root disease index than lateral root disease index. There was also a pattern for both tap and lateral root with higher levels of disease found in plants under high moisture (Fig. 42).

Table 18. Significant interactions from linear and generalised linear modelling of the effects of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables dried shoot weight (DSW), dried root weight (DRW), emergence, and tap root and lateral root disease indices (TDI, LDI) of three subterranean clover (*Trifolium subterraneum*) varieties inoculated with *Pythium irregulare* in a controlled environment experiment.

Model	*Significant interactions*	*df*	*P-value*	*Res. Dev.*	R^2	*RMSE*
DSW	Temp x soil x moisture x nutrition x variety	4	0.002	-	0.66	11.17
DRW	Temp x soil x moisture x nutrition x variety	4	0.001	-	0.61	11.58
Emergence	Temp x moisture x nutrition x variety	4	0.008	304.33 on 216	-	2.49
	Temp x soil x variety	4	>0.001			
TDI	Soil x moisture x nutrition x variety	2	0.04	33.106 on 175	-	16.28
	Temp x soil x moisture x nutrition	2	0.04			
LDI	Temp x soil x moisture x nutrition	2	0.001	30.228 on 175	-	15.50
	Soil x variety	2	0.003			

Table 19. Percent variable relative influence from boosted regression tree modelling of the effects of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables dried shoot weight (DSW), dried root weight (DRW), emergence, and tap root and lateral root disease indices (TDI, LDI) of three subterranean clover (*Trifolium subterraneum*) varieties inoculated with *Pythium irregulare* in a controlled experiment.

	Variable relative influence (%)						
Model	*Soil*	*Temp*	*Variety*	*Nutrition*	*Moisture*	*RMSE*	*Avg. test RMSE*
Diseased Plants							
DSW	40.65	21.20	12.16	16.64	9.35	1.91	2.27
DRW	30.69	20.54	12.92	19.28	16.57	1.58	2.18
Emergence	50.50	12.54	17.87	10.64	8.47	1.08	1.17
TDI	28.81	21.11	24.44	7.42	18.22	0.03	0.04
LDI	19.65	21.09	23.66	9.42	26.18	0.03	0.04
Differences in mean values for diseased and control plants							
DSW	21.39	17.52	13.44	43.21	4.44	-	-
DRW	15.73	43.10	8.62	27.10	5.45	-	-
Emergence	52.43	26.30	7.78	8.32	5.17	-	-

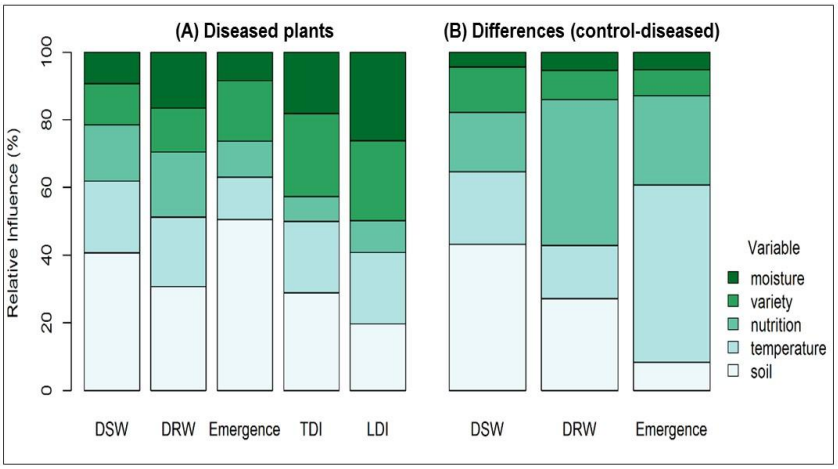


Fig. 38. Percent relative influence from boosted regression tree modelling of the effects of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables dried shoot weight (DSW), dried root weight (DRW), emergence, and tap root and lateral root disease indices (TDI,DI) of three subterranean clover (*Trifolium subterraneum*) varieties, **(A)** model results from plants inoculated with *Pythium irregulare*; **(B)** mean values between plants inoculated with *P. irregulare* versus control plants.

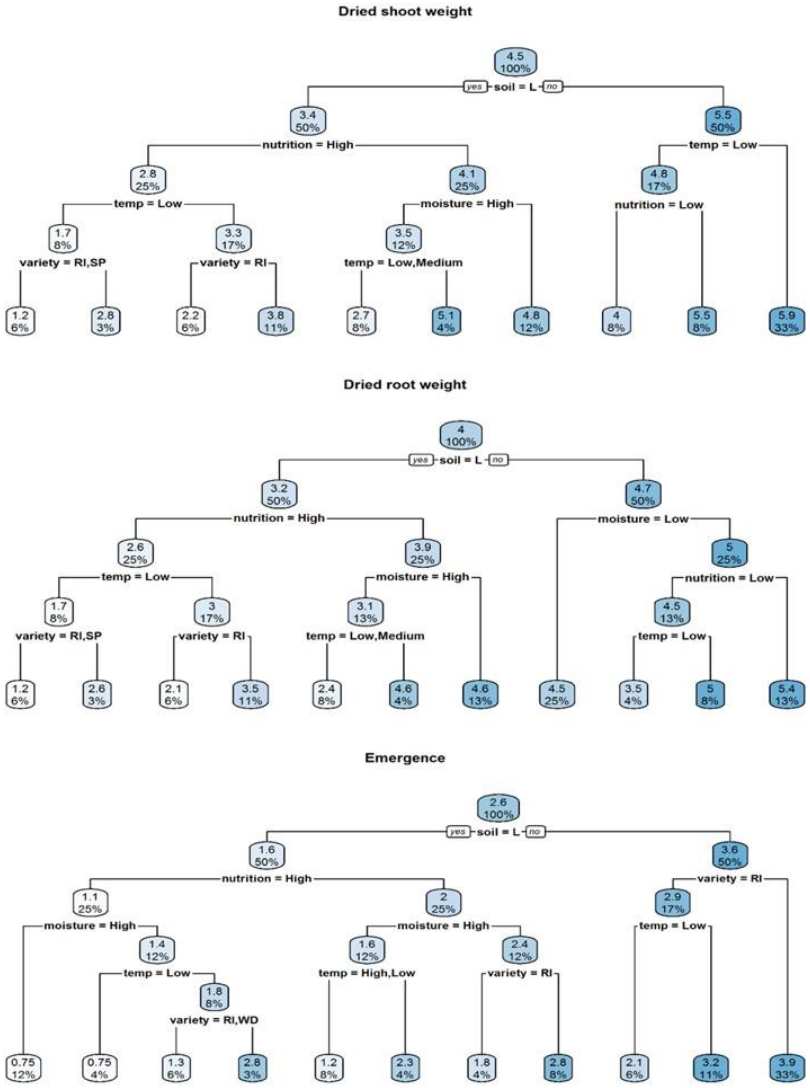


Fig. 39. Regression decision trees illustrating the influence of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables dried shoot weight, dried root weight and emergence of three subterranean clover (*Trifolium subterraneum*) varieties inoculated with *Pythium irregulare* in a controlled environment experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). The numbers and the shading in the boxes represent the mean value at each decision point; the percentages indicate the percentage of all values considered at that decision point.

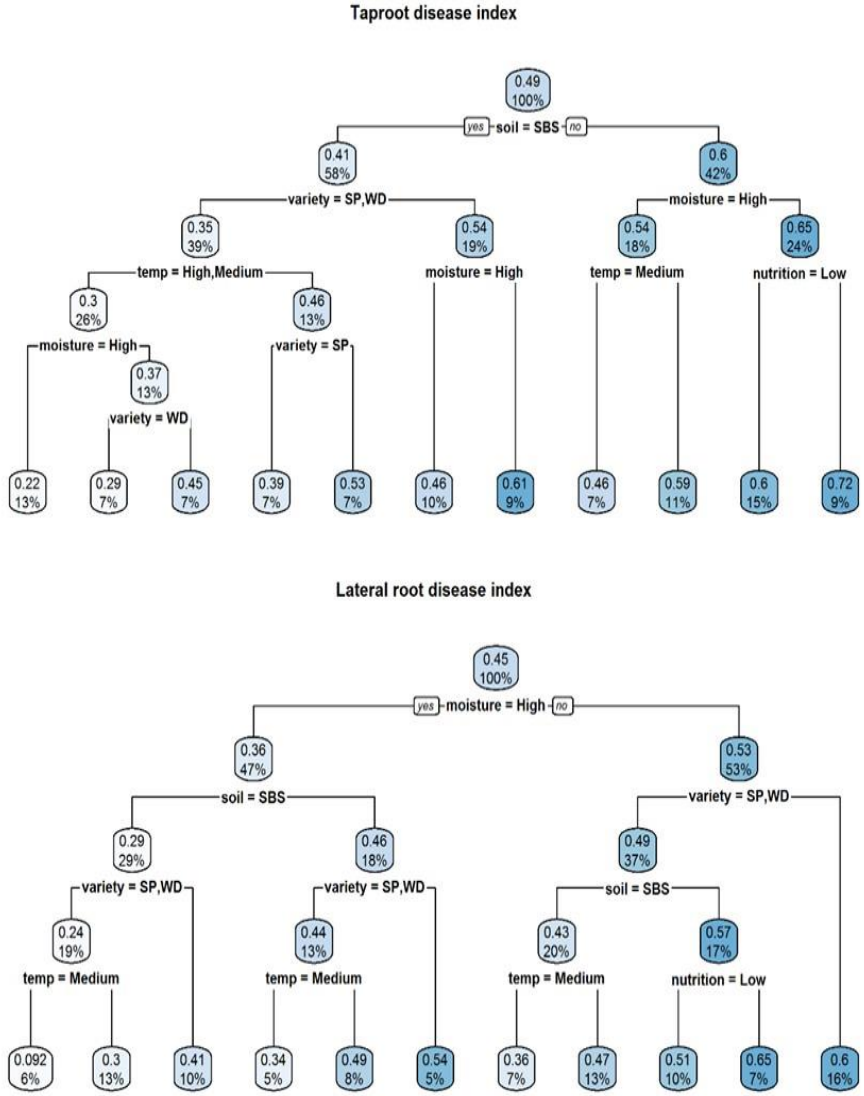


Fig. 40. Regression decision trees illustrating the influence of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables tap root and lateral root disease indices (TDI, LDI) of three subterranean clover (*Trifolium subterraneum*) varieties inoculated with *Pythium irregulare* in a controlled environment experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). The numbers and the shading in the boxes represent the mean value at each decision point; the percentages indicate the percentage of all values considered at that decision point.

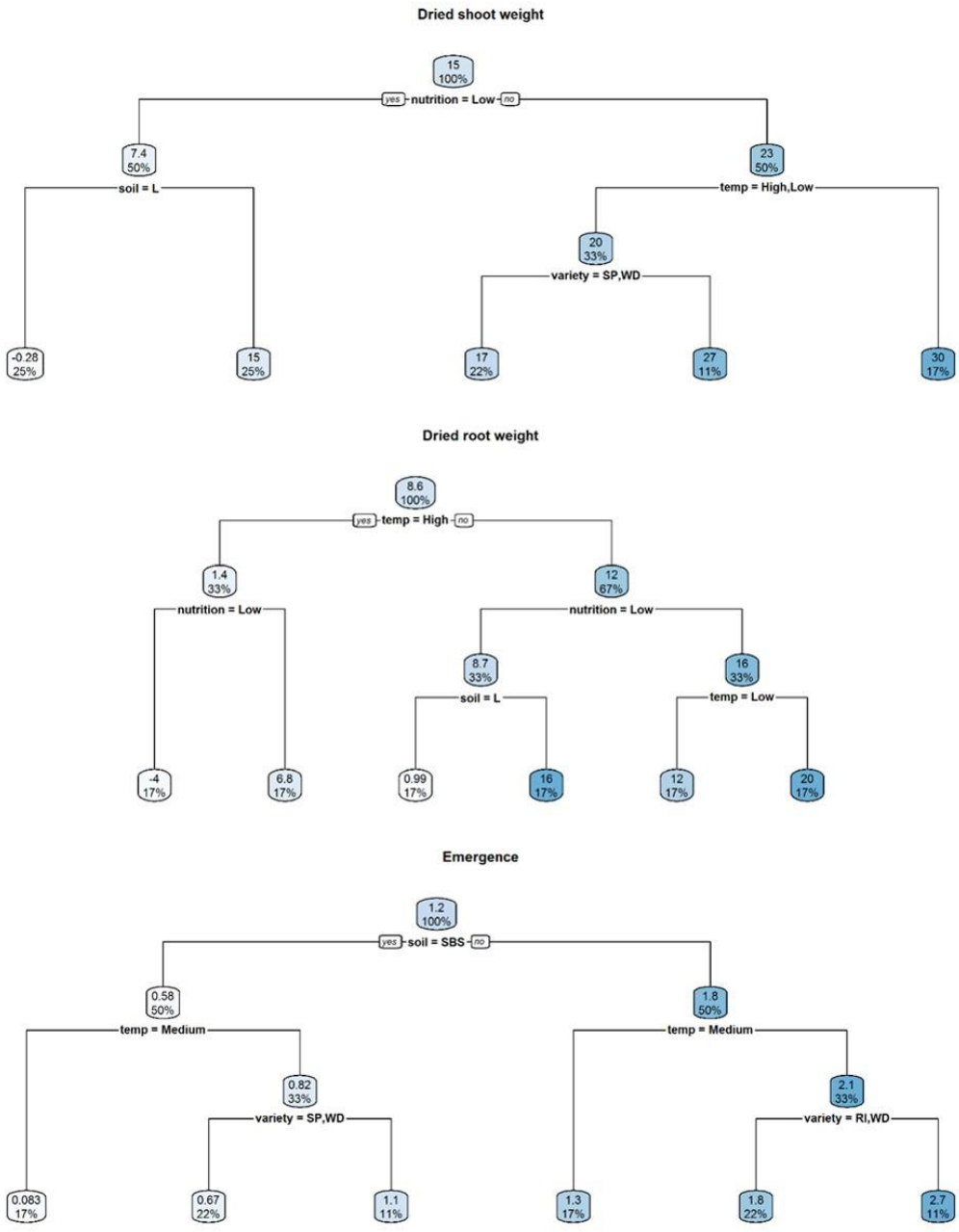


Fig. 41. Regression decision trees illustrating the influence of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the difference between mean dried shoot weight, dried root weight and emergence of three subterranean clover (*Trifolium subterraneum*) varieties inoculated with *Pythium irregulare* or control plants in a controlled environment experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). The numbers and the shading in the boxes represent the mean value at each decision point; the percentages indicate the percentage of all values considered at that decision point.

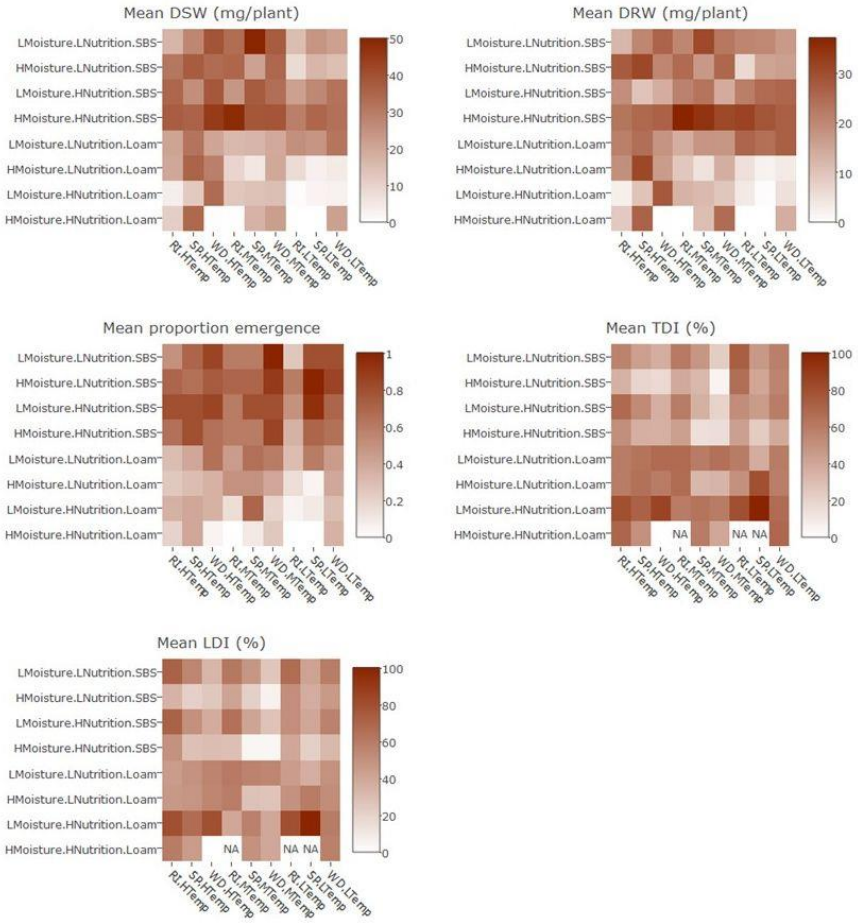


Fig. 42. Heat maps of mean dry shoot weight (DSW), dry root weight (DRW), emergence, and tap and lateral root disease indices (TDI, LDI) of three subterranean clover varieties (*Trifolium subterraneum*) inoculated with *Pythium irregulare* in a controlled environment experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). Darker shading indicates higher values, as per the colour scale bar. NAs values indicate missing data.

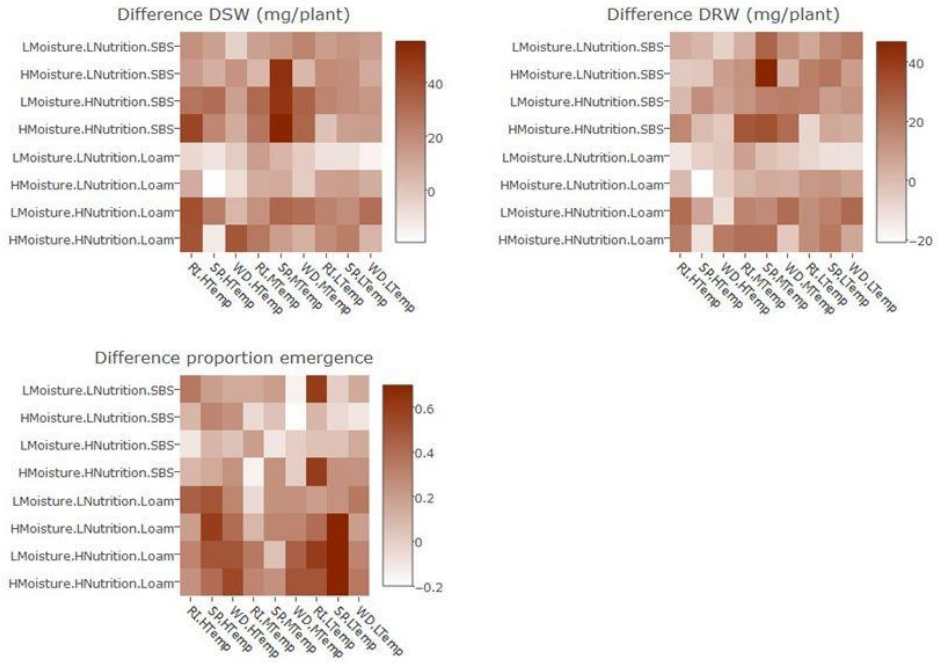


Fig. 43. Heat maps of the differences between mean dried shoot weight (DSW), dried root weight (DRW) and emergence for three subterranean clover (*Trifolium subterraneum*) varieties inoculated with *Pythium irregulare* or control plants in a controlled experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). Darker shading indicates higher values, as per the colour scale bar.

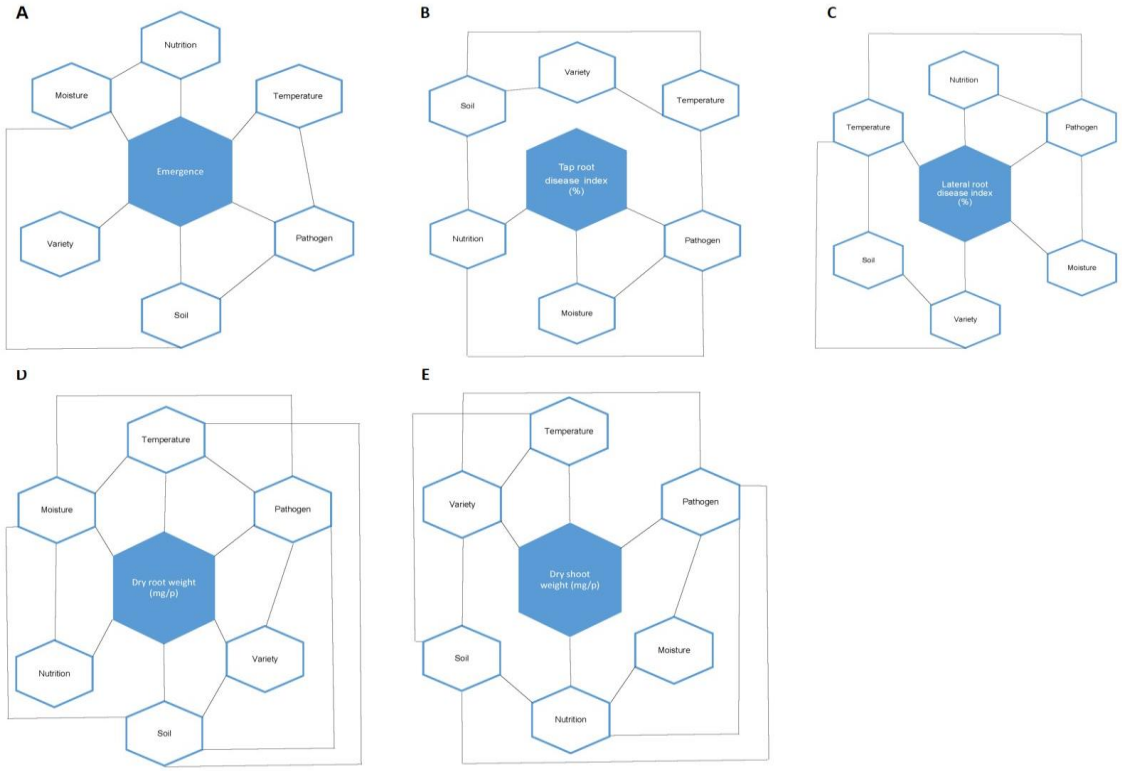


Fig. 44 A-E. Effect of environment explanatory factors (moisture, temperature, nutrition, soil type, variety) and their interactions on: **(A)** subterranean clover (*Trifolium subterraneum*) emergence rate (%), **(B)** tap root disease index (%), **(C)** lateral root disease index (%), **(D)** dry root weight (mg/p), and **(E)** dry shoot weight (mg/p) in the presence of the soilborne pathogen *Pythium irregulare*.

4.3.2 Environmental interactions – *Phytophthora*

Emergence (%): In the presence of *P. clandestina* under the conditions of low, medium and high temperature regimes (14/9^{o}C, 18/13^{o}C, and 22/17^{o}C day/night), high and low moisture, high and low nutrition and two soil types (sand and loam sand), % seedling emergence (i.e., survival) was significantly affected by single factors of moisture, pathogen, soil type, temperature and cultivar (Tables 20, 21). In brief, % emergence was greater under high moisture, in sand soil, at medium to high temperatures and, as expected, in the absence of *P. clandestina*. Cultivar Seaton Park had the greatest % emergence followed by Meteora and Riverina, while Woogenellup had lowest % emergence. Factors affecting % emergence and their interactions are detailed in Fig. 45.

Percent emergence was also affected by two-way interactions involving moisture and pathogen; moisture and soil type; pathogen and soil type; moisture and temperature; soil type and temperature; moisture and variety; pathogen and variety; and temperature and variety. For example, in the presence of *P. clandestina*, % emergence was significantly higher under high moisture in sand soil and under high temperature with high moisture. In contrast % emergence was lowest at low temperature with either high or low moisture; and low temperature in loam soil significantly reduced emergence. Percent emergence of Seaton Park and Woogenellup were significantly affected by temperature, with, for example, Seaton Park having greatest germination at intermediate temperature but lowest germination at low temperature. In contrast, Woogenellup germinated best at high temperature but poorest at low temperature.

Percent emergence was also significantly affected by three-way interactions involving moisture, nutrition and pathogen; nutrition, moisture and cultivar; nutrition, pathogen and cultivar; and nutrition, temperature and cultivar. For example, % emergence was lowest in the presence of *P. clandestina* under low moisture regardless of nutrition. A major driving factor of three-way interactions was the differences between the subterranean clover varieties. For example, in terms of interactions between nutrition, temperature and cultivar, Meteora and Seaton Park performed best at intermediate temperature without any impact of nutrition; Riverina performed best under high nutrition and low temperature; and Woogenellup performed best at high temperature and low nutrition conditions. Percent emergence was affected by four-way interactions involving moisture, nutrition, pathogen and soil; moisture, pathogen, soil and temperature; nutrition, pathogen, soil and cultivar; nutrition, pathogen, temperature and cultivar; and between nutrition, soil, temperature and cultivar In the presence of the pathogen, across the factors of moisture, temperature, soil type and pathogen, the greatest emergence was for the combination high moisture, high temperature and sand soil A major driving factor in these four-interactions were differences between the subterranean clover varieties. For example, in the presence of pathogen, best emergence was for Meteora and Seaton Park under high nutrition with medium temperature; for Riverina it was high nutrition with high temperature, and for Woogenellup it was low nutrition with high temperature.

Tap root disease index (%): Tap root disease index (%) was significantly affected by single factors of nutrition level, soil type, temperature and subterranean clover cultivar (Tables 20, 21). In brief, low nutrition, sand soil and high temperature lowered tap root disease index. Cultivar Seaton Park suffered least from tap root disease followed by Meteora and Riverina, while Woogenellup had the highest levels of tap root disease from *P. clandestina*. Factors affecting tap root disease index (%) and their interactions are detailed in Fig. 46.

Tap root disease was significantly affected by two-way interactions involving soil type and moisture; temperature and nutrition; soil type and temperature; soil type and cultivar; temperature and cultivar. For example, tap root disease was significantly lower for low moisture with sand soil; for

low nutrition with high temperature; for sand soil with high temperature; for sand soil with Seaton Park; and for high temperature with Seaton Park.

Tap root disease was significantly affected by three-way interactions involving moisture, soil and temperature; moisture, nutrition and cultivar; nutrition, soil and cultivar; and soil, temperature and cultivar., Tap root disease was significantly reduced for low moisture, high temperature with sand soil. A major driving factor in these interactions were differences between the subterranean clover varieties. For example tap root disease was significantly reduced for low or high moisture and low nutrition with Meteora, Riverina and Seaton Park (but for high moisture with low nutrition for Woogenellup); for low nutrition and sand soil for all varieties; for high temperature and sand soil for Meteora and Riverina; for high or low temperature with sand soil for Seaton Park; and for high or low temperature and loam or sand soil for Woogenellup. Tap root disease was also affected by four-way interactions involving moisture, nutrition, soil and cultivar; moisture nutrition, temperature and cultivar; moisture, soil, temperature and cultivar. Again, a major driving factor in these interactions were differences between the subterranean clover varieties. For example, tap root disease was reduced for low moisture, low nutrition and sand soil for Meteora, Riverina and Seaton Park; for high moisture, low nutrition and loam soil for Woogenellup; for high moisture, low nutrition and high temperature for Meteora; for high moisture, low nutrition and high or low temperature for Riverina; for high or low moisture, low nutrition and high or low temperature for Seaton Park; for high moisture, low nutrition and high temperature for Woogenellup; for high or low moisture, sand soil and high temperature for Meteora; for high moisture, sand soil and high temperature for Riverina; for high moisture or low moisture, sand soil and high temperature for Seaton Park; and, for high moisture, loam soil and high temperature for Woogenellup.

Lateral root disease index (%): Lateral root disease index (%) was significantly affected by single factors moisture, nutrition, soil type, temperature and cultivar. In brief, lateral root disease index was reduced at high moisture, by low nutrition, in sand soil, from high temperature and by using Seaton Park (Tables 20, 21). Factors affecting lateral root disease index (%) and their interactions are detailed in Fig. 47.

Lateral root disease index was significantly affected by two-way interactions involving moisture and soil, nutrition and temperature, soil and temperature, moisture and cultivar, soil and cultivar, and by temperature with cultivar. For example, lateral root disease index was reduced for low moisture with sand soil, for high temperature with low nutrition, for high temperature with sand soil, for high moisture with Seaton Park, for sand soil with Seaton Park and for temperature with Seaton Park.

Lateral root disease index was significantly affected by three-way interactions involving moisture, nutrition and soil; between moisture, nutrition and cultivar; between nutrition, soil and cultivar; between moisture, temperature and cultivar; and between nutrition, temperature and cultivar. For example, lateral root disease index was reduced for low moisture, low or high nutrition and sand soil; for high moisture, low nutrition and Seaton Park; for high nutrition, sand soil and Seaton Park; for high moisture, high or low temperature and Seaton Park; and, for low nutrition, high temperature and Seaton Park.

Lateral root disease index was significantly affected by four-way interactions involving moisture, nutrition, soil and temperature; between moisture, nutrition, soil and cultivar; between moisture, nutrition, temperature and cultivar; and between moisture, soil, temperature and cultivar. For example, lateral root disease index was significantly reduced for high moisture, low nutrition and high temperature in loam soil; for low moisture, low nutrition and sand soil for Meteora, Seaton Park and Riverina; for high moisture, low nutrition and loam soil for Woogenellup; for high moisture, high nutrition and high temperature for Meteora and Riverina; for high moisture, low nutrition and high temperature for Seaton Park; for high moisture, low nutrition and high

temperature for Woogenellup; for high or low moisture, sand soil and high temperature for Meteora; for low moisture, sand soil and high or medium temperature for Riverina; for low moisture, sand soil and all three temperatures for Seaton Park; and, for high moisture, loam soil and high temperature for Woogenellup. As with tap root disease, a major driving factor in these interactions were differences between the subterranean clover varieties.

Nodulation index (%): Although nodulation levels overall were generally very low (range for % nodulation indices of 0 – 25.6; data not shown), nodulation was significantly affected by single factors nutrition, pathogen, soil type, temperature and cultivar (Table 20). In brief, nodulation index was higher when soil nutrition was low; when *P. clandestina* was present; when sand soil was used; when temperature was high; and when Seaton Park was the cultivar used. Factors affecting nodulation index (%) and their interactions are detailed in Fig. 48. Nodulation was significantly affected by two-way interactions involving nutrition and pathogen, nutrition and soil, pathogen and soil, nutrition and temperature, pathogen and temperature, soil and temperature, moisture and cultivar, nutrition and cultivar, pathogen and cultivar, soil and cultivar, and temperature and cultivar (data not shown). for example, nodulation increased for low nutrition and the presence of *P. clandestina*, for low nutrition and sand soil, for presence of *P. clandestina* and sand soil, for high temperature and low nutrition, for high temperature and the presence of *P. clandestina*, for high temperature and sand soil, for high moisture and Seaton Park, for low moisture and Meteora or Riverina, for low nutrition with all tested varieties, for the presence of *P. clandestina* and all varieties; for sand soil and all varieties, and for high temperature and all varieties. Nodulation was significantly affected by three- and four-way interactions involving the different factors (data not shown). For example, nodulation index was increased by the three-way interactions involving low nutrition, the presence of *P. clandestina* and sand soil; for low nutrition, the presence of *P. clandestina* and high temperature; for low nutrition, sand soil and high temperature; for sand soil, high temperature and the presence of *P. clandestina*; for low moisture, the presence of *P. clandestina* and Meteora, Riverina (or for Woogenellup with high moisture), and for low moisture with the presence of *P. clandestina* on Seaton Park; for high moisture, sand soil and Seaton Park; for sand soil, the presence of *P. clandestina* and all varieties; for low moisture, high temperature and Meteora or Riverina (or high moisture, high temperature and Seaton Park); for low nutrition, high temperature and Meteora, Riverina and Seaton Park; for high temperature, the presence of *P. clandestina* and all varieties; and, for sand soil, high temperature and all varieties. Similarly, nodulation increased in situations involving four-interactions for low nutrition, the presence of *P. clandestina*, sand soil and high temperature; in particular, it increased for Meteora, Riverina and Seaton Park in situations involving the presence of *P. clandestina,* low nutrition, and sand soil.

Dry root weight (mg plant^{-1}): Dry root weight (mg plant^{-1}) was significantly affected by the single factors of moisture, nutrition, pathogen, soil, temperature and cultivar (Table 20, 21). In brief, dry root weight was increased by high moisture, by high nutrition, by the absence of *P. clandestina*, by sand soil, by high temperature, by using Meteora or Riverina. Factors affecting dry root weight and their interactions are detailed in Fig. 49.

Dry root weight was significantly affected by two-way interactions involving moisture and pathogen, nutrition and soil, moisture and temperature, nutrition and temperature, pathogen and temperature, moisture and cultivar, pathogen and cultivar, soil and cultivar, and temperature and cultivar. For example, dry root weight was increased for high moisture and the absence of *P. clandestina*, for high nutrition and sand soil, for high moisture and high or medium temperature, for high temperature and high nutrition, for high temperature and the absence of *P. clandestina*, for high moisture and Riverina or Seaton Park, for the absence of *P. clandestina* and all varieties and especially Woogenellup, for sand soil and all varieties and especially Meteora, Riverina and Seaton Park, and for high temperature and Meteora or Riverina.

Dry root weight was significantly affected by three-way interactions involving moisture, nutrition and pathogen; for moisture, nutrition and temperature; for nutrition, pathogen and temperature; for nutrition, soil and temperature; for pathogen, soil and cultivar; for pathogen, temperature and cultivar; and, for soil, temperature and cultivar. For example, dry root weight increased for high moisture, high nutrition and the absence of *P. clandestina*; for high moisture, high nutrition and high temperature; for high nutrition, the absence of *P. clandestina* and high temperature; for high nutrition, sand soil and high temperature; for the absence of *P. clandestina*, sand soil and all tested varieties and especially Woogenellup; for the absence of *P. clandestina*, high temperature and Woogenellup or Meteora or Riverina; and, for sand soil, high temperature and Meteora or Riverina.

Dry root weight was significantly affected by four-way interactions involving moisture, nutrition, pathogen and temperature; between moisture, pathogen, soil and cultivar; and between nutrition, soil, temperature and cultivar. for example, dry root weight increased for high moisture, high nutrition, the absence of *P. clandestina* and high temperature; for high moisture, the absence of *P. clandestina*, sand soil and Meteora or Riverina or Woogenellup; for high nutrition, sand soil, high temperature and Meteora or Riverina; and, for high moisture, low nutrition, the absence of *P. clandestina*; sand soil and high temperature.

Dry shoot weight (mg plant^{-1}): Dry shoot weight (mg/plant) was significantly affected by the single factors of nutrition, pathogen, soil, temperature and cultivar (Table 20, 21). In brief, dry shoot weight was increased by high nutrition; by the absence of *P. clandestina*; by sand soil; by high temperature; and by using Meteora. Factors effecting dry shoot weight and their interactions are detailed in Fig 50.

Dry shoot weight was significantly affected by two-way interactions involving nutrition and pathogen; for pathogen and soil; for moisture and temperature; between nutrition and temperature; for pathogen and temperature; for soil and temperature; and, for pathogen and cultivar. For example, dry shoot weight increased under the conditions of high nutrition and the absence of *P. clandestina*; sand soil and the absence of *P. clandestina*; under high moisture and high temperature; high nutrition and high temperature high temperature and the absence of *P. clandestina*; sand soil and high temperature; and the absence of *P. clandestina* and application of Woogenellup.

Dry shoot weight was significantly affected by three-way interactions involving moisture, nutrition and temperature; and, for pathogen, temperature and cultivar. For example, dry shoot weight increased for high nutrition, high temperature and high moisture; for absence of *P. clandestina*, with high temperature and Woogenellup; and, for absence of *P. clandestina* with medium temperature and Meteora.

Relationships between important variables: There was a significant and strong positive relationship between tap root disease index (%) and lateral root disease index (%) ($y = 1.0864x - 5.8244$; $P<0.001$, $n = 94$; $R^2 = 0.92$). There was a significant negative relationship between tap root disease index (%) and dry root weight (mg plant^{-1}) ($y = -0.3915x + 43.196$; $P<0.001$ $n = 94$; $R^2 = 0.34$). There was a significant negative relationship between lateral root disease index (%) and dry root weight (mg plant^{-1}) ($y = -0.3349x + 39.73$; $P<0.001$ $n = 94$; $R^2 = 0.33$). There was a significant negative relationship between tap root disease index (%) and dry shoot weight (mg plant^{-1}) ($y = -0.8204x + 85.916$; $P<0.001$ $n = 94$; $R^2 = 0.29$). There was a significant negative relationship between lateral root disease index (%) and dry shoot weight (mg plant^{-1}) ($y = -0.7324x + 80.646$; $P<0.001$ $n = 94$; $R^2 = 0.30$). There was a significant and strong positive relationship between dry root weight (mg plant^{-1}) and dry shoot weight (mg plant^{-1}) ($y = 2.194x - 1.4134$; $P<0.001$ $n = 94$; $R^2 = 0.78$).

Table 20. Statistical main effects and interactions of environmental factors on disease indices (%) of tap and lateral root rot, nodulation index (%), emergence (%), and dry weights (mg plant^{-1}) of root and shoot.

	TDI%		*LDI%*		*NI%*		*Emergence rate%*		*DRW (mg plant^{-1})*		*DSW (mg plant^{-1})*	
Factors	*p-value*	*LSD $_{0.05}$*	*p-value*	*LSD $_{0.05}$*	*p-value*	*LSD $_{0.05}$*	*p-value*	*LSD $_{0.05}$*	*p-value*	*LSD $_{0.05}$*	*p-value*	*LSD $_{0.05}$*
Moisture	Ns	0.985	<0.001	0.831	Ns	0.1738	0.006	2.045	<0.001	2.019	Ns	12.91
Nutrition	<0.001	0.985	<0.001	0.831	<0.001	0.1738	Ns	2.045	<0.001	2.019	<0.001	12.91
Pathogen	<0.001	0.985	<0.001	0.831	<0.001	0.1738	0.036	2.045	<0.001	2.019	<0.001	12.91
Soil	<0.001	0.985	<0.001	0.831	<0.001	0.1738	<0.001	2.045	<0.001	2.019	<0.001	12.91
Temperature	<0.001	1.207	<0.001	1.018	<0.001	0.2129	<0.001	2.504	<0.001	2.473	<0.001	15.81
Cultivar	<0.001	1.393	<0.001	1.175	<0.001	0.2458	<0.001	2.892	0.036	2.855	0.005	18.26
Interactions												
Moisture*nutrition	Ns	1.393	Ns	1.175	Ns	0.2458	Ns	2.892	Ns	2.855	Ns	18.26
Moisture*pathogen	Ns	1.393	<0.001	1.175	Ns	0.2458	0.048	2.892	<0.001	2.855	Ns	18.26
Nutrition*pathogen	<0.001	1.393	<0.001	1.175	<0.001	0.2458	Ns	2.892	Ns	2.855	0.003	18.26
Moisture*soil	<0.001	1.393	<0.001	1.175	Ns	0.2458	<0.001	2.892	Ns	2.855	Ns	18.26
Nutrition*soil	Ns	1.393	Ns	1.175	<0.001	0.2458	Ns	2.892	0.008	2.855	Ns	18.26
Pathogen*soil	<0.001	1.393	<0.001	1.175	<0.001	0.2458	0.009	2.892	Ns	2.855	<0.001	18.26
Moisture*temperature	Ns	1.706	Ns	1.44	Ns	0.301	<0.001	3.542	0.002	3.497	0.002	22.37
Nutrition*temperature	0.002	1.706	0.002	1.44	<0.001	0.301	Ns	3.542	<0.001	3.497	0.035	22.37
Pathogen*temperature	<0.001	1.706	<0.001	1.44	<0.001	0.301	Ns	3.542	<0.001	3.497	0.037	22.37
Soil*temperature	<0.001	1.706	<0.001	1.44	<0.001	0.301	<0.001	3.542	Ns	3.497	0.025	22.37
Moisture*cultivar	Ns	1.97	0.014	1.662	<0.001	0.3476	<0.001	4.09	0.016	4.038	Ns	25.83
Nutrition*cultivar	Ns	1.97	Ns	1.662	<0.001	0.3476	Ns	4.09	Ns	4.038	Ns	25.83
Pathogen*cultivar	<0.001	1.97	<0.001	1.662	<0.001	0.3476	<0.001	4.09	<0.001	4.038	<0.001	25.83
Soil*cultivar	<0.001	1.97	<0.001	1.662	<0.001	0.3476	Ns	4.09	0.003	4.038	Ns	25.83
Temperature*cultivar	<0.001	2.413	<0.001	2.036	<0.001	0.4257	<0.001	5.009	<0.001	4.945	Ns	31.63
Moisture*nutrition*pathogen	Ns	1.97	Ns	1.662	Ns	0.3476	Ns	4.09	0.024	4.038	Ns	25.83
Moisture*nutrition*soil	Ns	1.97	0.048	1.662	Ns	0.3476	Ns	4.09	Ns	4.038	Ns	25.83
Moisture*pathogen*soil	<0.001	1.97	<0.001	1.662	Ns	0.3476	Ns	4.09	Ns	4.038	Ns	25.83
Nutrition*pathogen*soil	Ns	1.97	Ns	1.662	<0.001	0.3476	Ns	4.09	Ns	4.038	Ns	25.83
Moisture*nutrition*temperature	Ns	2.413	Ns	2.036	Ns	0.4257	Ns	5.009	0.008	4.945	0.029	31.63
Moisture*pathogen*temperature	Ns	2.413	Ns	2.036	Ns	0.4257	Ns	5.009	Ns	4.945	Ns	31.63
Nutrition*pathogen*temperature	0.002	2.413	0.002	2.036	<0.001	0.4257	Ns	5.009	<0.001	4.945	Ns	31.63
Moisture*soil*temperature	0.047	2.413	Ns	2.036	Ns	0.4257	Ns	5.009	Ns	4.945	Ns	31.63
Nutrition*soil*temperature	Ns	2.413	Ns	2.036	<0.001	0.4257	Ns	5.009	0.029	4.945	Ns	31.63
Pathogen*soil*temperature	<0.001	2.413	<0.001	2.036	<0.001	0.4257	0.02	5.009	Ns	4.945	Ns	31.63
Moisture*nutrition*cultivar	<0.001	2.787	<0.001	2.351	Ns	0.4916	Ns	5.784	Ns	5.71	Ns	36.52
Moisture*pathogen*cultivar	Ns	2.787	Ns	2.351	<0.001	0.4916	0.045	5.784	Ns	5.71	Ns	36.52
Nutrition*pathogen*cultivar	Ns	2.787	Ns	2.351	<0.001	0.4916	Ns	5.784	Ns	5.71	Ns	36.52
Moisture*soil*cultivar	Ns	2.787	<0.001	2.351	<0.001	0.4916	Ns	5.784	Ns	5.71	Ns	36.52

Nutrition*soil*cultivar	0.04	2.787	0.006	2.351	<0.001	0.4916	Ns	5.784	Ns	5.71	Ns	36.52
Pathogen*soil*cultivar	<0.001	2.787	<0.001	2.351	<0.001	0.4916	Ns	5.784	<0.001	5.71	Ns	36.52
Moisture*temperature*cultivar	Ns	3.413	<0.001	2.879	<0.001	0.602	0.012	7.083	Ns	6.994	Ns	44.73
Nutrition*temperature*cultivar	Ns	3.413	0.002	2.879	<0.001	0.602	Ns	7.083	Ns	6.994	Ns	44.73
Pathogen*temperature*cultivar	<0.001	3.413	<0.001	2.879	<0.001	0.602	Ns	7.083	0.015	6.994	<0.001	44.73
Soil*temperature*cultivar	<0.001	3.413	Ns	2.879	<0.001	0.602	0.003	7.083	0.004	6.994	Ns	44.73
Moisture*nutrition*pathogen*soil	Ns	2.787	Ns	2.351	Ns	0.4916	Ns	5.784	Ns	5.71	Ns	36.52
Moisture*nutrition*pathogen*temperature	Ns	3.413	Ns	2.879	Ns	0.602	Ns	7.083	<0.001	6.994	Ns	44.73
Moisture*nutrition*soil*temperature	Ns	3.413	<0.001	2.879	Ns	0.602	0.005	7.083	Ns	6.994	Ns	44.73
Moisture*pathogen*soil*temperature	0.042	3.413	Ns	2.879	Ns	0.602	Ns	7.083	Ns	6.994	Ns	44.73
Nutrition*pathogen*soil*temperature	Ns	3.413	Ns	2.879	<0.001	0.602	Ns	7.083	Ns	6.994	Ns	44.73
Moisture*nutrition*pathogen*cultivar	<0.001	3.941	<0.001	3.325	Ns	0.6952	Ns	8.179	Ns	8.076	Ns	51.65
Moisture*nutrition*soil*cultivar	<0.001	3.941	<0.001	3.325	0.016	0.6952	Ns	8.179	Ns	8.076	Ns	51.65
Moisture*pathogen*soil*cultivar	Ns	3.941	<0.001	3.325	<0.001	0.6952	0.004	8.179	0.007	8.076	Ns	51.65
Nutrition*pathogen*soil*cultivar	0.038	3.941	0.005	3.325	<0.001	0.6952	Ns	8.179	Ns	8.076	Ns	51.65
Moisture*nutrition*temperature*cultivar	0.043	4.827	0.04	4.072	0.006	0.8514	Ns	10.018	Ns	9.89	Ns	63.26
Moisture*pathogen*temperature*cultivar	Ns	4.827	<0.001	4.072	<0.001	0.8514	0.02	10.018	Ns	9.89	Ns	63.26
Nutrition*pathogen*temperature*cultivar	Ns	4.827	0.001	4.072	<0.001	0.8514	Ns	10.018	Ns	9.89	Ns	63.26
Moisture*soil*temperature*cultivar	0.023	4.827	0.006	4.072	<0.001	0.8514	0.024	10.018	Ns	9.89	Ns	63.26
Nutrition*soil*temperature*cultivar	Ns	4.827	Ns	4.072	<0.001	0.8514	Ns	10.018	0.032	9.89	Ns	63.26
Pathogen*soil*temperature*cultivar	<0.001	4.827	Ns	4.072	<0.001	0.8514	0.047	10.018	Ns	9.89	Ns	63.26
Moisture*nutrition*pathogen*soil*temperature	Ns	4.827	<0.001	4.072	Ns	0.8514	Ns	10.018	0.022	9.89	Ns	63.26
Moisture*nutrition*pathogen*soil*cultivar	<0.001	5.573	<0.001	4.702	0.016	0.9831	Ns	11.567	Ns	11.421	Ns	73.05
Moisture*nutrition*pathogen*temperature*cultivar	0.045	6.826	0.045	5.759	0.006	1.2041	Ns	14.167	0.005	13.987	Ns	89.46
Moisture*nutrition*soil*temperature*cultivar	<0.001	6.826	<0.001	5.759	0.016	1.2041	Ns	14.167	Ns	13.987	Ns	89.46
Moisture*pathogen*soil*temperature*cultivar	0.021	6.826	0.006	5.759	<0.001	1.2041	Ns	14.167	Ns	13.987	Ns	89.46
Nutrition*pathogen*soil*temperature*cultivar	Ns	6.826	Ns	5.759	<0.001	1.2041	Ns	14.167	Ns	13.987	Ns	89.46
Moisture*nutrition*pathogen*soil*temperature*cultivar	<0.001	9.653	<0.001	8.144	0.019	1.7028	Ns	20.035	Ns	19.781	Ns	126.52

Table 21. Effects of environmental factors on disease indices (%) of tap and lateral roots, nodulation index (%), emergence (%), dry weights of root and shoot (mg plant^{-1}) (Note: tap and lateral root disease indices on nil were all 0's therefore not listed in this Table). 'Nil' refers to the treatments without presence of *Phytophthora clandestina*.

						Cultivar				
	Moisture	*Nutrition*	*Pathogen*	*Soil type*	*Temperature*	*Meteora*	*Riverina*	*Seaton Park*	*Woogenellup*	***Mean***
Tap root disease index %	High	High	Phytophthora	Loam soil	High	41.08	30.46	20.54	80.07	43.04
					Medium	27.87	29.79	19.33	85.00	40.50
					Low	55.04	55.83	26.21	86.75	55.96
				Sand soil	High	27.42	23.79	3.33	78.24	33.20
					Medium	50.25	46.83	11.63	82.50	47.80
					Low	41.67	45.83	4.58	70.12	40.55
		Low	Phytophthora	Loam soil	High	34.79	33.54	8.54	39.86	29.18
					Medium	35.37	37.50	17.83	85.00	43.93
					Low	49.87	49.38	4.63	78.74	45.66
				Sand soil	High	19.75	22.71	0.50	76.11	29.77
					Medium	36.00	37.42	15.50	77.50	41.61
					Low	27.79	32.79	2.38	59.81	30.69
	Low	High	Phytophthora	Loam soil	High	48.04	58.05	25.37	50.53	45.50
					Medium	41.67	51.67	32.71	87.50	53.39
					Low	63.33	59.17	50.42	83.23	64.04
				Sand soil	High	16.42	24.58	3.08	75.28	29.84
					Medium	21.33	35.00	12.00	76.25	36.15
					Low	29.79	49.50	3.13	70.40	38.21
		Low	Phytophthora	Loam soil	High	38.43	30.33	14.25	84.99	42.00
					Medium	45.75	36.00	15.58	87.50	46.21
					Low	47.50	44.58	18.00	73.33	45.85
				Sand soil	High	19.75	28.40	0.00	66.89	28.76
					Medium	23.12	20.83	0.00	87.50	32.86
					Low	23.54	35.58	0.63	59.81	29.89
Lateral root disease index %	High	High	Phytophthora	Loam soil	High	14.79	15.46	6.58	80.32	29.29
					Medium	12.87	26.46	8.00	85.00	33.08
					Low	49.77	57.92	7.29	79.99	48.74
				Sand soil	High	13.79	17.62	4.62	76.86	28.22
					Medium	29.46	44.17	4.62	87.50	41.44
					Low	47.63	48.54	1.88	79.85	44.48
		Low	Phytophthora	Loam soil	High	19.17	20.62	2.17	39.97	20.48
					Medium	36.71	33.33	8.33	87.50	41.47
					Low	41.25	52.71	2.50	68.82	41.32
				Sand soil	High	17.50	17.00	0.00	70.53	26.26
					Medium	30.00	31.21	6.50	77.50	36.30
					Low	28.88	47.92	1.25	79.82	39.47
	Low	High	Phytophthora	Loam soil	High	40.58	57.35	27.54	50.16	43.91
					Medium	54.17	66.46	33.25	90.00	60.97
					Low	70.00	66.04	50.12	83.25	67.35
				Sand soil	High	13.92	14.58	0.00	75.47	25.99
					Medium	15.54	19.58	1.62	77.50	28.56
					Low	32.50	35.50	0.00	80.02	37.01

			Phytophthora		High	35.84	33.00	4.50	87.74	40.27
				Loam soil	Medium	45.92	51.17	11.96	87.50	49.14
					Low	56.25	60.42	25.12	79.97	55.44
		Low			High	18.00	15.61	0.00	55.23	22.21
				Sand soil	Medium	23.75	13.33	0.00	87.50	31.15
					Low	12.54	17.25	0.00	76.01	26.45
					High	0.00	0.00	0.00	0.03	0.01
			Nil	Loam soil	Medium	0.00	0.00	0.00	0.00	0.00
					Low	0.00	0.00	0.01	0.00	0.00
					High	0.00	0.00	0.00	0.00	0.00
				Sand soil	Medium	0.00	0.00	0.00	0.00	0.00
		High			Low	0.00	0.00	0.02	0.00	0.00
					High	0.00	0.00	0.00	0.00	0.00
			Phytophthora	Loam soil	Medium	0.00	0.00	0.00	0.00	0.00
					Low	0.00	0.00	0.00	0.03	0.01
					High	0.00	0.00	5.38	0.01	1.35
				Sand soil	Medium	0.00	0.00	0.00	0.00	0.00
	High				Low	0.00	0.00	0.00	0.01	0.00
					High	0.00	0.00	0.00	0.00	0.00
			Nil	Loam soil	Medium	0.00	0.00	0.00	0.02	0.00
					Low	0.00	0.02	0.00	0.00	0.00
					High	0.00	0.00	0.00	0.00	0.00
				Sand soil	Medium	0.00	0.00	0.00	0.02	0.00
		Low			Low	0.00	0.00	0.00	0.01	0.00
					High	0.00	0.00	0.50	0.10	0.15
			Phytophthora	Loam soil	Medium	0.00	0.00	0.63	0.00	0.16
					Low	0.00	0.00	0.00	0.02	0.01
					High	5.25	2.50	25.63	0.00	8.34
				Sand soil	Medium	0.00	0.00	0.00	0.00	0.00
Nodulation index %					Low	0.00	0.00	0.00	0.03	0.01
					High	0.00	0.03	0.00	0.00	0.01
			Nil	Loam soil	Medium	0.00	0.00	0.00	0.00	0.00
					Low	0.00	0.00	0.00	0.00	0.00
					High	0.00	0.00	0.00	0.02	0.00
				Sand soil	Medium	0.00	0.00	0.00	0.00	0.00
		High			Low	0.00	0.00	0.00	0.00	0.00
					High	0.83	0.00	0.00	0.09	0.23
			Phytophthora	Loam soil	Medium	0.00	0.00	0.00	0.00	0.00
					Low	0.00	0.00	0.00	0.06	0.02
					High	0.00	0.00	0.00	0.00	0.00
				Sand soil	Medium	0.00	0.00	0.00	0.00	0.00
	Low				Low	0.00	0.00	0.00	0.09	0.02
					High	0.00	0.00	0.00	0.00	0.00
			Nil	Loam soil	Medium	0.02	0.00	0.00	0.00	0.00
					Low	0.00	0.08	0.00	0.02	0.02
					High	0.00	0.06	0.01	0.00	0.02
				Sand soil	Medium	0.00	0.00	0.00	0.00	0.00
		Low			Low	0.00	0.00	0.00	0.04	0.01
					High	0.00	0.00	0.63	0.00	0.16
			Phytophthora	Loam soil	Medium	0.00	0.00	2.50	0.00	0.63
					Low	0.00	0.00	0.00	0.00	0.00
					High	8.92	13.32	14.21	0.00	9.11
				Sand soil	Medium	0.00	0.00	0.00	0.00	0.00

					Low	0.00	0.00	0.00	0.00	0.00
					High	70.00	62.50	80.00	28.56	60.27
				Loam soil	Medium	65.00	60.00	65.00	17.50	51.88
			Nil		Low	52.50	67.50	35.00	20.00	43.75
					High	75.00	57.50	85.00	47.50	66.25
				Sand soil	Medium	82.50	62.50	95.00	35.00	68.75
		High			Low	70.00	65.00	70.00	30.00	58.75
					High	72.50	72.50	85.00	21.39	62.85
				Loam soil	Medium	70.00	45.00	90.00	17.50	55.63
			Phytophthora		Low	52.50	57.50	67.50	12.50	47.50
					High	72.50	65.00	85.00	34.82	64.33
				Sand soil	Medium	80.00	65.00	77.50	20.00	60.63
	High				Low	72.50	62.50	82.50	17.50	58.75
					High	72.50	60.00	85.00	40.00	64.38
				Loam soil	Medium	65.00	47.50	82.50	35.00	57.50
			Nil		Low	65.00	47.50	37.50	15.00	41.25
					High	72.50	72.50	75.00	52.50	68.13
				Sand soil	Medium	80.00	47.50	100.00	27.50	63.75
		Low			Low	82.50	77.50	72.50	22.50	63.75
					High	60.00	60.00	85.00	33.79	59.70
				Loam soil	Medium	75.00	42.50	92.50	17.50	56.88
			Phytophthora		Low	75.00	65.00	82.50	5.00	56.88
					High	85.00	72.50	87.50	39.91	71.23
				Sand soil	Medium	55.00	52.50	82.50	22.50	53.13
Emergence rate%					Low	77.50	67.50	85.00	7.50	59.38
					High	77.50	42.84	72.50	33.43	56.57
				Loam soil	Medium	72.50	57.50	95.00	45.00	67.50
			Nil		Low	62.50	42.50	87.50	10.00	50.63
					High	75.00	45.00	65.00	48.45	58.36
				Sand soil	Medium	82.50	62.50	90.00	20.00	63.75
		High			Low	75.00	67.50	75.00	22.50	60.00
					High	62.50	51.46	82.50	20.72	54.30
				Loam soil	Medium	75.00	47.50	90.00	10.00	55.63
			Phytophthora		Low	55.00	55.00	75.00	12.50	49.38
					High	50.00	62.50	75.00	36.71	56.05
				Sand soil	Medium	67.50	50.00	95.00	20.00	58.13
	Low				Low	65.00	50.00	75.00	10.00	50.00
					High	65.00	47.50	95.00	40.23	61.93
				Loam soil	Medium	70.00	52.50	92.50	25.00	60.00
			Nil		Low	57.50	20.00	85.00	17.50	45.00
					High	45.64	40.17	57.67	47.50	47.75
				Sand soil	Medium	72.50	47.50	92.50	27.50	60.00
		Low			Low	85.00	75.00	87.50	20.00	66.88
					High	76.74	60.00	80.00	26.14	60.72
				Loam soil	Medium	70.00	60.00	82.50	17.50	57.50
			Phytophthora		Low	55.00	47.50	80.00	17.50	50.00
					High	50.00	29.57	75.00	30.48	46.26
				Sand soil	Medium	70.00	60.00	87.50	15.00	58.13
					Low	60.00	62.50	67.50	15.00	51.25
					High	51.04	60.43	39.35	74.20	56.26
				Loam soil	Medium	40.98	40.00	31.75	38.31	37.76
					Low	24.75	30.17	43.20	42.81	35.23
			Nil		High	67.34	73.97	45.26	82.81	67.35

				Sand soil	Medium	60.28	70.11	39.34	55.56	56.32
					Low	46.92	45.17	38.08	45.05	43.81
		High			High	34.03	37.46	45.66	28.34	36.37
				Loam soil	Medium	27.81	34.03	28.96	27.38	29.55
					Low	9.18	13.18	14.35	9.71	11.61
			Phytophthora		High	73.58	68.11	62.86	9.05	53.40
				Sand soil	Medium	36.74	25.20	54.38	7.88	31.05
					Low	13.28	14.51	35.41	24.01	21.80
	High				High	40.30	41.33	39.95	51.31	43.22
				Loam soil	Medium	24.50	31.35	24.37	31.95	28.04
					Low	20.88	39.88	26.13	34.33	30.31
			Nil		High	72.24	70.69	58.25	86.55	71.93
				Sand soil	Medium	56.75	43.73	49.30	52.63	50.60
					Low	35.55	45.02	52.65	41.94	43.79
		Low			High	14.63	14.52	23.65	10.99	15.95
				Loam soil	Medium	12.96	17.79	18.77	8.56	14.52
			Phytophthora		Low	10.58	9.94	13.61	1.91	9.01
					High	25.36	23.45	42.22	20.74	27.94
				Sand soil	Medium	36.09	34.35	50.31	14.29	33.76
Dry root weight (mg/plant)					Low	9.95	17.64	24.59	18.88	17.77
					High	48.01	65.41	28.21	59.34	50.24
				Loam soil	Medium	29.71	19.28	18.93	26.42	23.59
			Nil		Low	33.66	38.96	28.04	36.01	34.17
					High	56.60	55.10	42.63	49.61	50.99
				Sand soil	Medium	41.56	29.53	27.36	31.51	32.49
					Low	53.32	46.21	45.78	95.09	60.10
		High			High	32.23	20.56	28.31	44.17	31.32
				Loam soil	Medium	21.20	15.04	23.36	11.25	17.71
			Phytophthora		Low	11.23	12.12	10.42	7.14	10.23
					High	58.01	40.54	35.12	17.50	37.79
				Sand soil	Medium	49.50	43.09	39.07	14.88	36.64
					Low	12.50	19.94	34.10	12.57	19.78
	Low				High	39.94	33.68	26.93	29.97	32.63
				Loam soil	Medium	33.04	26.20	24.25	33.25	29.19
			Nil		Low	15.73	27.34	17.75	17.65	19.62
					High	54.90	46.82	40.64	69.13	52.87
				Sand soil	Medium	51.19	47.16	40.91	37.81	44.27
					Low	29.04	33.59	34.67	40.23	34.38
		Low			High	17.55	8.10	23.01	0.00	12.17
				Loam soil	Medium	20.63	15.98	22.69	17.50	19.20
			Phytophthora		Low	9.85	11.84	10.52	15.54	11.94
					High	67.67	46.07	25.40	4.26	35.85
				Sand soil	Medium	44.54	30.90	37.29	9.38	30.53
					Low	27.70	25.42	33.23	13.73	25.02
					High	183.60	201.40	133.40	278.90	199.33
				Loam soil	Medium	114.50	101.40	88.50	68.70	93.28
			Nil		Low	102.60	85.10	62.90	107.30	89.48
					High	313.80	289.20	200.80	467.20	317.75
				Sand soil	Medium	178.20	180.30	107.30	129.20	148.75
					Low	141.70	116.10	90.00	112.90	115.18
		High			High	90.10	85.20	105.30	10.70	72.83
				Loam soil	Medium	50.30	58.60	64.90	37.80	52.90
					Low	21.00	29.00	30.60	25.30	26.48

Dry shoot weight (mg/plant)	High		Phytophthora	Sand soil	High	181.90	139.40	220.90	22.40	141.15
					Medium	58.70	53.30	111.10	7.90	57.75
					Low	42.20	46.50	54.50	29.50	43.18
		Low	Nil	Loam soil	High	70.70	68.10	61.70	69.80	67.58
					Medium	44.60	48.10	34.40	49.10	44.05
					Low	56.70	62.50	40.50	53.10	53.20
				Sand soil	High	228.40	164.50	149.40	282.10	206.10
					Medium	144.90	97.30	103.70	130.30	119.05
					Low	112.60	106.40	118.40	102.30	109.93
			Phytophthora	Loam soil	High	31.60	21.10	52.10	30.40	33.80
					Medium	24.40	35.30	31.70	21.40	28.20
					Low	18.50	22.90	19.80	17.30	19.63
				Sand soil	High	89.50	65.10	93.70	18.90	66.80
					Medium	51.40	55.60	80.50	16.90	51.10
					Low	33.20	34.90	40.50	28.60	34.30
	Low	High	Nil	Loam soil	High	96.90	151.70	78.30	147.30	118.55
					Medium	103.30	78.60	62.30	96.50	85.18
					Low	87.80	82.10	77.40	66.50	78.45
				Sand soil	High	206.20	138.40	136.90	212.10	173.40
					Medium	192.20	87.30	92.00	133.60	251.28
					Low	139.90	121.70	100.80	127.80	122.55
			Phytophthora	Loam soil	High	67.90	44.00	75.70	40.50	57.03
					Medium	50.10	35.90	46.20	27.70	39.98
					Low	28.20	25.40	34.90	27.30	28.95
				Sand soil	High	147.70	93.00	128.40	48.80	104.48
					Medium	110.50	83.80	107.30	23.90	81.38
					Low	60.40	49.50	56.30	35.60	50.45
		Low	Nil	Loam soil	High	87.50	66.00	49.50	47.80	62.70
					Medium	59.30	47.20	50.40	55.00	52.98
					Low	37.90	26.80	30.90	23.30	29.73
				Sand soil	High	139.30	126.20	92.10	245.30	150.73
					Medium	129.90	100.30	84.70	79.60	98.63
					Low	70.60	74.80	74.20	77.00	74.15
			Phytophthora	Loam soil	High	30.80	23.70	45.90	0.00	25.10
					Medium	39.90	34.50	35.80	25.80	34.00
					Low	30.60	22.40	23.50	15.10	22.90
				Sand soil	High	155.40	81.30	54.70	26.80	79.55
					Medium	79.60	50.40	85.70	21.40	59.28
					Low	69.60	47.00	60.80	31.90	52.33

Fig. 45. Emergence (%) as affected by environment factors and their interactions.

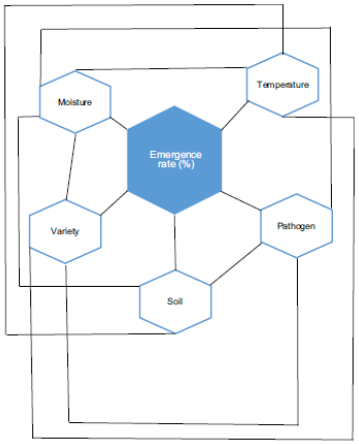


Fig. 46. Tap root disease index (%) as affected by environment factors and their interactions.

Fig. 47. Lateral root disease index (%) as affected by environment factors and their interactions.

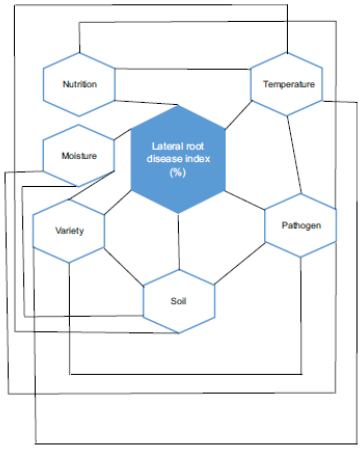


Fig. 48. Nodulation index (%) as affected by environment factors and their interactions.

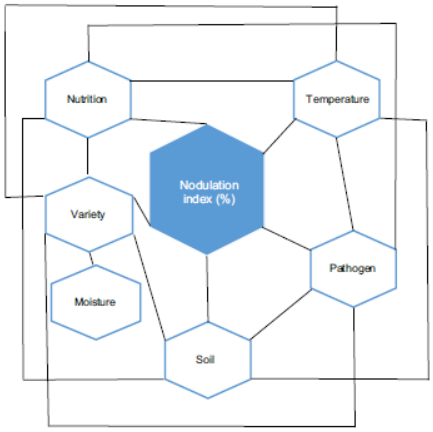


Fig. 49. Dry root weight (mg/p) as affected by environment factors and their interactions.

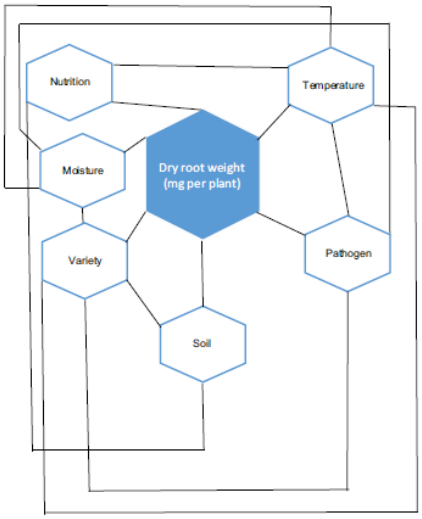
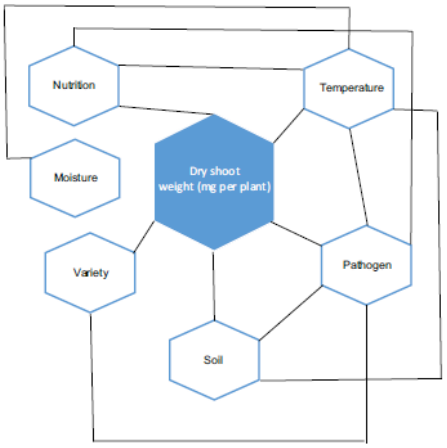


Fig. 50. Dry shoot weight (mg/p) as affected by environment factors and their interactions.



4.3.3 Environmental interactions – *Rhizoctonia*

***Germination*:** Germination (%) was extremely low at the two cooler of the three temperature regimes used in these studies (viz. 14/9 and 18/13ºC). Germination at the highest temperature regime (viz. 22/17°C) was significantly affected by moisture (P<0.05), pathogen (P<0.001) and variety (P<0.001). It was also significantly affected by two way interactions of moisture with nutrition P<0.01), of moisture with pathogen (P<0.001), of nutrition with pathogen (P<0.001), of nutrition with soil type (P<0.05), of pathogen with soil type (P<0.001), of pathogen with variety (P<0.005), and of soil type with variety P<0.05). It was significantly affected by three way interactions of moisture, nutrition and pathogen (P<0.05), of moisture, nutrition and soil type (P<0.05), of moisture, pathogen and soil type (P<0.001), of nutrition, pathogen and soil type (P<0.05). (Fig. 51a; Tables 22, 23).

***Tap root disease index*:** While germination (%) was extremely low at the two cooler of the three temperature regimes used in these studies (viz. 14/9 and 18/13ºC) there were sufficient plants surviving to enable assessment of tap root disease on these particular seedlings before their subsequent death from damping-off at 3-5 weeks post-emergence. Tap root disease index on surviving seedlings at the highest temperature regime (viz. 22/17°C) was significantly affected by moisture (P<0.001), nutrition (P<0.001), pathogen (P<0.001) and soil type (P<0.001). It was significantly affected by two way interactions of moisture with nutrition (P<0.001), of moisture with pathogen (P<0.001), of nutrition with pathogen (P<0.005), of moisture with soil type (P<0.001), of nutrition with soil type (P<0.05) and of pathogen with soil type (P<0.001). It was significantly affected by three way interactions of moisture, nutrition and pathogen (P<0.001), of moisture, nutrition and soil type (P<0.05), of moisture, pathogen and soil type (P<0.001), of nutrition, pathogen and soil type (P<0.05), of nutrition, soil type and variety. (Fig. 51b; Tables 22, 23).

***Lateral root disease index*:** While germination (%) was extremely low at the two cooler of the three temperature regimes used in these studies (viz. 14/9 and 18/13ºC) there were sufficient plants surviving to enable assessment of lateral root disease on seedlings before their subsequent death from damping-off at 3-5 weeks post-emergence. Lateral root rot disease index on surviving seedlings at the highest temperature regime (viz. 22/17°C) was effected by moisture (P<0.001), nutrition (P<0.001), pathogen (P<0.001) and soil type (P<0.001). It was significantly affected by two way interactions of moisture with nutrition (P<0.005), of moisture with pathogen (P<0.001), of nutrition with pathogen (P<0.005), of moisture with soil type (P<0.001), of nutrition with soil type (P<0.05) and of pathogen with soil type (P<0.001). It was significantly affected by three way interactions of moisture, nutrition and pathogen (P<0.001), of moisture, nutrition and soil type (P<0.05), of moisture, pathogen and soil type (P<0.001), of nutrition, pathogen and soil type (P<0.05) and of nutrition, soil type and variety (P<0.05). (Fig. 51c; Tables 22, 23).

***Dry root weight*:** Root dry weight on surviving seedlings at the highest temperature regime (viz. 22/17°C) was affected by moisture (P<0.001), nutrition (P<0.001) and pathogen (P<0.001). It was significantly affected by two way interactions of moisture with nutrition (P<0.05), of moisture with pathogen (P<0.05), of nutrition with soil type (P<0.05), of pathogen with soil type (P<0.001), of moisture with variety (P<0.05). It was significantly affected by three way interactions of moisture, pathogen and soil type (P<0.005 (Fig 51d; Tables 22, 23).

***Dry shoot weight*:** Shoot dry weight on surviving seedlings at the highest temperature regime (viz. 22/17°C) was effected by moisture (P<0.001), nutrition (P<0.001), pathogen (P<0.001), soil type (P<0.005), and variety (P<0.05). It was significantly affected by two way interactions of moisture with nutrition (P<0.05), of moisture with pathogen (P<0.05), of pathogen with soil type (P<0.001). It was significantly affected by three way interactions of moisture, nutrition and pathogen (P<0.01), of moisture, pathogen and soil type (P<0.01) (Fig. 51e; Tables 22, 23).

Table 22. Statistical main effects and interactions of environmental factors on disease indices (%) of tap and lateral root rot, nodulation index (%), emergence (%), and dry weights (mg plant^{-1}) of root and shoot. Data is only for the high 22/17°C day/night temperature regime as germination was at best minimal at the lower two temperature regimes of 14/9; 18/13°C day/night. TDI (%) = Tap Root Disease Index (%); LDI% = Lateral Root Disease Index (%); DRW (mg/p) = Dry Root Weight (mg/plant); Dry Shoot Weight (mg/plant).

	Germination %		TDI (%)		LDI%		DRW (mg/p)		DSW (mg/p)	
Factor	***P*-value**	**LSD at *P*=0.05**	***P*-value**	**LSD at *P*=0.05**	***P*-value**	**LSD at *P*=0.05**	***P*-value**	**LSD at *P*=0.05**	***P*-value**	**LSD at *P*=0.05**
Moisture	0.048	4.14	<.001	3.45	<.001	3.70	<.001	3.00	<.001	4.30
Nutrition	0.321	4.14	<.001	3.45	<.001	3.70	<.001	3.00	<.001	4.30
Pathogen	<.001	4.14	<.001	3.45	<.001	3.70	<.001	3.00	<.001	4.30
Soil type	0.842	4.14	<.001	3.45	<.001	3.70	0.925	3.00	0.004	4.30
Variety	<.001	5.07	0.691	4.23	0.832	4.54	0.061	3.67	0.03	5.27
Interaction										
Moisture x Nutrition	0.006	5.85	<.001	4.88	0.003	5.24	0.011	4.24	0.031	6.08
Moisture x Pathogen	<.001	5.85	<.001	4.88	<.001	5.24	0.024	4.24	0.015	6.08
Nutrition x Pathogen	<.001	5.85	0.001	4.88	0.003	5.24	0.469	4.24	0.087	6.08
Moisture x Soil type	0.234	5.85	<.001	4.88	<.001	5.24	0.221	4.24	0.181	6.08
Nutrition x Soil type	0.03	5.85	0.015	4.88	0.012	5.24	0.041	4.24	0.201	6.08
Pathogen x Soil type	<.001	5.85	<.001	4.88	<.001	5.24	<.001	4.24	<.001	6.08
Moisture x Variety	0.127	7.16	0.672	5.98	0.542	6.42	0.044	5.19	0.101	7.45
Nutrition x Variety	0.180	7.16	0.268	5.98	0.404	6.42	0.194	5.19	0.327	7.45
Pathogen x Variety	0.001	7.16	0.575	5.98	0.534	6.42	0.828	5.19	0.765	7.45
Soil type x Variety	0.017	7.16	0.799	5.98	0.563	6.42	0.933	5.19	0.322	7.45
Moisture x Nutrition x Pathogen	0.011	8.27	<.001	6.90	<.001	7.41	0.050	5.99	0.005	8.60
Moisture x Nutrition x Soil type	0.048	8.27	0.012	6.90	0.035	7.41	0.291	5.99	0.383	8.60
Moisture x Pathogen x Soil type	<.001	8.27	<.001	6.90	<.001	7.41	0.002	5.99	0.006	8.60
Nutrition x Pathogen x Soil type	0.018	8.27	0.017	6.90	0.036	7.41	0.901	5.99	0.406	8.60
Moisture x Nutrition x Cultivar	0.616	10.13	0.256	8.46	0.14	9.07	0.675	7.34	0.851	10.54
Moisture x Pathogen x Variety	0.107	10.13	0.592	8.46	0.824	9.07	0.968	7.34	0.928	10.54

Nutrition x Pathogen x Variety	0.151	10.13	0.197	8.46	0.143	9.07	0.59	7.34	0.412	10.54
Moisture x Soil type x Variety	0.066	10.13	0.812	8.46	0.818	9.07	0.909	7.34	0.738	10.54
Nutrition x Soil type x Variety	0.202	10.13	0.014	8.46	0.037	9.07	0.333	7.34	0.055	10.54
Pathogen x Soil type x Variety	0.090	10.13	0.878	8.46	0.814	9.07	0.657	7.34	0.648	10.54

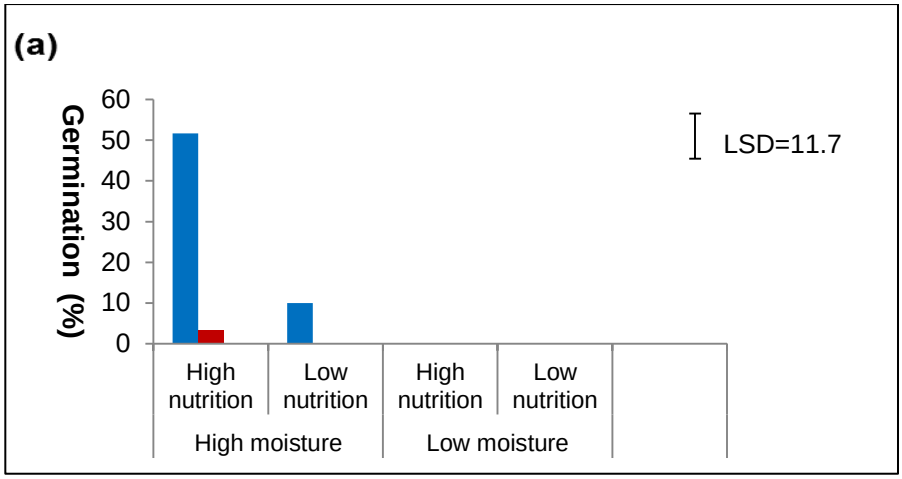
Table 23. Effects of environmental factors on disease indices (%) of tap and lateral roots, nodulation index (%), emergence (%), dry weights of root and shoot (mg plant^{-1}) (Note: tap and lateral root disease indices on nil controls were all 0's therefore not listed in this Table). 'Nil' refers to the treatments without presence of *Rhizoctonia solani* ZG6. Data is only for the high 22/17°C day/night temperature regime as germination was at best minimal at the lower two temperature regimes of 14/9; 18/13°C day/night.

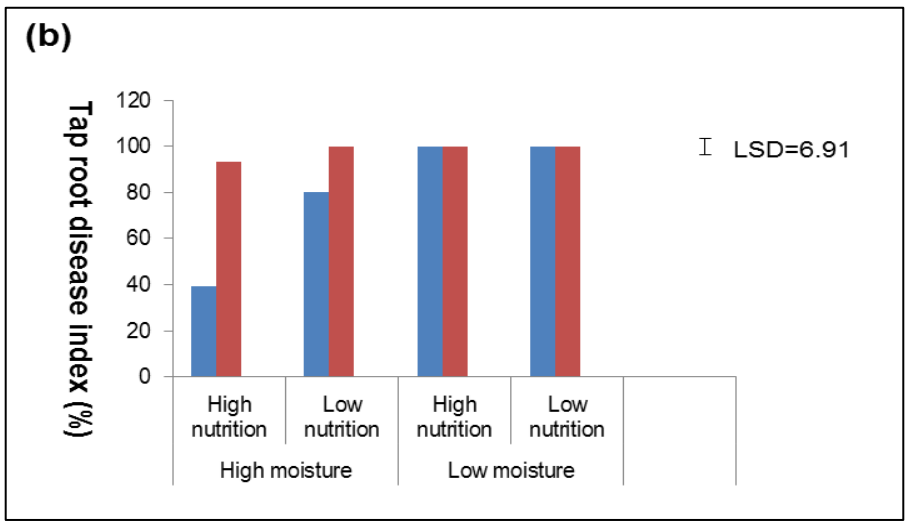
	Moisture	*Nutrition*	*Pathogen*	*Soil type*	*Riverina*	*Seaton Park*	*Woogenellup*	*Mean*
Emergence (%)	High	High	Nil	Gingin loam	45.0	80.0	60.0	***61.7***
				Potting mix	75.0	95.0	90.0	***86.7***
			Rhizoctonia	Gingin loam	10.0	60.0	85.0	***51.7***
				Potting mix	10.0	0.0	0.0	***3.3***
		Low	Nil	Gingin loam	45.0	90.0	75.0	***70.0***
				Potting mix	80.0	95.0	100.0	***91.7***
			Rhizoctonia	Gingin loam	15.0	0.0	15.0	***10.0***
				Potting mix	0.0	0.0	0.0	***0.0***
	Low	High	Nil	Gingin loam	65.0	90.0	85.0	***80.0***
				Potting mix	70.0	90.0	90.0	***83.3***
			Rhizoctonia	Gingin loam	0.0	0.0	0.0	***0.0***
				Potting mix	0.0	0.0	0.0	***0.0***
		Low	Nil	Gingin loam	75.0	90.0	95.0	***86.7***
				Potting mix	85.0	90.0	100.0	***91.7***
			Rhizoctonia	Gingin loam	0.0	0.0	0.0	***0.0***
				Potting mix	0.0	0.0	0.0	***0.0***
Mean					***35.9***	***48.8***	***49.7***	***44.8***
	High	High	Nil	Gingin loam	0.0	0.0	0.0	***0.0***
				Potting mix	0.0	0.0	0.0	***0.0***
			Rhizoctonia	Gingin loam	60.0	27.5	30.0	***39.2***
				Potting mix	80.0	100.0	100.0	***93.3***
		Low	Nil	Gingin loam	2.5	0.0	0.0	***0.8***
				Potting mix	0.0	0.0	0.0	***0.0***

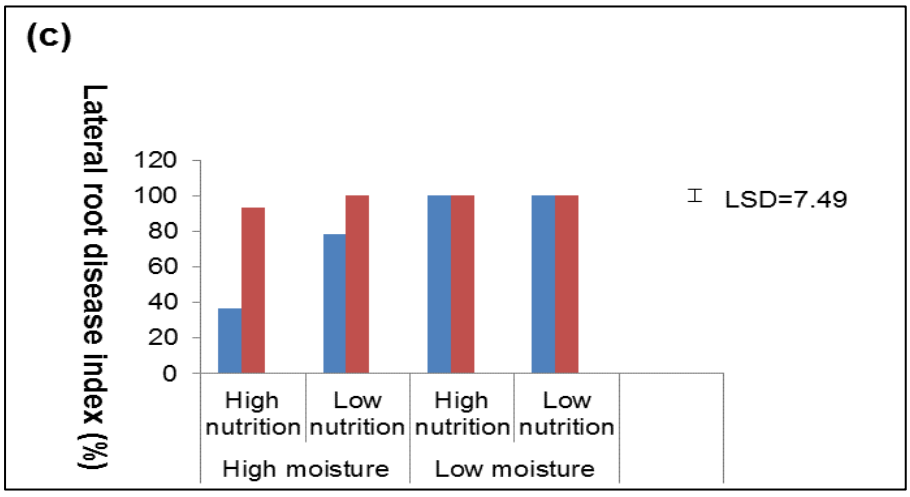
Tap root disease index (%)			Rhizoctonia	Gingin loam	55.0	100.0	85.0	***80.0***
				Potting mix	100.0	100.0	100.0	***100.0***
	Low	High	Nil	Gingin loam	0.0	0.0	0.0	***0.0***
				Potting mix	0.0	0.0	0.0	***0.0***
			Rhizoctonia	Gingin loam	100.0	100.0	100.0	***100.0***
				Potting mix	100.0	100.0	100.0	***100.0***
		Low	Nil	Gingin loam	0.0	0.0	0.0	***0.0***
				Potting mix	0.6	0.0	1.0	***0.5***
			Rhizoctonia	Gingin loam	100.0	100.0	100.0	***100.0***
				Potting mix	100.0	100.0	100.0	***100.0***
Mean					***43.6***	***45.5***	***44.8***	***44.6***
Lateral root disease index (%)	High	High	Nil	Gingin loam	0.0	0.0	0.0	***0.0***
				Potting mix	0.0	0.0	0.0	***0.0***
			Rhizoctonia	Gingin loam	60.0	25.0	25.0	***36.7***
				Potting mix	80.0	100.0	100.0	***93.3***
		Low	Nil	Gingin loam	0.0	0.0	0.0	***0.0***
				Potting mix	0.0	0.0	0.0	***0.0***
			Rhizoctonia	Gingin loam	55.0	100.0	80.0	***78.3***
				Potting mix	100.0	100.0	100.0	***100.0***
	Low	High	Nil	Gingin loam	0.0	0.0	0.0	***0.0***
				Potting mix	0.0	0.0	0.0	***0.0***
			Rhizoctonia	Gingin loam	100.0	100.0	100.0	***100.0***
				Potting mix	100.0	100.0	100.0	***100.0***
		Low	Nil	Gingin loam	10.2	0.0	0.0	***3.4***
				Potting mix	0.8	0.0	0.0	***0.3***
			Rhizoctonia	Gingin loam	100.0	100.0	100.0	***100.0***
				Potting mix	100.0	100.0	100.0	***100.0***
Mean					***44.1***	***45.3***	***44.1***	***44.5***
			Nil	Gingin loam	28.6	19.7	19.6	***22.6***

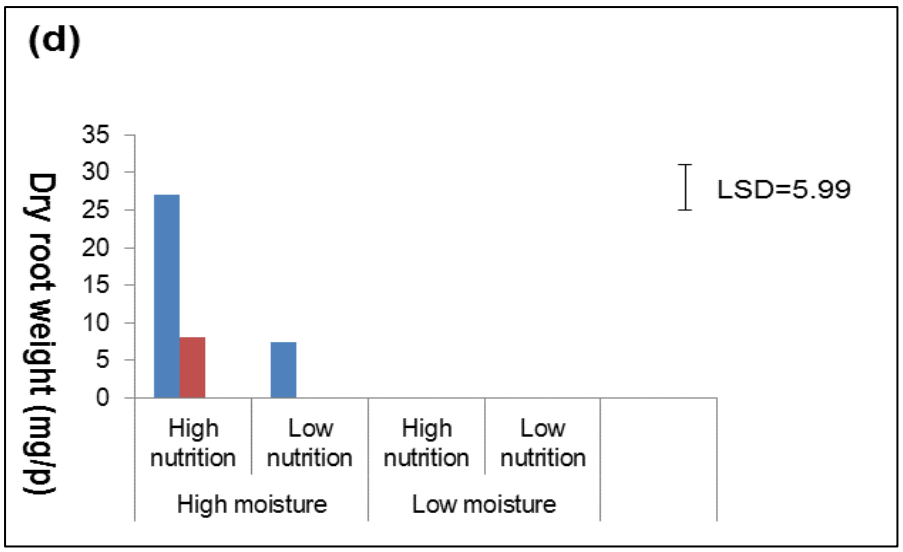
Dry root weight (mg/p)	High	High	High		Potting mix	39.2	25.3	21.9	***28.8***
			Rhizoctonia		Gingin loam	26.3	27.8	27.2	***27.1***
					Potting mix	24.0	0.0	0.0	***8.0***
		Low	Nil		Gingin loam	18.8	10.3	11.1	***13.4***
					Potting mix	23.3	27.7	29.3	***26.7***
			Rhizoctonia		Gingin loam	15.8	0.0	6.3	***7.4***
					Potting mix	0.0	0.0	0.0	***0.0***
	Low	High	Nil		Gingin loam	26.8	17.0	18.0	***20.6***
					Potting mix	19.8	23.8	21.2	***21.6***
			Rhizoctonia		Gingin loam	0.0	0.0	0.0	***0.0***
					Potting mix	0.0	0.0	0.0	***0.0***
		Low	Nil		Gingin loam	8.5	18.2	14.6	***13.8***
					Potting mix	20.7	22.2	19.5	***20.8***
			Rhizoctonia		Gingin loam	0.0	0.0	0.0	***0.0***
					Potting mix	0.0	0.0	0.0	***0.0***
Mean						***15.7***	***12.0***	***11.8***	***13.2***
Dry shoot weight (mg/p)	High	High	High		Gingin loam	50.0	28.8	38.4	***39.0***
					Potting mix	80.7	56.9	53.7	***63.8***
			Rhizoctonia		Gingin loam	27.5	44.4	48.3	***40.1***
					Potting mix	38.3	0.0	0.0	***12.8***
		Low	Nil		Gingin loam	28.7	16.0	20.9	***21.9***
					Potting mix	45.5	45.1	50.4	***47.0***
			Rhizoctonia		Gingin loam	19.8	0.0	6.8	***8.9***
					Potting mix	0.0	0.0	0.0	***0.0***
	Low	High	Nil		Gingin loam	44.2	36.7	38.6	***39.8***
					Potting mix	62.6	56.0	51.2	***56.6***
			Rhizoctonia		Gingin loam	0.0	0.0	0.0	***0.0***
					Potting mix	0.0	0.0	0.0	***0.0***
		Low	Nil		Gingin loam	14.3	21.2	18.7	***18.0***

	Low		Potting mix	40.3	40.0	34.9	***38.4***
		Rhizoctonia	Gingin loam	0.0	0.0	0.0	***0.0***
			Potting mix	0.0	0.0	0.0	***0.0***
Mean				***28.2***	***21.6***	***22.6***	***24.1***


(a)
Germination (%)
60
50
40
30
20
10
0
LSD=11.7
High nutrition
Low nutrition
High nutrition
Low nutrition
High moisture
Low moisture


(b)
Tap root disease index (%)
120
100
80
60
40
20
0
LSD=6.91
High nutrition
Low nutrition
High nutrition
Low nutrition
High moisture
Low moisture


(c)
Lateral root disease index (%)
120
100
80
60
40
20
0
LSD=7.49
High nutrition
Low nutrition
High nutrition
Low nutrition
High moisture
Low moisture



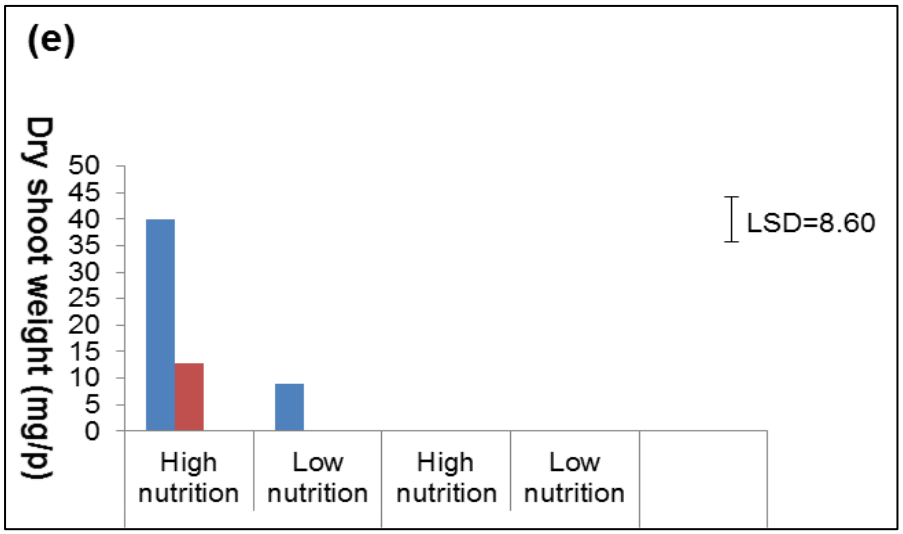


Fig. 51 a-e Effect of *Rhizoctonia solani* ZG6 under different moisture, nutrition and soil type conditions on the subterranean clover (*Trifolium subterraneum*) (**a**) germination (%), (**b**) tap root disease index (%), (**c**) lateral root disease index, (**d**) root dry weight per plant (mg), and (**e**) shoot dry weight per plant (mg). Note: Blue colour columns = Gingin loam; Red colour columns = sand soil. Data is only for the high 22/17°C day/night temperature regime as germination was at best minimal at the lower two temperature regimes of 14/9; 18/13°C day/night.

4.3.4 Environmental interactions – *Aphanomyces*

***Overview and outcomes from modelling approaches*:** Modelling found complex relationships between environmental variables and the presence of Aphanomyces root rot. Linear modelling identified multiple significant interactions for each dependent variable (dry shoot weight, dry root weight, emergence, tap root disease index, and lateral root disease index) (Table 24). Furthermore, all environmental variables were found significant as part of some interaction or as main effects within these models (temperature, soil, moisture, nutrition, variety).

The second approach to modelling, using boosted regression trees, provided support for the complex nature of the relationships found in linear models. For diseased plants, nutrition had the

lowest relative influence on all dependent variables, while temperature and either soil or variety were the most influential. When considering the difference between diseased and control plants, soil type and nutrition had most influence on root and shoot weights, while soil and moisture had the most influence on emergence. (Table 25 and Fig. 52).

The unpruned decision trees provided a visual representation of the complex relationships identified in linear and boosted regression tree modelling (Fig. 53, 54, 55). . For diseased plants, both dry shoot weight and dry root weight initially split with variable soil, while tap root disease index and lateral root disease index both split initially with temperature (Figures 53, 54). This was consistent with outputs from boosted regression trees, which showed soil to have the greatest or second greatest relative influence on dry shoot weight and dry root weight and temperature to have the greatest influence on tap root disease index and lateral root disease index. For diseased-control difference data, all models initially split with the same variables found most influential in boosted regression trees (Fig. 55).

Heat maps illustrating the mean actual values across explanatory variable combinations provided a complementary visual representation of the complex relationships for diseased plant and difference data (Fig. 56, 57). For dry shoot weight, dry root weight, tap root disease index and lateral root disease index, a trend was found for soil type and/or temperature, with sand-based soil and high and medium temperatures having either higher weights or lower disease indices. Lowest weights as well as higher disease indices were found for loam soil and low temperature. Patterns in emergence did not show a clear trend. Models of difference data for dry shoot weight and dry root weight showed similar patterns to diseased data models (Fig. 56, 57). Greater differences between diseased and control plants were found in sand-based soil and high or medium temperatures for dry shoot weight, and sand-based soil for dry root weight. Heat maps showing the predicted values from linear modelling and boosted regression trees showed similar trends.

An overview of relationships between factors (moisture, temperature, nutrition, soil type, variety) and their interactions influencing emergence (%), tap root disease index (%), lateral root disease index (%), dry root weight (mg/p), and dry shoot weight (mg/p) in the presence of *A. trifolii* are illustrated in Fig. 58 a-e.

***Explanatory variables and dry shoot weight and dry root weight**:* Modelling found complex relationships between dried shoot weights and dried root weights of plants inoculated with *A. trifolii* and environmental variables. Linear modelling found multiple significant interactions involving all environmental variables. For dry root weight there was a significant 4-way interaction between soil, moisture, nutrition and variety. For dry shoot weight there were two similar 3-way interactions, between moisture, nutrition and variety and soil, nutrition and variety (Table 24).

BRT models provided further support for the influence of all environmental variables in influencing dry shoot weight and dry root weight (Table 25 and Fig. 52), with soil, temperature and variety being most influential, and accounting for over 80% of the relative influence in models. This pattern was also illustrated in decision tree models, where soil and temperature were the initial and second splitting variables (Fig. 53). In these models plants with the highest dry shoot weight were planted in sand-based soil, under high or medium temperatures and low nutrition, and were of varieties Riverina or Woogenellup. Similarly plants with the highest dry root weight were planted in sand-based soil, under medium temperature and were of varieties Seaton Park and Riverina. For both dry shoot weight and dry root weight, plants that had the smallest weights were grown in loam soil, under high or low temperatures and high moisture.

Heat maps showed a clear increase in dry shoot weight for plants grown in sand-based soil, in medium or high temperatures. A similar pattern was evident for dry root weight, but less obvious. Lowest values of dry shoot weight and dry root weight were found for loam soils, particularly under low temperatures. (Fig. 56).

Results of modelling the differences between mean emergence of diseased plants and control plants were less clear and not consistent with previous models, with nutrition and soil having the strongest relative influence on differences in dry shoot weight and dry root weight (Table 25) and both decision trees splitting initially with nutrition (Fig. 55).

Explanatory variables and emergence: Linear modelling identified two significant interactions for explaining the relationship between emergence and environmental variables, a 4-way interaction between temperature, moisture, nutrition and variety, and a 3-way interaction between temperature soil and variety (Table 1). Relative influence of these variables from BRT modelling found variety had the highest influence on emergence, but all variables had at least 13% influence (Table 25 and Fig. 52).

Decision trees appeared to support results from BRT modelling, as the full tree had 11 splits and included all environmental variables. Plants with the highest emergence were of variety Woogenellup, grown in high moisture, while the lowest emergence proportions were for plants of variety Riverina, grown in loam soil (Figure 3). Heat maps did not show a clear pattern (Fig. 56).

Results of modelling the differences between mean emergence of diseased plants and control plants showed soil to be the most influential variable in BRT models and decision trees (Table 25 and Fig. 55). The heat map of the differences in emergences also showed smaller differences for plants grown in loam soil with high moisture and high nutrition (Fig. 56).

***Explanatory variables and tap root and lateral root disease index*:** Less complex interactions were identified in models for root disease indexes (tap root disease index and lateral root disease index), and some environmental variables were only found significant as main effects (Table 1). Boosted regression trees found similar relative influence for tap root disease index and lateral root disease index, with Temperature having almost 50% relative influence in both models (Table 25). Decision trees also both split initially with temperature, and here plants with the highest disease indices were grown under low temperatures, with this being the only split. Splitting for the lowest disease indices was not consistent, however. For tap root disease index plants with the lowest indices were of variety Woogenellup and grown under high temperatures, while for lateral root disease index the lowest values were for plants grown under medium or high temperatures, under low moisture, were of variety Woogenellup and were grown in sand-based soil (Fig. 54). Heat maps also showed higher disease indices for low temperatures (Fig. 56).

Table 24. Significant interactions from linear and generalised linear modelling of the effects of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables dried shoot weight (DSW), dried root weight (DRW), emergence, and tap root and lateral root disease indices (TDI, LDI) of three subterranean clover (*Trifolium subterraneum*) varieties (Riverina, Seaton Park and Woogenellup) inoculated with *Aphanomyces trifolii* in a controlled environment experiment.

Model	*Significant interactions*	*df*	*p-value*	*Res. Dev. on df*	*R^2*	*RMSE*
DSW	Moisture x nutrition x variety	2	>0.001			
	Soil x nutrition x variety	2	0.049	-	0.54	9.07
	Temp x moisture x nutrition	2	0.048			
DRW	Soil x moisture x nutrition x variety	2	0.032	-	0.50	9.16
	Temp x variety	4	>0.001			
Emergence	Temp x moisture x nutrition x variety	4	0.011	250.43 on 216	-	0.41
	Temp x soil x variety	4	>0.001			
TDI	Soil x variety	2	0.005			
	Soil x nutrition	1	0.019	30.437 on 256	-	15.20
	Moisture	1	>0.001			
	Temp	2	>0.001			
LDI	Soil x variety	2	0.020			
	Temp x variety	4	0.038	28.666 on 256	-	14.68
	Moisture	1	>0.001			

Table 25. Percent variable relative influence from boosted regression tree modelling of the effects of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables dried shoot weight (DSW), dried root weight (DRW), emergence, and tap root and lateral root disease indices (TDI, LDI) of three subterranean clover (*Trifolium subterraneum*) varieties (Riverina, Seaton Park and Woogenellup) inoculated with *Aphanomyces trifolii* in a controlled experiment.

	Variable relative influence (%)						
Model	*Soil*	*Temp*	*Variety*	*Nutrition*	*Moisture*	*RMSE*	*Avg. test RMSE*
Diseased Plants							
DSW	30.67	29.37	20.96	7.76	11.24	0.88	1.00
DRW	31.02	34.62	17.04	5.44	11.88	0.12	0.12
Emergence	17.45	22.71	29.50	13.19	17.15	0.98	1.10
TDI	13.37	48.67	14.16	10.29	13.37	0.02	0.02
LDI	12.47	47.99	16.76	7.38	15.40	0.02	0.02
Differences in mean values for diseased and control plants							
DSW	33.49	10.68	15.38	35.89	4.57	-	-
DRW	25.61	20.06	19.26	25.79	9.28	-	-
Emergence	33.81	9.95	20.95	8.60	26.70	-	-

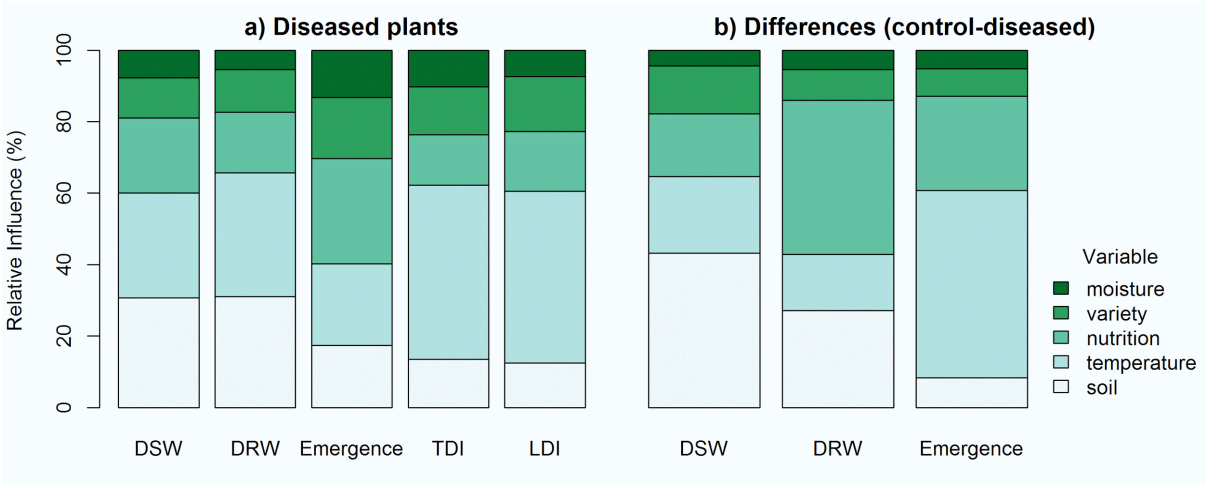


Fig. 52. Percent relative influence from boosted regression tree modelling of the effects of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables dried shoot weight (DSW), dried root weight (DRW), emergence, and tap root and lateral root disease indices (TDI, LDI) of three subterranean clover (*Trifolium subterraneum*) varieties (Riverina, Seaton Park and Woogenellup). a) model results from plants inoculated with *Aphanomyces trifolii*; b) mean values between plants inoculated with *P. irregulare* versus control plants

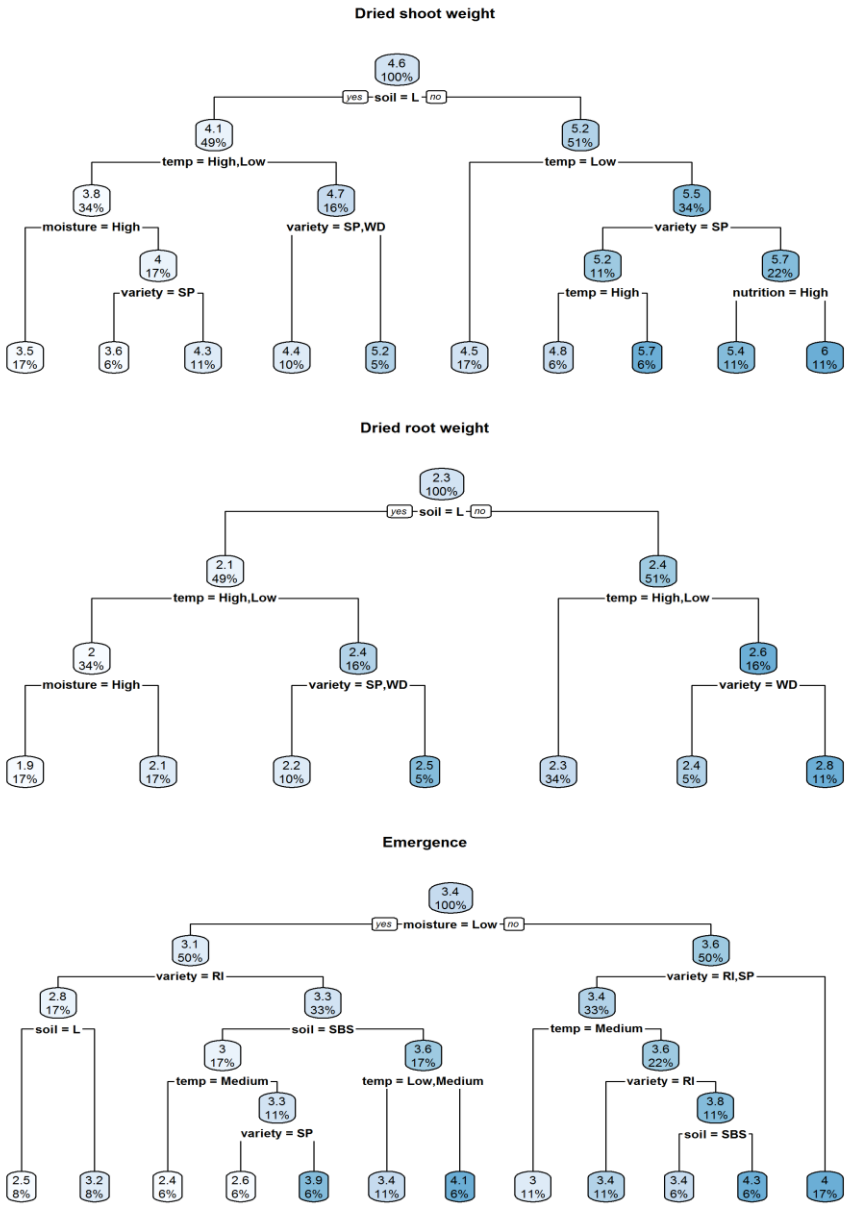


Fig. 53. Regression decision trees illustrating the influence of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables dried shoot weight, dried root weight and emergence of three subterranean clover (*Trifolium subterraneum*) varieties (Riverina, Seaton Park and Woogenellup) inoculated with *Aphanomyces trifolii* in a controlled environment experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). The numbers and the shading in the boxes represent the mean value at each decision point; the percentages indicate the percentage of all values considered at that decision point

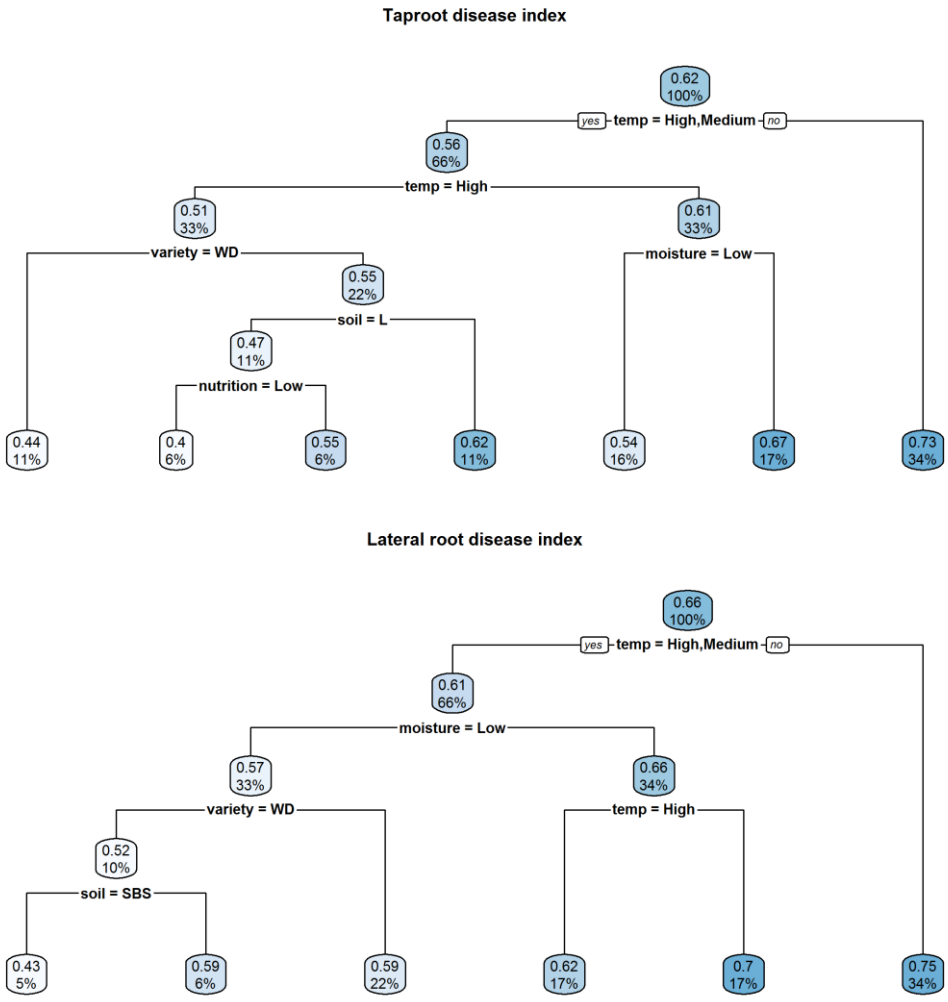


Fig. 54. Regression decision trees illustrating the influence of environmental explanatory variables (moisture, temperature, nutrition, soil type, variety) on the dependent variables tap root and lateral root disease indices (TDI, LDI) of three subterranean clover (*Trifolium subterraneum*) varieties (Riverina, Seaton Park and Woogenellup) inoculated with *Aphanomyces trifolii* in a controlled environment experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). The numbers and the shading in the boxes represent the mean value at each decision point; the percentages indicate the percentage of all values considered at that decision point.

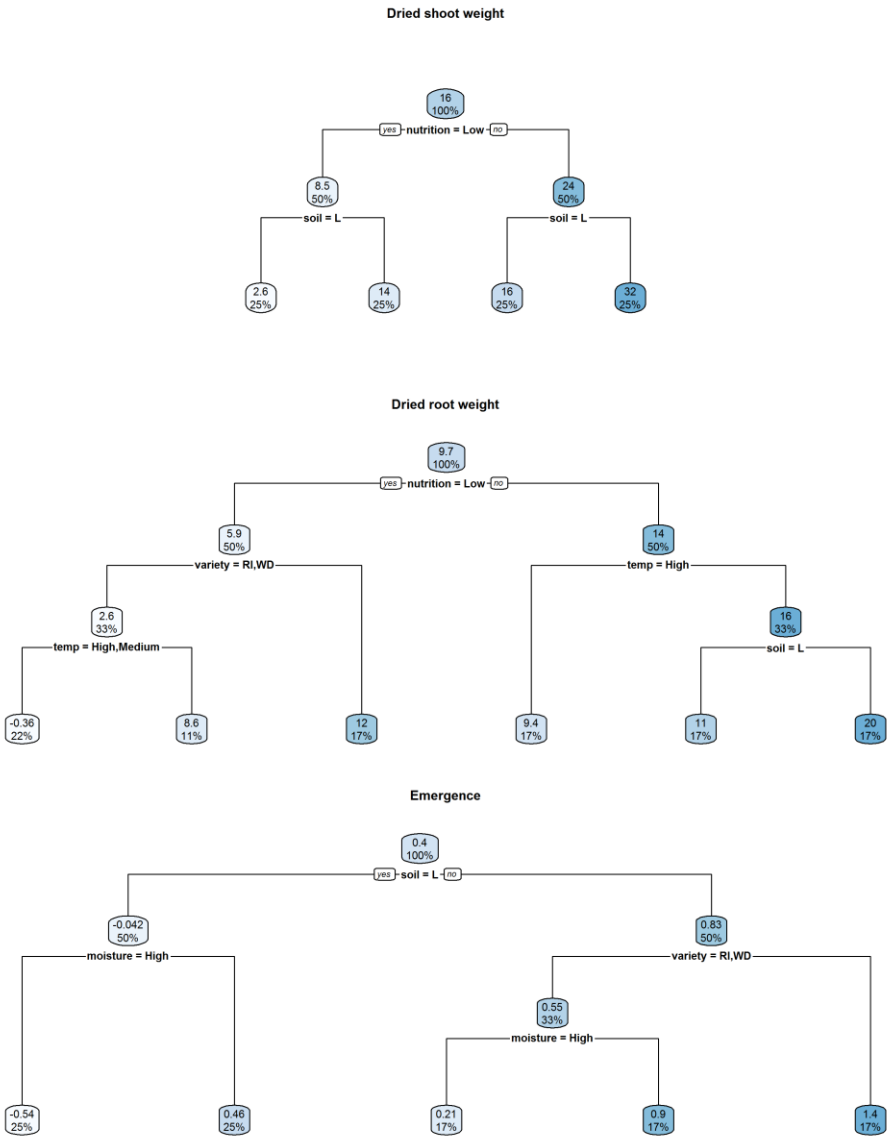


Fig. 55. Regression decision trees illustrating the influence of environmental explanatory variables(moisture, temperature, nutrition, soil type, variety) on the difference between dependent variables mean dried shoot weight, dried root weight and emergence of three subterranean clover (*Trifolium subterraneum*) varieties (Riverina, Seaton Park and Woogenellup) inoculated with *Aphanomyces trifolii* or control plants in a controlled environment experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). The numbers and the shading in the boxes represent the mean value at each decision point; the percentages indicate the percentage of all values considered at that decision point.

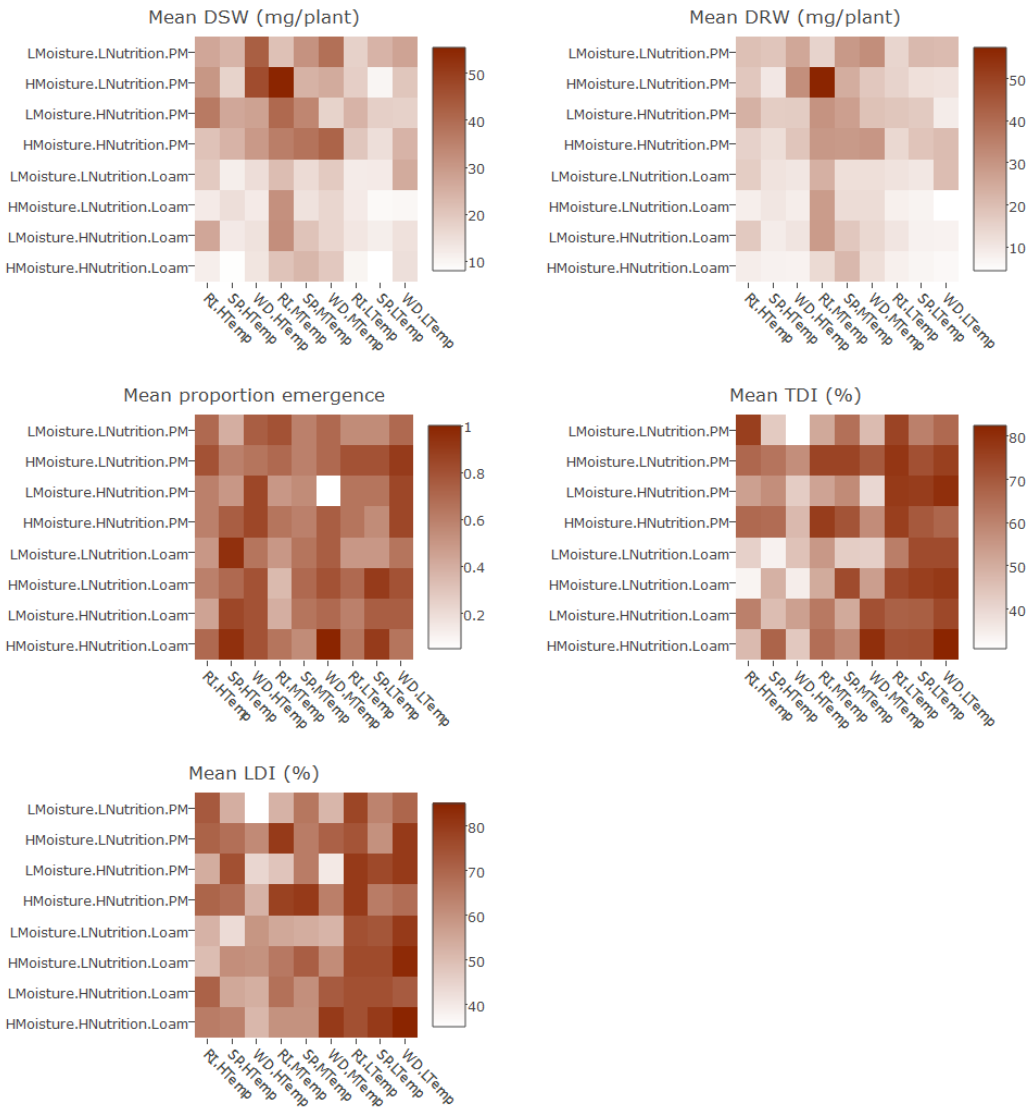


Fig. 56. Heat maps of mean dried shoot weight (DSW), dried root weight (DRW), emergence, and tap root and lateral root disease indices (TDI, LDI) of three subterranean clover (*Trifolium subterraneum*) varieties (Riverina, Seaton Park and Woogenellup) inoculated with *Aphanomyces trifolii* in a controlled environment experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). Darker shading indicates higher values, as per the colour scale bar.

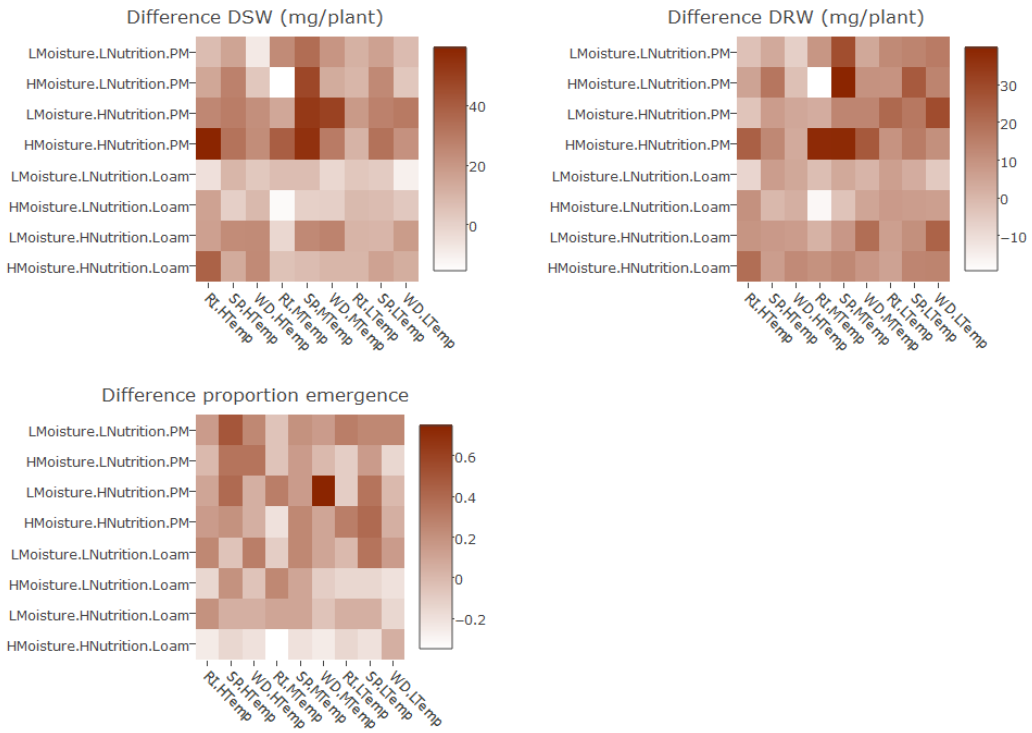


Fig. 57. Heat maps of the differences between mean dried shoot weight (DSW), dried root weight (DRW) and emergence for three subterranean clover (*Trifolium subterraneum*) varieties (Riverina, Seaton Park and Woogenellup) inoculated with *Aphanomyces trifolii* or control plants in a controlled experiment and grown under combinations of high (H) and low (L) moisture and nutrition and in sand-based soil (SBS) or loam soil (Loam). Darker shading indicates higher values, as per the colour scale bar.

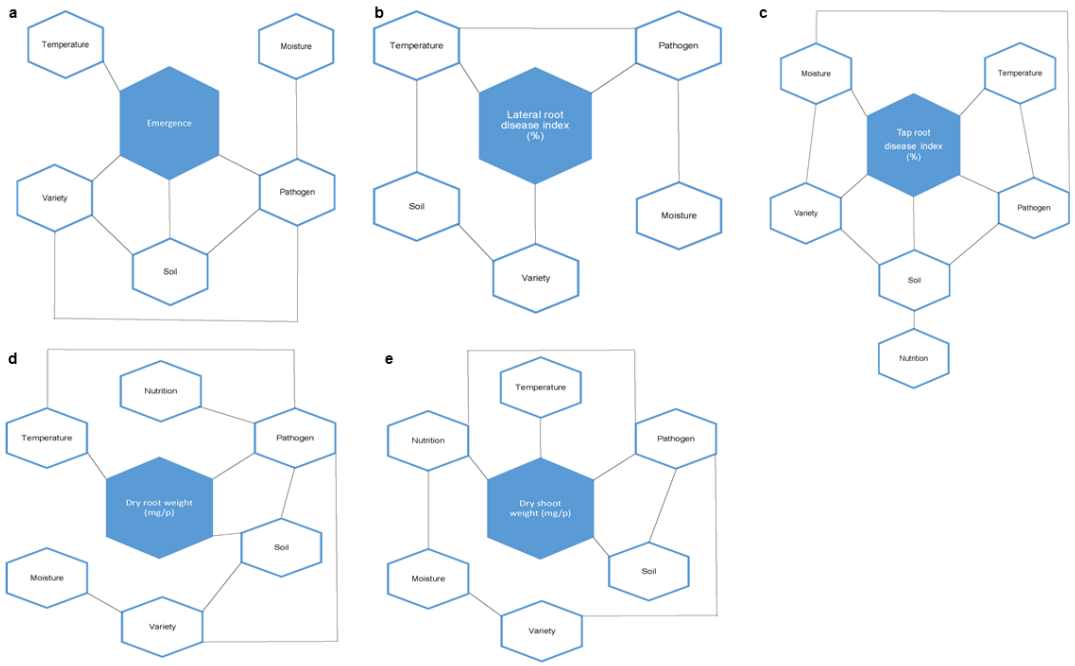


Figure 58 a-e. Effect of environmental explanatory factors (moisture, temperature, nutrition, soil type, variety) and their interactions on dependent variables. **a**) Subterranean clover (*Trifolium subterraneum*) emergence rate (%), **b**) lateral root disease index (%), **c**) tap root disease index (%), **d**) dry root weight (mg/p), and **e**) dry shoot weight (mg/p) in the presence of the soilborne pathogen *Aphanomyces trifolii.*

4.4 DNA testing for soilborne pasture diseases

4.4.1 Development of DNA tests for *Aphanomyces*

Using disease surveys followed by standard soil-baiting methodologies, cultures of six isolates of *Aphanomyces trifolii* were provided to SARDI in 2013. Subsequently, for each isolate, DNA was extracted using the SARDI in-house extraction protocol and the ITS region was sequenced. Sequences were aligned and compared to sequences of *A. trifolii* and closely related species publicly available in the database (*A. euteiches* and *A. cladogamus* are genetically the closest to *A. trifolii*). The sequences of the six WA isolates were almost identical to the *A. trifolii* sequences already published. Based on the sequence data, two qPCR assays (primers and probe) were tentatively designed for the specific detection of *A. trifolii*. Both assays detected *A. trifolii* but one showed a better sensitivity for the detection of *A. trifolii* and did not detect *A. euteiches*. DNA of *A. cladogamus* is still to be obtained to confirm the specificity of the assay. The assay with the best sensitivity and specificity was then selected and a standard will be prepared to allow for quantification of the target DNA. The test will also be validated for the detection of *A. trifolii* in soil samples. This was a significant achievement and benefit not only for livestock producers needing an accurate and reliable assessment of the full status of soilborne disease in their pastures for better management of soil-borne diseases in pastures, but also allowing molecular DNA assays of soil-borne pathogens to be used as effective research monitoring tools.

4.4.2 Critical evaluation of DNA tests

The development, calibration and validation of Predicta B DNA assays were successful. Table 26 shows twenty field soil samples from WA and two sites (4 samples) sampled for the BIGG participatory research trials in South Australia that were used to validate DNA assays. These confirm the outstanding success of a first ever DNA test developed for *A. trifolii*. *A. trifolii* was found in 10 out of 20 sites, and nine of these sites were from WA. *A. trifolii* DNA copies/g of soil ranged from 312 to as extremely high at 95285. In summary, DNA assays for identifying *A. trifolii* have been validated and meaningful application of DNA Predicta B test findings is now possible for the first time for pastures. Importantly, *A. trifolii* clearly constitutes a much more widespread and serious threat to subterranean clover productivity than previously believed.

The development and confirmation of a successful DNA test for *A. trifolii* is a very significant outcome of this project. It will allow, for the first time, meaningful interpretation of DNA test results for the full range of soilborne pathogens in pastures. Previously, in contrast to pasture DNA tests, DNA tests available for crop soilborne pathogens were able to be well interpreted in terms of just what the test results mean in terms of the expected expression of soilborne crop diseases. However, until now this is not the case for soilborne diseases of pastures as there is no comparative data on the interpretation of DNA test results where environmental factors vary widely between different locations and situations across southern Australia. This pathogen clearly is a much more serious threat to subterranean clover productivity than previously thought.

However, critical evaluation studies of DNA versus disease levels in relation to *Pythium* and *Rhizoctonia*, showed that the quantity of either pathogen in the soil [expressed as DNA weight (pg/g soil)] when using subterranean clover composition at 20% and 100% subterranean clover by "Predicta B" significantly differed in relation to differences in pasture composition, pathogen, subterranean clover variety, and also their interactions. For example, tap and lateral root disease indices and DNA weight were lower in 100% than 20% subterranean clover composition for all tested varieties. These relationships were variety-dependent, for example, Seaton Park and Riverina suffered lest *Pythium* root rot and contained least *Pythium* DNA weight at 10% subterranean clover than at 100% subterranean clover composition. However, it was encouraging that both *Pythium* and *Rhizoctonia* DNA weights were positively correlated with tap and lateral root disease. Further, while it was particularly encouraging that "Predicta B" tests gave about 70 to 80% indication for *Pythium* root disease it was only 40% for *Rhizoctonia*. The percent subterranean clover in the pasture also strongly influenced the level of relevance of the Predicta B tests. For example, at 10% subterranean clover, root rot disease indices were only about 10-15% when DNA weight was around 500 pg /g soil for *Pythium* but root rot disease indices were 60-80% when DNA weight was only about 100pg /g soil for *Rhizoctonia*. Therefore, it is clear not only that for the level of root disease from *Rhizoctonia* is determined by other factors than just its Predicta B DNA weight, but that even a relatively low 100pg DNA weight is sufficient for *Rhizoctonia* to cause severe root rot.

Table 26. Predicta B test results for sixteen field soil samples from Western Australia and 4 samples from South Australia for the BIGG participatory research trials in South Australia that were used to validate DNA assays.

	Aphanomyces trifolii
Sample No	*Copies / g sample*
BA52775	0
BA52776	453
BA52777	62889
BA52778	39883
BA52779	312
BA52780	559
BA52781	0
BA52782	6346
BA52783	95285
BA52784	8371
BA55191	0
BA55192	0
BA55193	0
BA55194	0
BA55195	26290
BA55196	0
BA56210	0
BA56211	0
BA56212	348
BA56213	0
BA38185	1755
BA38186	374
BA38187	3766
BA38188	1887

4.5 Identification of host resistance to *Aphanomyces*

Varieties Guildford-D, Campeda, Urana, and Antas, along with Dalkeith, Riverina and York are the first varieties identified with resistance to *A. trifolii*. However, there were strong indications of multiple pathogen races with different host genes controlling resistance to damping-off *vs* tap *vs* lateral root disease from *A. trifolii*. DNA assays for identifying *A. trifolii* have been field validated and meaningful application of DNA Predicta B test findings is now possible for the first time for pastures. *A. trifolii* clearly constitutes a much more widespread and serious threat to subterranean clover productivity than previously thought. In more detail, *A. trifolii* significantly reduced subterranean clover germination rate and root and shoot dry weight. It also caused severe tap and lateral root disease [up to 62.2% and 76.6% for tap and lateral root disease indices (Table 28)]. The most resistant varieties in terms of least reduction of germination rate and least tap and lateral root disease when confronted with mixed isolates of *A. trifolii* were Guildford-D, Campeda, Urana, and Antas. The most susceptible varieties were Bindoon and York. Others such as Uniwager and Northam showed lower tap and lateral diseases but still suffered high reductions in germination rate. Cultivar Coolamon had high reduction of germination rate but relatively low or medium severity of tap and lateral root disease,

respectively (Table 27). In addition to resistance shown in this particular study, high level resistance in Dalkeith, Riverina and York were also identified in related studies. These are the first studies to show the existence of effective resistance among available subterranean clover varieties to this newly emerged pathogen casing serious root disease. The fact that some varieties showed less reduction of germination rate but high level of tap or lateral root diseases such as Tallarook and Izmir suggests that there different genes control pre-emergence damping-off compared with tap or with lateral root disease. Further, there have been strong indications in related studies of the existence of different distinct races of *A. trifolii,* a serious complication for breeding for effective resistance against this pathogen until the race situation is better defined.

Table 27. The effects of *Aphanomyces trifolii* on tap root disease index, lateral root index, dry root and shoot weights and germination rate of forty-six subterranean clover varieties

	Tap root disease index %		*Lateral root disease index %*		*Dry root weight (mg)*		*Dry shoot weight(mg)*		*Germination rate （%）*	
Cultivar	*Inoculated*	*Uninoculated*	*Inoculated*	*Uninoculated*	*Inoculated*	*Uninoculated*	*Inoculated*	*Uninoculated*	*Inoculated*	*Uninoculated*
Antas	21.2	0	47.2	0	29.83	27.89	63.64	104.43	80	73.33
Bacchus Marsh	34.3	0	49	0	31.01	27.65	83.99	94.25	76.67	86.67
Bindoon	62.2	0	67.2	0	25.49	24.72	52.49	92.63	43.33	63.33
Campeda	35	0	59.1	1.1	37.5	31.26	60.89	93.1	63.33	73.33
Clare	29.2	0.7	58.8	0.7	27.27	34.79	72.4	127.64	93.33	83.33
Coolamon	32.2	0	57.8	0	27.51	32.89	47.96	72.08	43.33	66.67
Daliak	46.5	0	67.4	0	23.51	26.31	36.85	69.12	60	70
Dalkeith	44.2	0	64.8	0.7	27.96	32.63	53.76	85.14	86.67	80
Denmark	47.7	0	69.3	3.3	22.68	25.96	54.19	81.48	70	93.33
Dinninup	37.3	0	44	0	22.84	26.02	43.8	72.52	63.33	70
Dwalganup	43.7	0	52.2	0	20.96	24.37	41.79	79.49	86.67	86.67
Enfield	36.7	0	46.7	0	27.2	26.81	59.71	69.02	96.67	96.67
Esperance	37.2	0	56.7	1.1	28.99	30.65	47.87	78.63	80	73.33
Geraldton	51.3	0	62.7	0	17.66	20.96	41.04	76.34	83.33	90
Gosse	26.1	0	46.7	4.7	35.2	41.59	67.71	102.46	83.33	90
Goulburn	47.8	0	64.4	0	22.15	22.33	40.27	58.61	56.67	73.33
Green Range	44	0	62.5	3.3	31.06	31.14	71.01	93.27	80	93.33
Guildford-D	16.7	0	40	0	37	31.92	64.25	78.75	26.67	26.67
Howard	45.2	6	70.8	10.7	28.81	40.22	61.97	118.83	76.67	70
Izmir	55.7	0	68.7	0	19.38	22.61	42.36	81.08	86.67	83.33
Junee	45.9	0.1	68.7	0.6	32.04	31.93	61.05	83.8	80	70
Karridale	35.9	0	61.1	0	26.93	40.59	68.46	98.76	76.67	86.67
Larisa	44.9	0	62.6	0	32.64	30.78	58.65	77.95	83.33	80
Leura	33.3	0	48.3	0	21.69	17.89	39.89	44.03	70	63.33
Losa	45.3	0	50.0	0	19.79	23.45	42.68	80.09	90	96.67
Meteora	41.1	0	73.3	6.7	32.29	41.19	55.13	110.72	50	63.33

Midland-B	35.4	0	54.2	0	19.01	13.93	29.78	48.3	66.67	63.33
Mintaro	38.3	0	62.5	3.3	28.57	30.2	60.59	98.93	83.33	90
Monti	39.4	0	72.8	3.3	30.33	35.64	55.9	81.84	76.67	80
Mt Barke	58.4	0	76.1	0	21.0	31.47	44.36	90.09	76.67	70
Nangeela	54	0	70.3	0	23.01	25.7	54.06	98.53	76.67	63.33
Napier	31.4	0	51.7	0	31.7	30.41	66.29	78.45	66.67	66.67
Narrikup	48.6	0	71.4	0	31.89	25.55	62.51	84.34	56.67	60
Northam	26.1	0	42.3	0	33.85	26.75	85.84	93.4	70	86.67
Nuba	43.8	0	65.7	0	26.06	30.17	58.55	104.08	90	76.67
Nungarin	43.1	0	43.3	0	26.13	32.32	62.47	89.88	20	43.33
Riverina	36.4	0	65.6	2.5	38.35	40.26	61.31	87.37	53.33	86.67
Rosedale	40.3	0	60.3	0	40.26	35.36	87.94	112.62	80	86.67
Seaton Park	44.8	0	49.4	0	33.32	34.05	60.75	83.61	86.67	90
Tallarook	48.7	0	67.3	0	19.8	29.11	46.77	89.31	100	86.67
Trikkala	44.8	0	76.6	4.2	36.09	39.81	54.73	124.19	36.67	46.67
Uniwager	30.7	0	30.5	0	25.39	26.69	39.15	58.77	26.67	40
Urana	21.6	0	34.8	0	26.88	26.65	53.57	71.81	63.33	60
Woogenellup	50.2	0	70.0	3.3	25.88	26.24	49.45	88.4	83.33	73.33
Yarloop	49.7	0	70.6	3.3	27.23	46.96	66.67	132.33	90	83.33
York	56.1	0	74.4	0	29.4	27.74	55.38	84.4	56.67	66.67

Table 28. Statistical significances of main effects and interactions for root rot pathogen *Aphanomyces trifolii* in relation to tap and lateral root disease indexes, dry root weight and dry shoot weight (mg) of 46 screened subterranean clover varieties.

Factors	*Tap Root PDI (%)*		*Lateral Root PDI (%)*		*Dry Root Weight (mg)*		*Dry Shoot Weight (mg)*		*Germination rate (%)*	
	p-value	l.s.d =0.05	p-value	l.s.d =0.05	p-value	l.s.d =0.05	p-value	l.s.d.=0.05	p-value	l.s.d at=0.05
Main effects										
Varieties	0.22	13.76	0.014	14.17	<0.001	8.037	<0.001	17.437	<.001	15.626
Inoculation	<0.001	2.81	<0.001	2.89	0.014	1.641	<0.001	3.559	0.037	3.19
Interactions										
Cultivar*inoculation	0.14	19.47	0.054	20.03	0.651	11.366	0.048	24.66	0.476	22.098

4.6 Australia wide field trials 2015

For full details of the impact of chemical and cultural control measures for soil-borne disease in 2015 national field trials, please see Appendices 1 – 4 for details for South Australia, Victoria, New South Wales and Western Australia, respectively. A summary of main overall findings is as follows:

i. Fungicide seed treatment were able to reduce pre-damping off (i.e., increase germination rate) caused by these targeted pathogens by up to 30%.

ii. Fungicide spray treatments were able to reduce root disease up to 30%.

iii. Addition of complete fertilizer application was able to increase productivity expressed in root dry weight by up to 90% and as expressed as dry shoot weight up to 149%. This was particularly dramatic on poorer and less fertile soils.

iv. Cultivation was able to increase productivity expressed in root dry weight by up to 66% and expressed as shoot dry weight by up to 76% in soils dominated by combination of *Pythium*, *Phytophthora* or *Aphanomyces*, either individually of by a combination of any two of these pathogens.

v. Using more disease-resistant or disease-tolerant varieties was able to significantly increase plant productivity by up to 4 to 5 fold. Identification of cultivar resistances to each of the pathogens offers the best and most cost-effective, long term, and economically feasible means of managing damping-off and root diseases in subterranean clover across southern Australia.

vi. The main pathogens: *Phytophthora clandestina, Pythium* spp., especially *P. irregulare, Rhizoctonia solani* and *Aphanomyces trifolii*; generally complexes of 2-4 different pathogens.

vii. Soilborne pathogens reduce germination by up to 70%; reduce root systems up to 90%; reduce shoot systems up to 85%. Subclover in severely affected pastures does not persist!

viii. *Pythium* reduces germination by up to 60% and up to a 50% loss in plant productivity.

ix. *Phytophthora* reduces germination by up to 25% and a 4.5 fold loss in plant productivity.

x. *Rhizoctonia* reduces germination by up to 90% and up to 75-80% loss in shoot and root production.

xi. *Aphanomyces* reduces germination by up to 14% and up to 50-55% loss in shoot and root systems.

xii. Two or more pathogens result in losses up to 100% germination failure and total loss of root and shoot production.

xiii. 'Environmental factors' including soil moisture, type, temperature and nutrition, determine severity and impact of soilborne root diseases; specific roles of individual factors vary depending upon the prevailing pathogen(s).

4.7 Australia wide field trials 2016

For full details of the impact of chemical and cultural control measures for soil-borne disease in 2016 national field trials, please see Appendices 5 – 8 for details for South Australia, Victoria, New South Wales and Western Australia, respectively. A summary of main findings is as follows:

There were again 8 field trials across southern Australia in 2016 to cover fungicide seed and spray treatments, cultivar, and fertilizer and cultivation options. All 8 sites suffered severe subterranean clover root diseases (> 60% disease severity) caused by the soilborne pathogen complex involving various complexes between *P. clandestina, P. irregulare, A. trifolii* and *R. solani*. At such high disease

pressure, choice of variety and cultivation showed significant reductions in tap and lateral root disease, and significant increases in root nodulation, germination rate and in both root and shoot dry weight. Varieties with root disease tolerance showed significant positive performance on shoot dry weight, such as Riverina, Meteora and Dalkeith. Mixed fungicide for seed treatment and complete fertilizer had significant positive effect on shoot dry weight at some sites. Chemical spray treatment only showed positive effect for Phos-jet at one site (Denmark) which is the site with known high populations of *Phytophthora* and severe root disease at both sampling times at 2016. Overall, these results showed an urgent need for development of varieties with tolerance to overcome subterranean clover root disease and consequent poor performance and decline and disappearance of once productive subterranean clover pastures. While many farmers are now aware of the nature and severity of root disease in their declining/disappearing pastures, many others are still unaware and there is a need for increased awareness and implementation of management options. However, it is clear that the most effective and cost-effective long term option will be the development of subterranean clover varieties with both combined field tolerance and resistance, with up to a 4.5 fold increase in productivity available for producers with severely diseased subterranean clover pastures if this occurs.

NSW/ACT site: Overall, variety had significant effect on root disease level, nodulation level and germination rate as well as root and shoot dry weights. At first sampling time, variety had significant effect on tap and lateral root disease and nodulation level as well as germination rate but no significant effect on root and shoot dry weights as plants were still very small. At second sampling time, varieties again showed significant differences on root and shoot dry weights, as well as tap root disease and nodulation level, but not necessarily the same varieties that were best at the first sampling time. Although, variety Riverina suffered severe root disease it still maintained it yield potential by having high shoot dry weight indicating it has natural field tolerance ability against soilborne disease. In contrast, varieties Naofil elite null and Novostar elite null suffered most severe root disease and least shoot and root dry weights. Cultivation resulted in significant reductions in both tap and lateral root disease indices and increase in nodulation indices. Cultivation resulted in significantly less tap and lateral root disease and higher nodulation indices at first sampling time and less lateral root disease at second sampling time. Fertilizer had significant effect on increasing root and shoot dry weights at first sampling time but had no effect at second sampling time.

Summary for Barossa SA site: Overall, variety had significant effect on the expression of root disease severity (on tap or lateral roots or both) and nodulation index for first and second sampling and a significant effect on root and shoot dry weight at second sampling. Variety Brachy yield highest shoot and root dry weights and Riverina also showed higher shoot and root dry weights than the other test varieties. Cultivation significantly reduced tap or lateral root disease at both first and second sampling times compared with non-cultivated areas.

Summary for Denmark WA sit: Denmark site was severely water logged in 2016. Variety significantly affected lateral root disease severity, nodulation index, and also root and shoot dry weight at both sampling times. Variety also significantly affected germination rate. Variety Riverina showed good shoot and root dry weight despite severe lateral root disease. Cultivation showed significantly reduced lateral root disease and increased root and shoot dry weight at both sampling times. Cultivated treatment had less root disease and higher root and shoot dry weight than non-cultivated areas. There was no significant effect of seed treatment. However, the Phos-jet spray treatment significant increased shoot dry weight.

Summary for South Eastern SA site: Variety had significant effect on tap and lateral root disease levels, nodulation index, and root and shoot dry weights at both sampling times. Variety also had significant effect on germination rate. Varieties Seaton Park and 'Early' showed highest shoot dry weight at the first sampling time but had lost competitive advantage by the second sampling time

where Woogenellup had largest shoot dry weight (this is indicative of an absence or very low Phytophthora population in this site). At the first sampling time, cultivation significantly reduced root and shoot dry weight, and significantly increased dry root and shoot weight. For the seed treatments, the mixed fungicide treatment provided the best protection and resulted in the lowest root disease, the highest germination rate and the highest nodulation index at the first sampling time.

Summary for NSW site: Despite a severe level of both tap and lateral root disease across all tested varieties at this site, variety still showed a significant effect on tap and lateral root disease and on nodulation indices as well as on root and shoot dry weight at both sampling times. At first sampling time, Riverina showed greatest shoot dry weight but had a low germination rate and variety 'Early' showed highest germination rate. At the second sampling time, variety 'Early' showed greatest shoot dry weight with relatively lower root disease levels (tap and lateral). Other treatments did not show any significant effects, indicating that where very severe disease occurs, choice of the most resistant variety has the greatest effect in reducing impact of soilborne disease.

Summary for Bendigo region Victoria site: Variety showed significant effect on tap and lateral root disease and on nodulation indices and also on root and shoot dry weights on both sampling time. More resistant varieties showed lower root disease and higher root and shoot dry weight at both sampling times. While Riverina suffered severe root disease at both sampling times, it had high shoot dry weight especially at second sampling time, its shoot dry weight the greatest among all tested varieties, indicative of a general field tolerance against the soilborne root disease complex. Variety Mounty also showed tolerance to the root disease yield second highest shoot dry weight. Seed treatment only had short term effect evident only at first sampling time. The fungicide mix treatment significantly increased shoot dry weight and reduced tap and lateral root disease and resulted in the lowest disease levels this was not statistically significant. Cultivation significantly reduced lateral root disease and increased root dry weight the first sampling time. Though statistically not significant, there appeared to be differences between cultivated and non-cultivated treatments at second sampling time, again with the cultivated treatment showing lower tap and lateral root disease levels and higher root and shoot dry weights compared with the non-cultivated control comparison. Fungicide spray did not show any significant effects.

Summary for Western Victoria: Variety had a significant effect on tap and lateral root disease and on nodulation indices and also upon root and shoot dry weight at both sampling times. Variety also had significant effect on germination rate. Varieties Dalkeith and Meteora suffered from medium to severe root disease at first and second samplings, but shoot dry weight was greatest for Dalkeith at both sampling times and for Meteora at the second sampling time. Cultivation significantly reduced both tap and lateral disease and increased nodulation indices and also root and shoot dry weights at second sampling time; and while there were no significant effects of cultivation at first sampling time there was a clear similar trend as occurred at the second sampling time.

***Summary for Wagin WA site*:** Variety had a significant effect on tap and lateral root disease and upon nodulation indices and also on root and shoot dry weight at both sampling times. Variety also significantly affected germination rate. Varieties Mt. Barker, Meteora and Riverina all suffered similar levels of root disease and had similar root and shoot dry weights. Seed treatment significantly increased nodulation index and root dry weight at first sampling time and also increased shoot dry weight at both sampling times. The fungicide seed treatment mix was the second best treatment in terms of shoot dry weight at the first sampling time but was the best treatment at second sampling time. For shoot productivity (expressed as shoot dry weight), the mixed fungicide seed treatment and the application of the complete fertilizer both showed good potential. Cultivation significantly reduced tap root disease and increased nodulation indices and also increased germination rate as well as increasing root and shoot dry weights. The cultivated treatment had less tap root disease and

higher germination rate, higher nodulation index and greater root and shoot dry weight at first sampling time. Again, while there were no similar significant differences at second sampling time, the same trend was evident as at the first sampling time.

4.8Modelling field environmental and other data against soilborne disease severity across southern Australia

Results for Tap root rot % incidence (TI%)

For TI%, the best model indicates that the presence of sand and clay; increased March and May rainfall; increased January, March and June temperatures; and greater longitudes were all correlated with a reduced TI%. On the other hand, increased April rainfall and increased February and July temperatures were correlated with increased TI%. The full analysis also provided some evidence that more waterlogging may decrease TI% and the presence of gravel may increase TI% (Figs 59, 60).

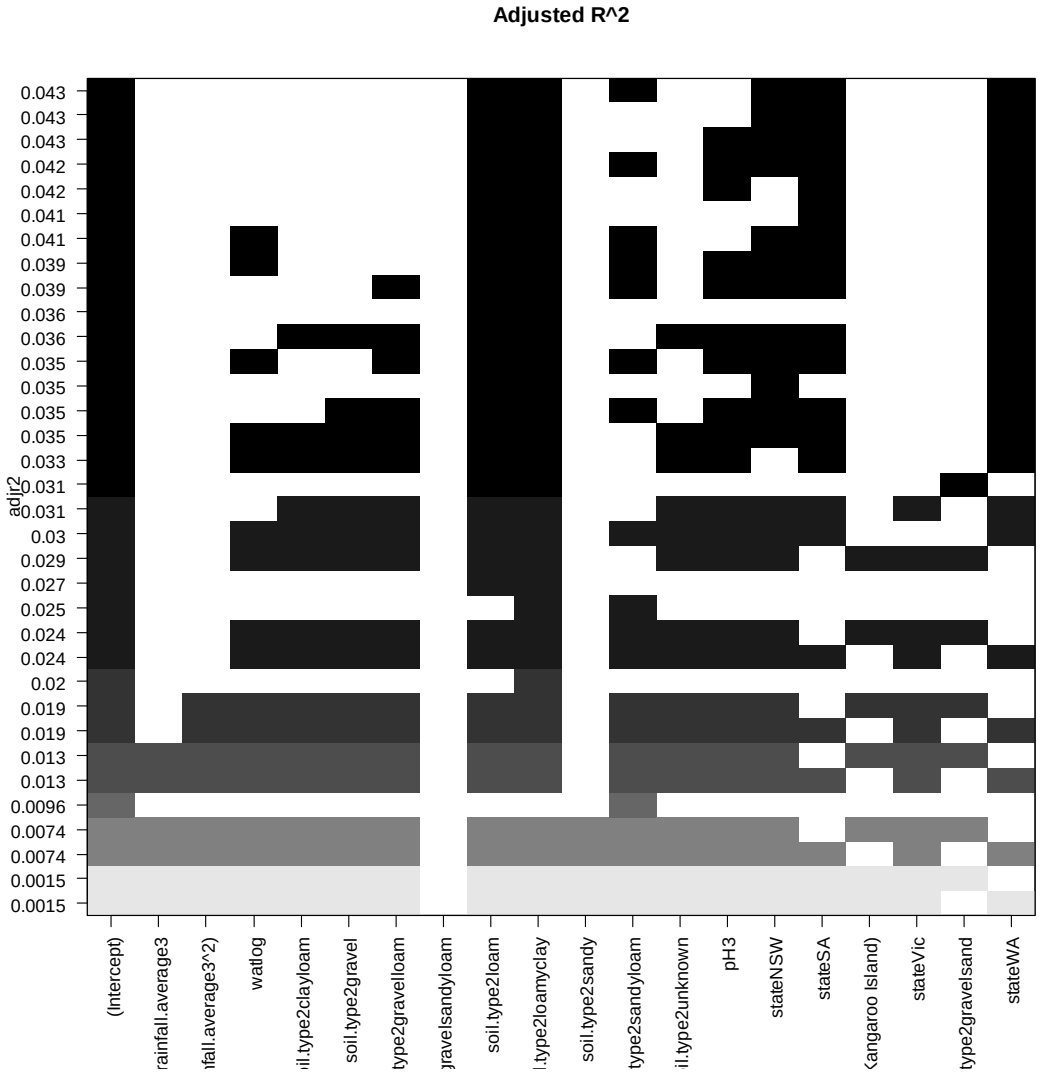


Fig. 59. Results of full subsets multiple regression for TI%, including original soil classifications as potential categorical explanatory variables. The plot shows which explanatory variables were included in each of the best two models for every number of possible explanatory variables, based on adjusted R-squared value. All these models are ranked (and shaded) based on adjusted R-squared value.

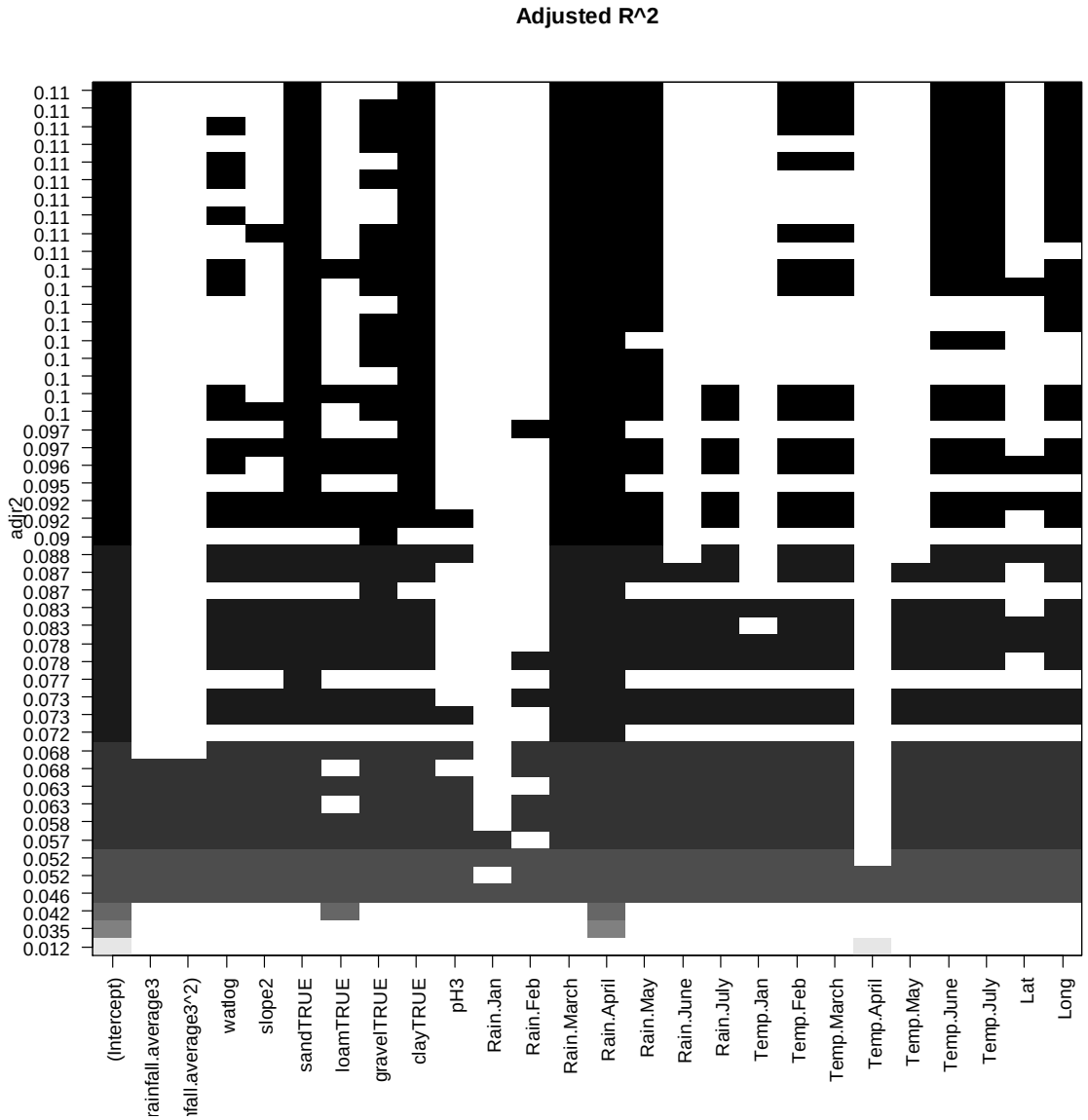


Fig. 60. Results of full subsets multiple regression for TI%, including recoded binary soil classifications as potential categorical explanatory variables. The plot shows which explanatory variables were included in each of the best two models for every number of possible explanatory variables, based on adjusted R-squared value. All these models are ranked (and shaded) based on adjusted R-squared value.

Results for Lateral root disease % incidence (LI%)

For LI%, the best model indicates that the presence of sand, loam and gravel; increased March and June rainfall; increased January temperatures; and greater longitudes were all correlated with a reduced LI%. On the other hand, increased February and April rainfall were correlated with increased LI%. The full analysis also provided some evidence that higher July rainfall; lower February and higher March temperatures and the absence of clay may also be related to higher LI% (Figs 61, 62).

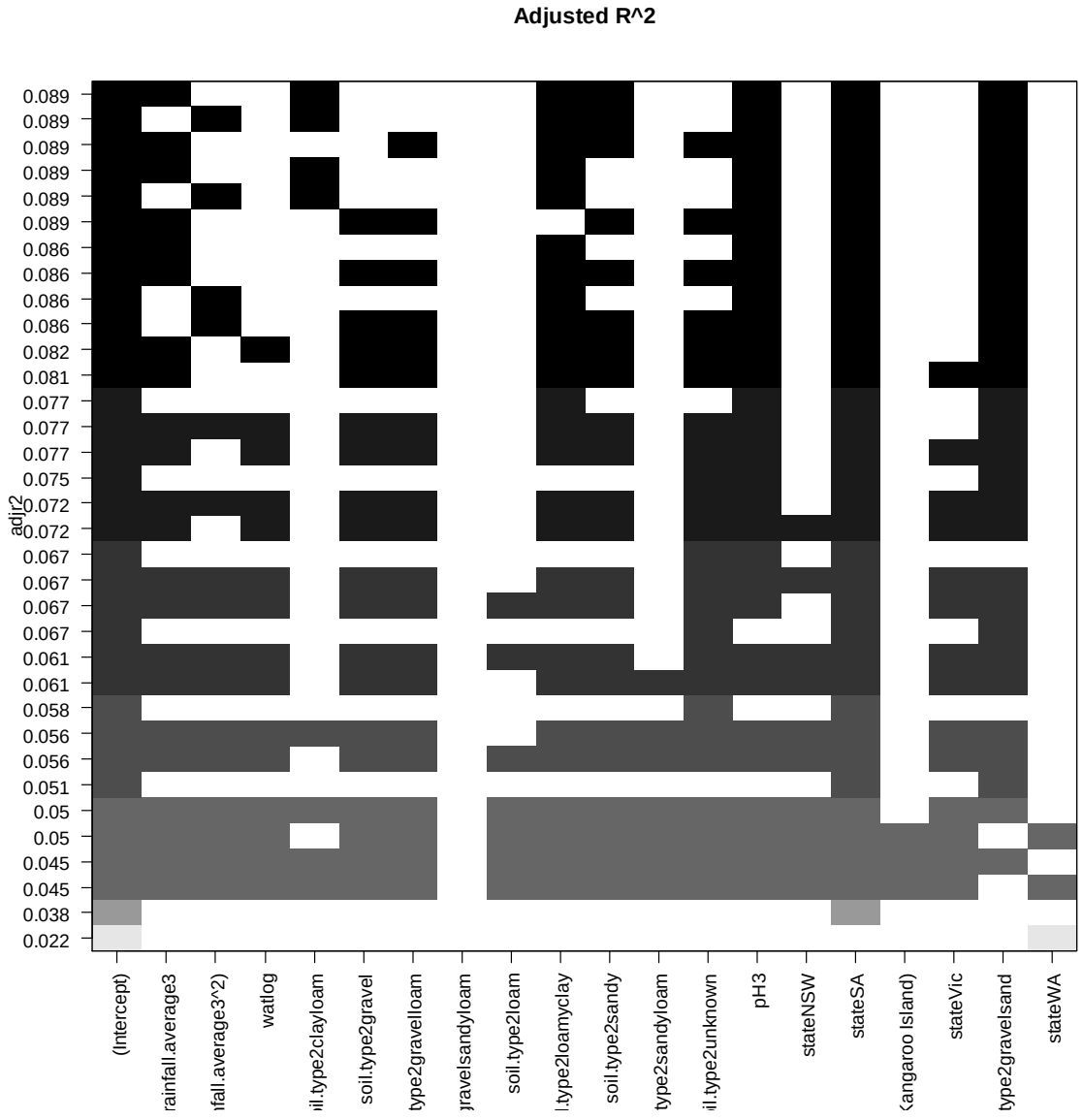


Fig. 61. Results of full subsets multiple regression for LI%, including original soil classifications as potential categorical explanatory variables. The plot shows which explanatory variables were included in each of the best two models for every number of possible explanatory variables, based on adjusted R-squared value. All these models are ranked (and shaded) based on adjusted R-squared value.

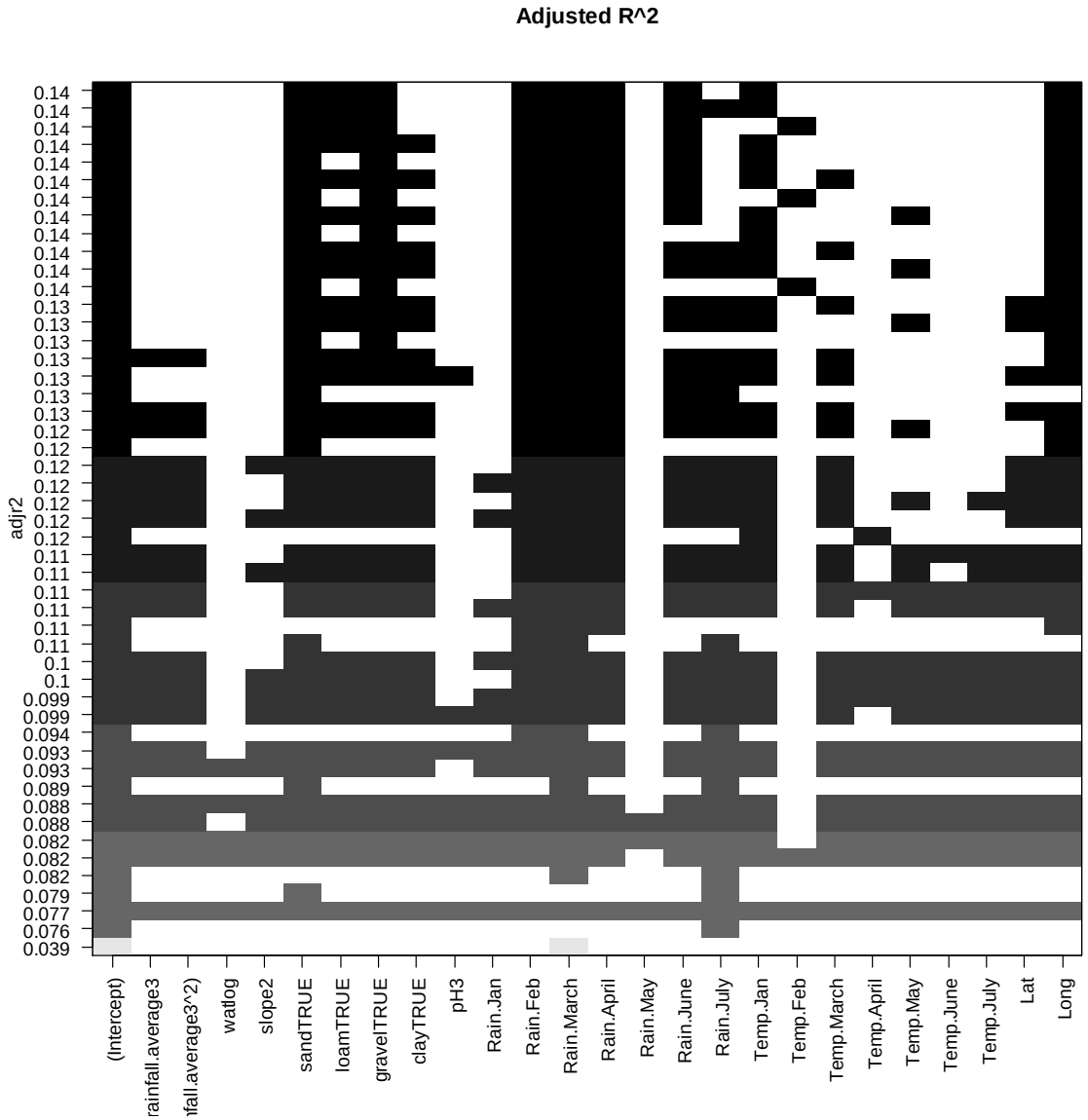


Fig. 62. Results of full subsets multiple regression for LI%, including recoded binary soil classifications as potential categorical explanatory variables. The plot shows which explanatory variables were included in each of the best two models for every number of possible explanatory variables, based on adjusted R-squared value. All these models are ranked (and shaded) based on adjusted R-squared value.

Results for nodulation (N)

For Nodulation %, the best model indicates that higher waterlogging; increased Feb, April and June rainfall; and increased June temperatures were all correlated with a reduced Nodulation %. On the other hand, increased slope; increased January and July rainfall; increased April temperatures; and greater longitudes were correlated with increased Nodulation %. The full analysis also provided some evidence that lower average annual rainfall; smaller latitude; and the presence of loam and gravel may also be related to higher Nodulation % (Figs 63, 64).

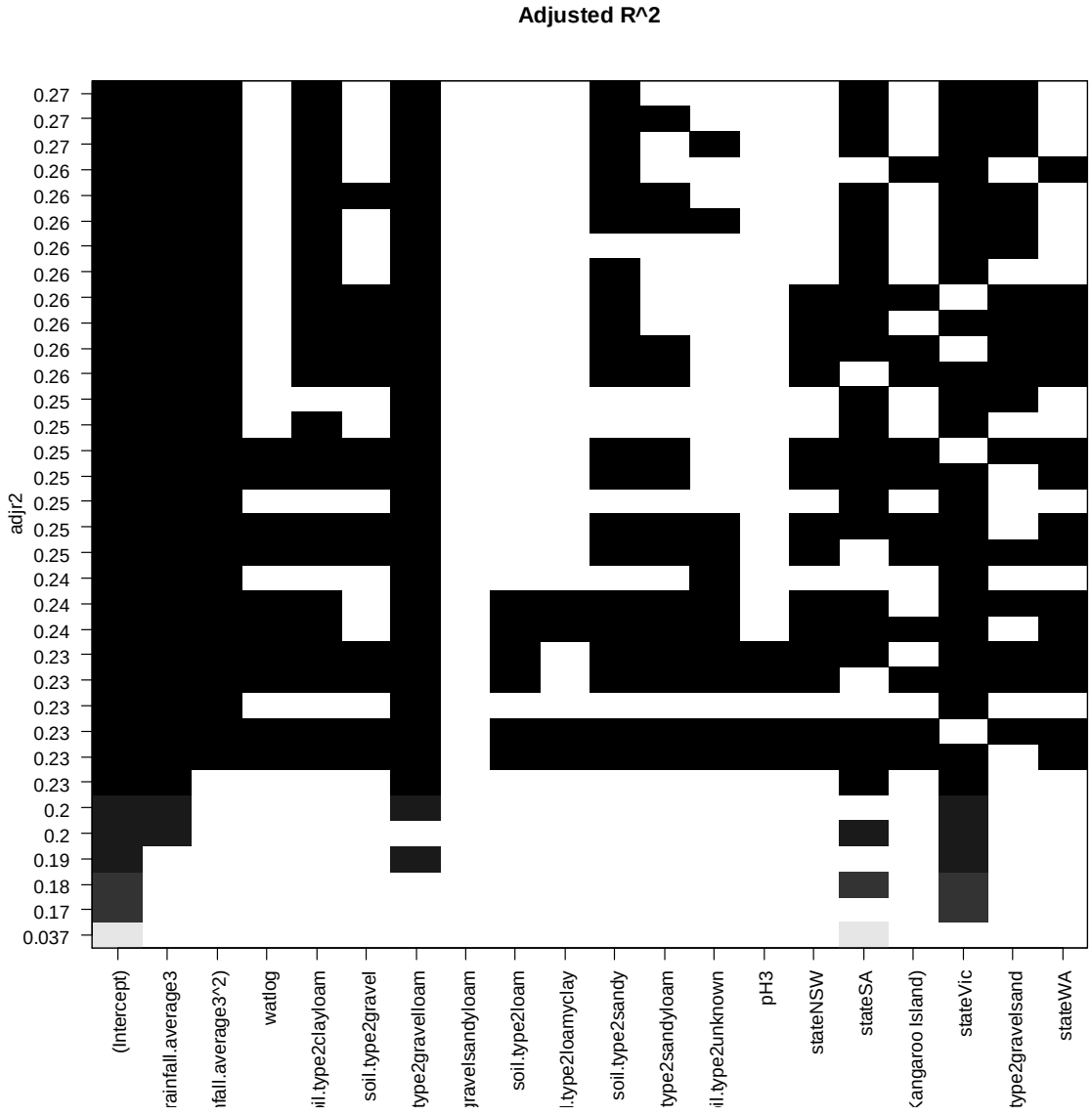


Fig. 63. Results of full subsets multiple regression for Nodulation %, including original soil classifications as potential categorical explanatory variables. The plot shows which explanatory variables were included in each of the best two models for every number of possible explanatory variables, based on adjusted R-squared value. All these models are ranked (and shaded) based on adjusted R-squared value.

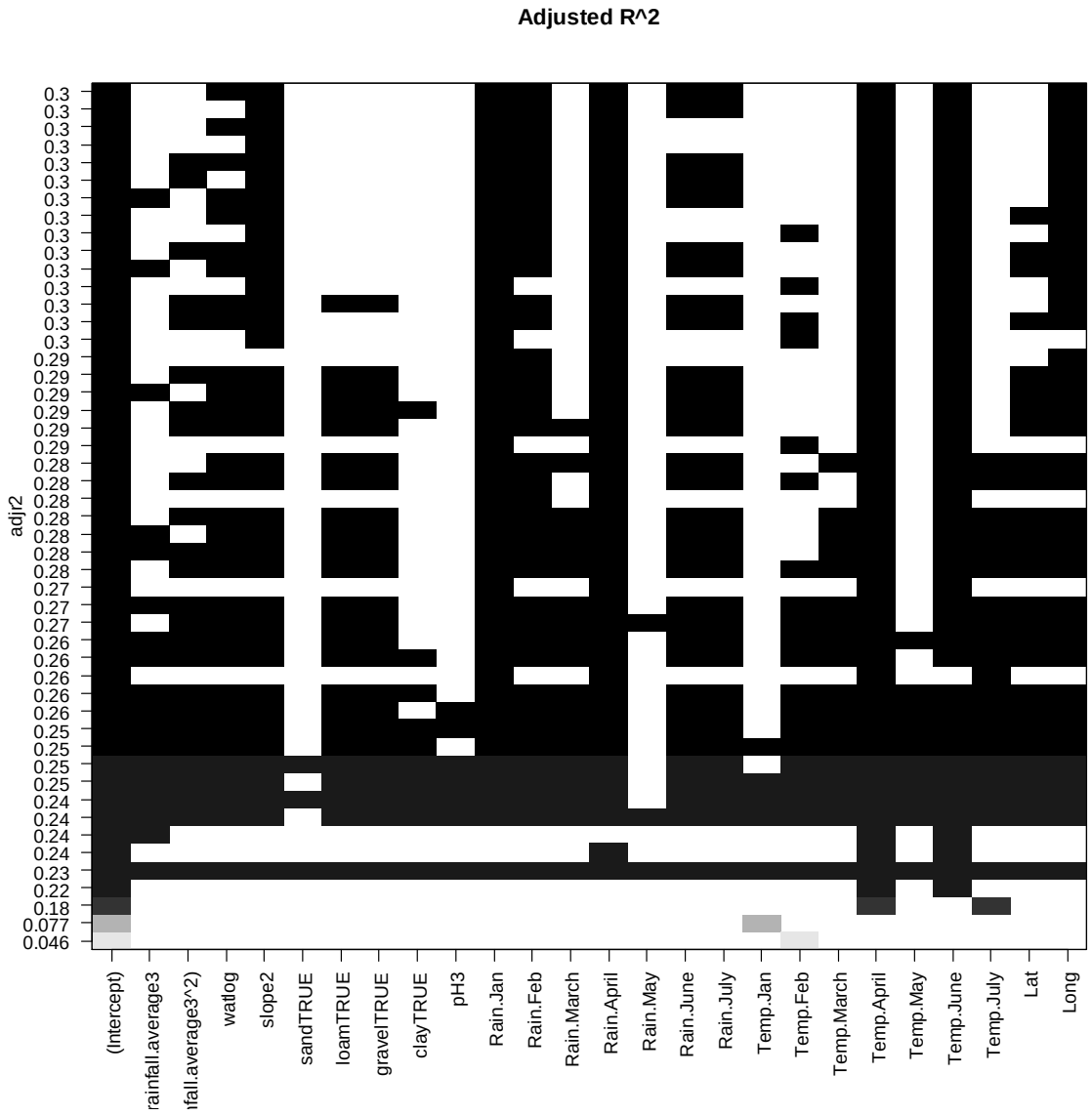


Fig. 64. Results of full subsets multiple regression for Nodulation %, including recoded binary soil classifications as potential categorical explanatory variables. The plot shows which explanatory variables were included in each of the best two models for every number of possible explanatory variables, based on adjusted R-squared value. All these models are ranked (and shaded) based on adjusted R-squared value.

4.9 Securing pathology skills and expertise for pastures into the future

Pathology skills and expertise for pastures currently, and if there is ongoing industry support into the future, has been secured by the appointment of a post-doctoral researcher (Dr Ming Pei You) to this project from its commencement. This appointment and training has enhanced and consolidated the technical and knowledge capacity on pasture plant pathology - soil biology available for current and potentially future research and extension in southern Australian pasture systems. In addition to this MLA project, Dr You also closely collaborated and work closely with two Producer Site Research Projects in South Australia and one in Western Australia, attended MLA and many other producers field days and project meetings. The outcome is a highly skilled and knowledgeable pastures plant

pathologist to meet both current and, if ongoing funding support is available, also future pathology requirements of the livestock industry.

5 Discussion

5.1 Fungicide seed and spray treatments

In MLA project glasshouse trials, tests of seed dressing fungicides against *Aphanomyces trifolii* showed Dividend, Rovral, Rancona Dimension, Syn-A1684F and Metalaxyl all reduced pre-emergence damping-off. Syn-A16874F, Fluquinconazole and Rancona Dimension reduced lateral root disease across two or more varieties. Rovral, Thiram and Syn-A16874F increased shoot dry weight. For foliar chemical spray treatments, Phos-Inject 200 was best at preventing productivity losses from *A. trifolii*.

In MLA project field trials, fungicide seed treatments increased germination by up to 30%, while fungicide sprays reduced root disease by up to 30% at some sites. However, there have been some spectacular visual responses where some producers have applied a fungicide spray across paddocks as test strips. Best for producers to try test strips before embarking on paddock-scale operation and some producers are already pleased with the outcome from this approach.

5.2 Cultural control treatments

5.2.1 Cultivation – field cores in glasshouse and additional field trials

Using field cores from five different farms in MLA project studies, overall, tap and lateral root disease were less severe and dry root and shoot weights greater, following simulated cultivation compared with control comparison cores left undisturbed. With exception of Woogenellup from three sites, simulated cultivation of soil suppressed tap root disease across all tested varieties on soils from all sites. Varieties Meteora and Riverina showed significantly lower tap root disease in cultivated soil across field sites, while Seaton Park showed the same for some sites. Varieties Riverina and Seaton Park have some resistance to *P. irregulare* (You et al. 2005b) and all three varieties have some resistance *to F. avenaceum* (You et al. 2005b). However, Seaton Park has high level resistance across all 10 defined races of *P. clandestina* in Western Australia (You et al. 2005a,c), whereas both Riverina and Meteora are also highly resistant to many but not all races of *P. clandestina* (You et al. 2005a,c). Hence it is likely that the difference between varieties relates to their relative resistance(s) to *P. irregulare* that was prevalent across all field sites from which field cores were taken.

The two in-field trials also confirmed a significant role of cultivation in reducing root disease severity, increasing germination rate in two of the three varieties and increasing plant productivity (expressed in dry weights of root and shoot). Overall, the best treatment was cultivation plus fumigation that reduced tap and lateral root disease and increased nodulation and root and shoot dry weights across the three tested varieties. Cultivation + fumigation also increased germination rate for Woogenellup and Seaton Park. That there were strong negative relationships between tap root disease and both root and shoot dry weights and between lateral root disease and both root and shoot dry weights confirms that the increases in plant productivity are directly due to reductions in root disease, supporting earlier findings in controlled environment studies (e.g.,). Such a conclusion is further supported by the strong positive correlation between shoot and root dry weights in the field trials.

This study confirms that devastating levels of severe tap and lateral root disease can be greatly mitigated by cultivation and that cultivation offers significant flexibility to producers to better manage situations where autumn-winter feed shortages aggravated by severe root disease. Root

rots are typically most severe on well-established pastures, and least severe or absent on recently cultivated soils in some dryland pastures (Kellock 1975*;* MacNish et al. 1976*).* Smiley et al. (1986) found that simulated cultivation of soil in cores also reduced root rots in the dryland pasture soil that had little surface litter, but not in the irrigated pasture soil which had high levels of organic debris (and pathogen inocula) distributed through the surface 0-11 cm). Similarly, Barbetti and MacNish (1984) found that both tap and lateral root rot levels were reduced by cultivation on its own or in combination with other cultural practice options. While they showed in field experiments in 1975 and 1976 in south-western Western Australia that the best treatments for reducing root disease in subterranean clover were those of fallowing an area from August to March before cultivation and reseeding, or spring cultivation before sowing to oats followed by a March cultivation and reseeding; cultivation treatments *per se* were also effective in that study, particularly if applied in conjunction with the arrival of the opening seasonal rains. While Barbetti (1984a) showed that a reduction in the severity of tap root rot was much more important than a reduction in the severity of lateral root rot, we found in the current field study disease that reductions for both were similar, not unexpected as tap and lateral root disease levels were closely correlated in the current study. There are several possible reasons for the reductions in root disease and/or increase in plant productivity from cultivation in the field or simulated; for example, due to the plants' ability to withstand disease; depression of pathogen populations; increased incidence or activity of competing saprophytic organisms; increased aerobic metabolism; decreased root exudation; more rapid root growth; and stimulation following N mineralisation and improved water infiltration (Rittenhouse and Hale 1971). Together, these include a combination of possible factors that can not only discourage active growth of soilborne pathogens, for example, that of the oomycete pathogen *P. irregulare* in the rhizosphere (Stovold 1974a,b), but also improve root growth as continuous heavy stocking of permanent pastures without cultivation may be conducive to compaction and poor root development, poor seed burial, seed set and intolerance to dry periods.

In the previous field trials of Barbetti and MacNish (1984) the reduction resulting from treatments involving cultivation were of relative short duration, failing to persist beyond the second year after treatment and, for some treatments, even failing to persist beyond the first year. At the time, this lack of persistence was put down to the widespread presence of high levels of *P. irregulare* (Barbetti and MacNish 1978), perhaps the most common *Pythium* species present in soils (Hendrix and Campbell 1970) and one that can infect a very wide range of hosts making its elimination from soils by crop rotation unfeasible (Stovold 1974). Barbetti and MacNish (1978) also highlighted the challenge that clover density was much lower in plots cultivated after the break of season than in other treatments involving cultivation and clearly more studies are warranted on timing of cultivation to maintain the prevailing subterranean clover seed-base while maximizing reductions in damping-off and root disease and increases in plant productivity. Such complexities in those earlier studies meant that no practical cultivation practice was recommended to farmers as a means of reducing root rot severity at the time of the Barbetti and MacNish (1978) studies. However, the results from the current MLA project studies and extensive field survey observations of vastly improved pasture productivity and corresponding reduced damping-off and root disease levels and increases in nodulation following cultivation by farmers of subterranean clover pastures suggest further studies are warranted.

In the field trials, there were strong negative correlations between both tap and lateral root disease with nodulation. This study highlights the close relationship between increased root disease and reduced nodulation in subterranean clover. Microbial antagonism and/or interactions within the rhizosphere, including those involving soilborne fungal and oomycete root pathogens, are a known factor associated with the failure of inoculation of subterranean clover (e.g., Hely et al. 1957). The current study provides an important explanation, at least in part, for the widespread poor nodulation observed for subterranean clover pastures over many decades (e.g., Gibson, 1968),

clearly an ongoing issue as evidenced in a recent survey of subterranean clover pastures across southern Australia highlighting the widespread low nodulation rates among seedlings (Foster et al. 2016).

The MLA project studies confirm that severe tap and lateral root disease in subterranean clover can be significantly moderated by cultivation. It is noteworthy that the level of reduction of root disease from cultivation was significantly greater at the Wagerup site where it was a low productivity pasture that had been uncultivated and unrenovated for more than 15 years compared with the Coolup site that was a highly productive pasture that had been regularly renovated and resown predominantly to Italian rye grasses every few years. Clearly, cultivation will be of greatest benefit in situations where little if any cultivation has occurred for some years. While an effect of in-field cultivation had previously been demonstrated by Barbetti and MacNish (1984) for a single field site in Western Australia, the current MLA studies confirm that greatly reduced root disease can be achieved by application of cultivation across wider locations. Further, in the glasshouse and in-field MLA studies, there was no evidence of any 'adverse secondary effects' from cultivation as had been observed in the previous study of Barbetti and MacNish (1984) where production losses occurred from increased damage from root knot nematodes following cultivation at that particular experimental site. This opens the way for wider recommendation of cultivation for control of root disease and increased productivity of subterranean clover, particularly in situations where farmers face an inherent 'feed-gap' shortage in autumn-winter period. As such, cultivation can provide producers additional flexibility in choice of control options they can utilise, and while taking into account livestock prices, potential productivity of pasture(s), and degree of feed shortage as associated with their individual situation. Ideally, the long-term sustainable management in the situation across southern Australia where pathogen complex composition is highly variable should come from identification of general tolerance in subterranean clovers to a wide cross-section of the pathogen complex to provide the basis of developing new varieties with general tolerance as a foundation for building durable resistance. However, in the interim, strategic application of cultivation offers significant flexibility to producers to better manage situations where feed is particularly short due to severe damping-off and root disease, and at a seasonal time when any additional feed is very high priced. Further, the close relationship in MLA project studies between increased root disease and reduced nodulation in subterranean clover is indicative that reduction of root disease by cultivation likely offers significant redress for farmers from the widespread poor nodulation observed for diseased subterranean clover pastures over many decades.

5.2.2 Cultural control treatments – others

Effective pasture nutrition could increase shoot productivity by 1.5 times in the MLA project studies and this confirms earlier studies by O'Rourke et al. (2012) that there is significant potential to mitigate the adverse impact of soilborne diseases by ensuring fertilizer applications are maintained and plant requirements fully met in situations where soilborne disease is prevalent. In addition, farmers need to carry out regular soil tests an address nutritional deficiencies through strategic fertiliser applications, to optimise growth. Applying fertiliser as test strips in a paddock will indicate a potential deficiency and highlight the benefits of additional fertilizer application before committing to such on a larger scale.

While grazing, particularly 'crash grazing' or continuous grazing, leads to more severe tap and lateral root disease, poorer nodulation and smaller plants in terms of both roots and shoots, reducing grazing pressure offers potential to significantly increase sub-clover pasture productivity during the autumn feed-gap period from decreased root disease and increased nodulation associated with less intensive grazing of root rot-affected sub-clover pastures as shown in these MLA project studies. These responses largely relate to the ability/inability of plants in terms of their capacity/incapacities, respectively, to grown new roots to replace those damaged or lost from soilborne root disease.

However, as the most severe root disease coincides with the autumn-winter feed gap, farmers may have little practical opportunity to manipulate grazing pressure across this period.

Swards where clover content is high also demonstrated better disease tolerance than grass-dominated pastures in these MLA project studies. Although this is contrary to common thinking, it does explain, for the first time, the accelerating rate of increasing soilborne disease levels and rates of overall subterranean clover decline occurring in pastures.

5.3 Environmental interactions with soilborne diseases

5.3.1 Environmental interactions – *Pythium*

These MLA project studies are the first to use a comprehensive modelling approach to define the importance of environmental conditions occurring across southern Australian subterranean clover forage pastures upon the severity of *Pythium* damping-off and root disease and forage productivity. All explanatory variables (temperature, soil, moisture, nutrition, variety) significantly affected severity of pre-emergence damping-off (i.e., emergence), root disease and root and shoot productivity and all environmental factors were found significant as part of some interaction within these models. Relationships between environmental factors and the presence of *Pythium* damping-off and root disease were complex, with linear modelling identifying high-level (4 or 5-way) significant interactions for each dependent variable (dry shoot and root weight, emergence, tap and lateral root disease index). For example, a significant five-way interaction between all factors was found on both dry shoot and root weights, and a four way interaction between temperature, soil, moisture, and nutrition was found on both tap and lateral root disease indices. A second approach to modelling using boosted regression trees provided support for the complex nature of the relationships found in linear models, with all explanatory variables showing at least 5% relative effect on each of the five dependent variables (temperature, soil, moisture, nutrition, variety). For all models a clear trend was the difference in soil type, with the sand-based soil having either higher weights, greater emergence, or lower disease indices; while lowest weights and less emergence, as well as higher disease indices were for loam soil and low temperature.

Specifically in relation to emergence, linear modelling highlighted significant interactions between temperature, soil and variety, and between temperature, moisture, nutrition and variety. Relative influence of these explanatory variables from BRT modelling showed a 50% influence of soil type on emergence, followed by variety, temperature, nutrition, and moisture. When Wong et al. (1984) examined the effects of temperature and moisture on pathogenicity of *P. irregulare*, they similarly found that specific combinations of multiple explanatory variables resulted in the greatest pre-emergence damping-off. For example, they found that temperature/moisture combinations of 10°C + 45%WHC or 15°C + flooding caused greatest pre-emergence damping-off. Similarly, for another oomycete pathogen, *P. clandestina*, You and Barbetti (2017a) showed significant interactions involving temperature, moisture, variety and soil type in terms of emergence, with cultivar resistance, high moisture, high or medium temperature, high nutrition and sand soil all contributing towards less pre-emergence damping-off and tap and lateral root disease and to greater clover productivity. That decision trees showed highest emergence for Woogenellup and Seaton Park grown in sand-based soil, but lowest emergence in loam, in high nutrition and high moisture is not surprising as Seaton Park is highly susceptible and Woogenellup moderately susceptible to damping-off by *P. irregulare* (Nichols et al. 2014). However, while Riverina is overall considered moderately resistant to root disease *per se* (Nichols et al. 2014), it showed poorest emergence, in line with field observations that in soilborne disease-conducive situations this latter variety is extremely susceptible to pre-emergence damping-off with consequent very poor emergence (MP You and MJ Barbetti, unpubl.).

Heat maps and modelling differences between mean emergence of diseased plants and control plants demonstrated and confirmed the strong influence from soil type with less pre-emergence damping-off in sand-based soil vs loam soil, similar as demonstrated with *P. clandestina* (You and Barbetti 2017a). While *P. irregulare* is a serious pathogen across diverse soil types throughout southern Australia, from coarse sand to heavier loam or even clay based soils, it is a particularly devastating pre-emergence pathogen across the widely prevalent, impoverished and nutrient-deficient soils across south west of Western Australia (Barbetti et al. 2006b), soils that predispose plants to soilborne pathogens, particularly as microbial competition is minimal in such soils (Sivasithamparam, 1993). There, seedling losses in the field from damping-off can exceed 90% where *P. irregulare* dominates (Wong et al., 1985a) and the current study confirms that the importance of *P. irregulare* as a cause of extensive pre-emergence damping-off in subterranean clover across southern Australia (Barbetti and MacNish 1978; Greenhalgh and Lucas 1984; Wong et al. 1984, 1985a; Wong et al. 1986a,b). Any impedance of root extension, as occurs in heavier soils, can increase the extent of pre-emergence damping-off (MJ Barbetti and MP You, unpubl.). Establishing adequate seedling density is critical for early-season subterranean clover production as it closely correlates with seedling density (Donald 1951), and pre-emergence damping-off also reduces persistence of subterranean forages, the latter leading increased weedy content of forages (Barbetti et al. 2006b; Jones and Barbetti 2011). The widespread decline and failure of subterranean clover forages to persist has led to severe reductions in both the capacity to carry livestock and the overall whole-farm profitability across southern Australia (Barbetti et al. 1986; Nichols et al. 2014).

In relation to tap and lateral root disease, there were significant interactions between temperature, soil, moisture, and nutrition, and for tap root disease between soil, moisture, nutrition and variety and lateral root disease between soil and variety. That boosted regression trees found similar relative influence for both tap root and lateral root disease index of soil, temperature and variety was expected, as tap and lateral root disease severities are strongly and positively correlated across different situations, environments and even varieties in other studies (e.g., Barbetti and MacNish 1984). There have been other studies with subterranean clover to relate environmental factors to severity of damping-off and/or root disease, but these involved a single or only a very small number of explanatory variables. For example, simple analysis of climatic data along the Western Australian south coast across 1972-1975 showed that 1973, a particularly severe root disease year, had significantly heavier and more frequent rainfall in autumn compared with other years when there was a much lower severity of root disease (MacNish et al. 1976). In the current study, there were higher levels of tap and lateral root rot disease in high moisture treatments, to be expected as wet soil conditions strongly favour attack by oomycete pathogens such as *Pythium* or *Phytophthora* spp. on germinating seedlings and/or root systems of surviving plants (You and Barbetti, 2017a). Even brief periods of soil saturation from flooding promote infection of roots by *Pythium* spp. because they thrive and produce massive numbers of motile zoospores under such conditions (Yanar et al. 1997). Wong et al. (1984), however, examined the effects of temperature and moisture on pathogenicity of *P. irregulare*, showing that it was specific temperature/moisture combinations that resulted in the most severe root disease (e.g., flooding across 10, 15, 20, and 25°C). That there was an effect of nutrition level on root disease was not unexpected, as nutrient stress enhances the susceptibility of plants to disease (Graham 1983). O'Rourke et al. (2012) found that application of a complete nutrient solution to field soils decreased the severity of tap and lateral root disease in subterranean clover by approximately 45% and 32%, respectively; and that even amendment with either K or N alone reduced severity of tap root disease by >30%. In addition, application of a complete fertilizer (200 kg ha^{-1} of superphosphate containing Cu, Zn and Mo, plus 50 kg ha^{-1} of potash) in field trials across southern Australia in 2015 and 2016, increased productivity of subterranean clover in stands severely affected by soilborne root disease by up to 1.5 fold (MP You and MJ Barbetti, unpubl.). Taken together, improved nutrition likely offer considerable potential for alleviating the impact of soilborne root pathogens such as *P. irregulare*.

Subterranean clover variety was also an important factor. In decision trees, least disease was for Seaton Park and Woogenellup in sand-based soil under higher and medium temperatures and at higher moisture for tap roots. A similar pattern was found for least lateral root disease for Seaton Park and Woogenellup grown in sand-based soil under high moisture and medium temperature. The most severe tap root disease was in loam soil under low moisture and high nutrition, but for lateral root disease it was most severe under low moisture for Riverina. Heat maps showed greatest tap and lateral root disease for loam soil, particularly for tap than compared with lateral root disease. Variety of subterranean clover significantly affected severity of both tap and lateral root disease and also pre-emergence damping-off. In relation to strong interactions in the current study between variety with soil type, temperature and moisture, it is noteworthy that Seaton Park and Woogenellup had least tap and lateral root disease. This was a somewhat surprising outcome for Woogenellup as it is known to be very susceptible to Pythium root rot (Wong et al. 1984, 1985a, 1986a; You et al. 2005a), but it is known to be productive despite presence of *P. irregulare* providing conditions for rapid growth are present such as warmer spring temperatures. However, improved varietal host resistance remains the focus if root disease severity is to be reduced and productivity of subterranean clover forages is to be increased, particularly when environmental conditions are conducive for development of severe disease (Barbetti et al., 2007; Nichols et al. 2014; You et al. 2017a).

That *P. irregulare* played a significant role in shoot weight reduction (i.e., productivity) in this study was anticipated because historical studies involving *P. irregulare* demonstrated a strong negative correlation between root disease severity and productivity of subterranean clover as expressed by shoot dry weight (e.g., Barbetti 1984a,b, 1986b; Barbetti and MacNish 1978; Wong et al. 1984). Modelling found a complex relationship between dried shoot weights of plants infested with *P. irregulare* and explanatory variables, linear modelling highlighted significant interaction between all explanatory variables in terms of both dry shoot and dry root weight, and BRTs provided further support for the influence of all explanatory variables in differences in root weights, the latter showing that soil type most influenced dry weights, followed by temperature, nutrition, variety and moisture. The most productive plants, with the greatest dry shoot weight, were planted in sand-based soil, under high or medium temperatures, and plants with the greatest dry root weight were similarly grown in sand-based, but with high moisture and high nutrition. Similarly, heat maps showed a clear increase in dry shoot and root productivity for plants grown in sand-based, in medium or high temperatures; but least productivity was for loam soils, particularly under low temperatures. The findings in relation to greater productivity under warmer temperatures are in line expectations, as in the presence of *P. irregulare* Wong et al. (1984) showed largest subterranean clover shoots were produced at the warmer temperature conditions (e.g., 25°C) compared with cooler temperatures (e.g., 10°C). O'Rourke et al. (2012) highlighted the potential to incorporate nutrient amendments into an integrated and more sustainable approach to not only better manage root disease, as discussed above, but also to increase size of subterranean clover root systems and consequent shoot growth, particularly in the commonly occurring situations where soils are inherently nutrient deficient in one or more nutrients. Improving nutrition offers significant potential for mitigation of the adverse disease impact caused by *P. irregulare* on subterranean clover productivity.

Across southern Australia, producers face critical feed shortage across autumn–winter that coincides with severe attack by soilborne pathogens like *P. irregulare*, markedly decreasing the autumn–winter biomass production in regenerating stands. Hence, the need to determine and understand the role of environmental factors in *Pythium* damping-off and root disease. It is a clear that fluctuating temperature and moisture conditions, common under the southern Australian annual subterranean clover forages and as demonstrated in the current study, determine the severity and impact of soilborne disease epidemics. However, faced with warming temperatures across the

southern Australian forage and cropping zones (Barbetti et al. 2012; Jones and Barbetti 2012), not only will the relative importance of the different environmental factors likely change in association with these future climate scenarios, but the relative importance of pathogens, including soilborne pathogens such as *P. irregulare*, will also likely alter (Chakraborty et al. 1998), possibly becoming less severe under future predicted warmer growing season temperatures (Barbetti et al. 2012; Jones and Barbetti 2012) .

It is evident from the current MLA project studies that areas of sand-based soil will have greater emergence, less disease and greatest persistence and productivity and these may be productive irrespective of variety grown. In contrast, the most diseased, least persistent and least productive subterranean clover forages will likely be for heavier soils (e.g., loam soil) and when temperatures are low. There were higher levels of tap and lateral root rot disease in higher moisture situations. Where conditions are conducive for severe disease, affected forages would require application of a combination of relatively expensive cultural management techniques [e.g., cultivation (Barbetti and MacNish 1984; You et al. 2017b)] in conjunction with reseeding with more disease resistant varieties where they are available (Barbetti et al. 2006a,b, 2007). Improved host resistance offers the best long-term and cost-effective way to curtail losses where severe *Pythium* damping-off and root disease occurs, and would provide certainty of production even under seasonal variations in moisture, temperature, nutrition that favour severe *Pythium* damping-off and root disease. However, unfortunately, effective host resistance against *P. irregulare* is rare within Australian subterranean clover varieties, with only Karridale showing strong resistance and Dinninup, Enfield, Mt Barker and Urana showing only moderate resistance (Nichols et al. 2014). Despite this, the existence of high level resistance in some breeding lines (You et al. 2005a), the existence of some varieties with resistance to two or more of the main pathogens in the soilborne pathogen complex (You et al., 2005b), and the recent discovery of the first effective field tolerance against the entire soilborne disease complex (MP You and MJ Barbetti, unpubl.), together offer ample opportunity for *Pythium* damping-off and root disease to be cost-effectively managed in future for the first time providing there is funding support for such an approach.

5.3.2 Environmental interactions – *Phytophthora*

These MLA project studies are the first ones undertaken to comprehensively define the importance of environmental conditions occurring across southern Australian subterranean clover forage pastures on the severity of *Phytophthora* damping-off and root rot and on plant productivity. Environmental factors significantly affected severity of pre-emergence damping-off, root disease and root and shoot productivity; with high moisture, high or medium temperature, high nutrition, sandy rather than loamy soil and subterranean clover varieties with better resistance to *P. clandestina* all contributing towards less damping-off and root disease, and to greater subterranean clover productivity. There were significant interactions between temperature, moisture soil type and nutrition, particularly in relation to pre-emergence damping-off and tap and lateral root disease.

In the presence of *P. clandestina* the level of tap root disease was significantly affected by temperature and cultivar while the level of lateral root disease was significantly affected by both moisture and temperature.

In the presence of *P. clandestina* the level of both tap and lateral root disease was significantly affected by nutrition. In the presence of *P. clandestina,* seedling emergence (i.e., level of pre-emergence damping-off) was significantly affected by moisture, soil type, temperature and cultivar.

It has long been observed that the above ground symptoms of root rots in subterranean clover vary in different localities (Barbetti et al. 2006b, 2007), and while the above ground symptoms can be a consequence of different complexities of the different combinations of soilborne pathogens involved

(Barbetti et al. 2006b, 2007), it has been believed that this could be due to environmental differences (Barbetti and MacNish 1983), cultivar (Barbetti 1989; Barbetti et al. 1986) and nutritional influences (Barbetti et al. 2006b). For example, typical symptoms can include stunted yellow-green, or yellow-red, or red-purple plants (Burgess et al. 1973; Barbetti and MacNish 1983; Clarke 1983) either scattered among apparently healthy plants, or the affected areas may occur in distinct patches (Barbetti and MacNish 1983; Clarke 1983). However, it still remains to be proven that such symptom variations in the field are due to influence of environmental factors on soilborne pathogens rather than variations in the composition of different pathogens.

In the presence of *P. clandestina* the level of both tap and lateral root disease in the MLA project studies was significantly affected by cultivar, as occurred also in relation to pre-emergence damping-off discussed above. Subterranean clover cultivar host resistance was critical for reducing root disease severity and increasing productivity even when favorable environmental conditions for severe disease occurred. For example, in the presence of *P. clandestina,* Seaton Park performed best under a high temperature, high nutrition and high moisture combination, but showed poor productivity under conditions of low nutrition or lower temperature. A major driving factor in these interactions were differences between the subterranean clover varieties. Seaton Park is the only variety of the four chosen for this study that has resistance to race 173 (You et al. 2005a,b). In contrast to Seaton Park, less resistant Riverina and Meteora had less disease and greater productivity under low moisture conditions less favorable for *P. clandestina*. This is to be expected as You et al. (2005b) demonstrated that Meteora were moderately resistant to race 173 as used in the current study. This highlights the importance and advantages of breeding disease resistant varieties that can not only overcome disease problems *per se* but also unfavorable environmental conditions. Woogenellup is known to be highly susceptible to *P. clandestina* (Barbetti 1989; You et al. 2005a,b). However, a major challenge in managing this pathogen that remains is the pathogenic specialization of *P. clandestina*, specialization that changes in response to the cultivar or spectrum of varieties used in a given geographic region (You et al. 2006; Nichols et al. 2014).

Across southern Australia, producers usually face critical feed shortage across autumn–winter that coincides with severe attack by soilborne pathogens like *P. clandestina*, markedly decreasing the autumn–winter biomass production in regenerating stands. While the extent of disease and even the disease symptoms remain highly variable across southern Australia, these MLA project studies are the first to highlight the complexity of interactions driving such variability. In particular, while there is a strong relationship between early-season subterranean clover production and seedling density (Donald 1951), that soilborne pathogens like *P. clandestina* cause such fluctuating but often severe pre-emergence damping-off and/or decreases in seed yield resulting in subsequent failure of regenerating clover forages has remained unknown until now. Currently, severely affected forages require application of a combination of expensive cultural management techniques (e.g., cultivation) in conjunction with reseeding with more disease resistant varieties where they are available (Barbetti et al. 2006a,b, 2007). Improved host resistance offers the best long-term and cost-effective way to curtail losses where severe *Phytophthora* root rot and pre-emergence damping-off in subterranean clover occurs, particularly as the disease severity is clearly driven by moisture, temperature, nutrition soil type and even cultivar as shown in the current study. The current study demonstrates how variations in environmental factors such as soil type, nutrition, moisture and temperature, and also cultivar, individually and interactively, can have profound effects on the expression and severity of *Phytophthora* pre-emergence damping-off and root disease and the consequent level of productivity of subterranean clover forage. Locating and developing further improved host tolerance/resistance offers the best long-term and most cost-effective way to curtail losses where severe *Phytophthora* damping-off and root disease occurs in subterranean clover providing there is funding support for such an approach.

5.3.3 Environmental interactions – *Rhizoctonia*

These MLA project studies are the first to define the importance of environmental conditions occurring across southern Australian subterranean clover forage pastures on the severity of damping-off and root disease and consequent root and shoot productivity in the presence of *R. solani.* At the lower two temperature regimes (14/9°C and 18/13°C, day/night), seedling emergence was very low and survival extremely poor, an accurate representation of the complete seedling damping-off of subterranean clover observed in the field when there is a high incidence of this pathogen and temperatures approximate these test ranges (MP You and MJ Barbetti, unpubl.). Only at the warmest of the three temperature regimes tested (22/17°C) was there significant germination and seedling survival of subterranean clover and germination; dry root and shoot weights all increased conversely with decreased tap and lateral root disease under higher moisture, better nutrition and under 'heavier' soil conditions. Findings reflected field observations that damping-off and root disease from *R. solani* in subterranean clover are particularly severe when seasonal temperatures are low and demonstrate how variations in environmental factors like temperature in particular, but also soil type, nutrition, moisture and cultivar, individually and interacting, have profound effects on the expression and severity of *Rhizoctonia* damping-off and root disease and the consequent productivity of subterranean clover forages. In particular, the minimal germination at the lower two temperature regimes explains, for the first time, the severe devastation to subterranean clover pastures observed at emergence in the presence of *R. solani* when relatively cool seasonal conditions occur across the critical autumn feed-gap period; especially under relatively drier soil conditions for nutritionally impoverished sandy soils where there is little competition from other soil microbes, as is the case across large areas across southern Australia (Sivasithamparam 1993).

At the lower two temperature regimes of 14/9°C and 18/13°C, day/night, seedling emergence was very low and survival extremely low, while at (22/17°C) damping-off was significantly less. This somewhat contrasts with the findings of Wong et al. (1984) who found that damping-off of subterranean clover from *R. solani* was most severe at 20°C compared with lower temperatures (10, 15°C) or higher temperatures (25°C) in those studies. However, in the same study, Wong et al. (1984) found that damping-off and/or root rot caused by fungal combinations containing R. *solani* were generally most severe at 20 and 25°C. Similarly, Leach (1947) showed that damping-off by *R*. *solani* in spinach and silver beet was more severe at 20-25°C than at 4-12°C, while Sumner et al. (1976) showed that *R*. *solani* caused severe damping-off and root rot of spinach only at soil temperatures of 16°C and above. Yet, on wheat, Smiley and Uddin (1993) found, similar to the current study, that R, solani was more virulent and damaging to roots, plant growth and development at lower soil or ambient temperatures of 6-19°C than at warmer temperatures of 16-27°C.

Soil nutrients are known to affect diseases by their effects on the pathogen, on the nutrition of the plant host or on the pathogen and/or microbial antagonist(s) resident in the soil (Sivasithamparam 1996). However, in the current MLA project study it was not possible to apportion the effects of additional nutrients to any particular one of these 'factors'.

The current MLA project studies also demonstrate how small variations in environmental factors such as temperature, moisture, nutrition and soil type, individually and interactively, can have profound effects on the expression and severity of *Rhizoctonia* damping-off and root disease and the consequent level of productivity of subterranean clover forage. Across southern Australia, livestock producers usually face critical feed shortage across autumn–winter that coincides with severe attack by soilborne pathogens like *R. solani*, markedly decreasing the autumn–winter biomass production in regenerating pasture legume stands. There is a strong relationship between early-season

subterranean clover production and seedling density (Donald 1951), and as demonstrated in the current study, soilborne pathogens like *R. solani* can not only cause severe damping-off, root disease but likely also cause consequent decreases in seed yield. The latter in turn results in subsequent failure of regenerating subterranean clover forage pastures. Currently, severely affected subterranean clover forage pastures require application of a combination of expensive cultural management techniques (e.g., cultivation), but this can be particularly effective if undertaken in conjunction with reseeding with a more disease resistant cultivar such as Goulburn, Leura or York that all have moderate resistance to *R. solani* (Barbetti et al. 2006a,b, 2007; Nichols et al. 2014). Locating and developing further improved host resistance offers the best long-term and most cost-effective way to curtail losses where severe *Rhizoctonia* damping-off and root disease occurs in subterranean clover providing there is funding support for such an approach.

5.3.4 Environmental interactions – *Aphanomyces*

These MLA project studies are the first studies to utilise modelling approaches to reveal the complex nature of how fluctuating soil temperature, moisture and nutrition conditions, and soil type and variety, determine *Aphanomyces* damping-off and root disease severity in subterranean clover and the resultant adverse impacts on productivity of subterranean clover forages and their long term persistence. The outcomes are widely applicable across soilborne oomycete pathogens of forage legumes to the studies MLA project studies described above on other soilborne pathogens. However, these *A. trifolii* studies contrast with previous studies with other pathogens like *P. clandestina* and *R. solani* where only analyses of variance were utilized in an attempt to decipher these complex relationships (You and Barbetti 2017a,b). It is noteworthy in the current study that there were multiple significant interactions for each dependent variable (emergence, tap and lateral root disease, dry shoot and root weight) and all environmental explanatory variables were significant as part of some interaction or as main effects within these models in relation to dependent variables temperature, soil, moisture, nutrition and variety. Temperature and either soil or variety were the most influential, with high and medium temperatures having either higher shoot and/or root weight, or lower disease severity. Sand-based soil had either greater plant weight or lower disease severity, while smallest weights as well as higher disease severity were for loam soil. Modelling clearly revealed how fluctuating soil temperature, moisture, and soil type and variety are significant determinants of disease severity and consequent persistence and productivity of subterranean clover forages.

In these MLA project studies, plants showed lowest disease severity under high or medium temperatures, with low moisture, in sand-based soil and for variety Woogenellup. That heat maps confirmed least disease severity under warmer temperature is significant, as other studies showed less root disease from *A. trifolii* when temperature conditions were approximately 4°C warmer (You et al. 2016) or at warmer (25/20ºC, day/night) than at cooler (18/13ºC) temperatures (O'Rourke et al. 2010). Significantly, 18/13ºC represents a temperature range consistent with field temperatures in the high-rainfall areas of Western Australia during the early part of the growing season (Barbetti 1991) when *A. trifolii* is most damaging. It is possible that at warmer temperatures more rapid root growth assists plants to both 'escape' and/or compensate for already-diseased roots, and this is in line with Wong, Barbetti & Sivasithamparam (1984) who showed that most severe root rot in subterranean clover caused by *P. irregulare, R. solani, F. avenaceum, F. oxysporum* and *Phoma medicaginis* occurred at 10ºC, with less at 15, 20 and 25ºC. With the warming temperatures across the southern Australian forage and cropping zones (Barbetti et al. 2012; Jones and Barbetti 2012), it is likely the relative importance of the different environmental factors will also alter in association with these future climate scenarios (Chakraborty et al. 1998). Such climate change effects are already evident for other pathogens of subterranean clover, for example, the area affected by northern anthracnose (*Kabatiella caulivora*) has shrunk by more than 50% across south west Western Australia over the past 3-4 decades from increased temperature and reduced seasonal

rainfall (Jones and Barbetti 2012). In the same way, the relative importance and adverse impact of pathogens such as *A. trifolii* on subterranean clover will likely decrease in line with increasingly warmer temperatures and reduced seasonal rainfall from climate change, as both these climate parameters are continuing to become less favourable for *A. trifolii* as this pathogen prefers cool temperatures and high moisture situations. Historical analysis of climatic data for the south coast of Western Australia showed that a year with particularly severe root disease was a year with more frequent and heavier rainfall in comparison to years when root disease severity was much lower (MacNish et al. 1976). That interactions between environmental factors together determined root disease severity was expected, as for other oomycete pathogens of subterranean clover in Australia, such as *P. irregulare* and *P. clandestina* (Wong et al. 1984, 1986), it is specific temperature/moisture combinations that result in most severe disease, for example, flooding and 10°C. Clearly it is a similar situation for *A. trifolii*. In the current study, it is interesting that nutrition had the lowest relative influence on all dependent variables, especially as O'Rourke et al. (2012) had found a strong relationship between disease severity and nutrition in field soils infested with *A. trifolii*, *P. clandestina* and *P. irregulare*. Hence, the beneficial effect of supplying adequate nutrition may be specific only to other pathogens in the soilborne pathogen complex attacking subterranean clover, for example, as shown for *P. clandestina* by You and Barbetti (2017a).

There is a well-established strong relationship between early-season subterranean clover production and seedling density (Donald 1951), hence the adverse impact of pre-emergence damping-off from *A. trifolii*. In the current study, the low emergence for some factors, such as low emergence for variety Riverina grown in loam soil, leads to both lower persistence and productivity of subterranean forages. In higher rainfall areas *A. trifolii* is most damaging where soils more commonly experience saturation and/or periodic flooding events (O'Rourke et al. 2012, Ma et al. 2008). *A. trifolii* was observed on subterranean clover roots in more than a third of field survey samples across southern Australia in 2014 (You et al. 2016) but a commercial Predicta-B DNA test on the same samples showed a ~50% incidence of *A. trifolii* in the same field samples (You et al. 2016) and with the highest high level of *A. trifolii* DNA at a high rainfall site where subterranean clover forages had to be re-sown every 3-4 years due to collapse and decline from severe root disease (You et al. 2016). For another oomycete pathogen, *P. clandestina*, You and Barbetti (2017a) also showed significant interactions across similar multiple explanatory variables in terms of pre-emergence damping-off. In addition, for *P. irregulare,* Wong et al.1984) identified specific combinations of multiple explanatory variables maximizing pre-emergence damping-off, with temperature/moisture combinations of 10°C + 45% water holding capacity or 15°C + flooding causing greatest pre-emergence damping-off. Not only was soil the most influential variable in BRT models and decision trees, heat maps also demonstrated and confirmed the strong influence from soil type, with less pre-emergence damping-off in sand-based soil vs loam soil, similar as demonstrated with another oomycete pathogen, *P. clandestina* (You et al. 2017a). *A. trifolii, P. clandestina* and *P. irregulare* remain serious pathogens throughout a wide variety of different soil types across southern Australia, from coarse sand to heavier loam or even clay based soils, and have long been known to be particularly devastating in terms of pre-emergence damping-off throughout legume forages across south west of Western Australia. The current study confirms that *A trifolii* is likely responsible for extensive pre-emergence damping-off in subterranean clover across southern Australia, in the same way as occurs for *P. irregulare* (Wong et al. 1984, 1985, 1986, Barbetti and MacNish 1978, Greenhalgh and Lucas 1984).

That there were multiple interactions between environmental factors and plant productivity in these MLA project studies was also expected, as this consistently occurs in relation to other oomycete pathogens in Australia. For example, for *P. irregulare,* Wong et al. (1984) demonstrated the role of specific temperature/moisture combinations resulting in the smallest shoots (e.g., 10°C/45%WHC; 15°C/flooding). That *A. trifolii* played a significant role in shoot weight reduction (i.e., reduced productivity) in the current study confirms the strong negative relationship between root disease

severity and productivity of subterranean clover, as occurs with *P. irregulare* and *P. clandestina* (eg., Barbetti 1984a,b, Barbetti and MacNish 1984, Wong et al. 1984, Barbetti et al. 1986). In the current study, least productive plants were in loam soils, particularly under low temperatures, situations favouring severe disease as discussed above. A possible reason for better plant growth in the sand-based soil is its friable nature allowing more rapid root extension through soil, particularly under medium or high temperature conditions that favour faster plant growth *per se* and are less favourable to *A. trifolii* than cooler conditions (You et al. 2016).

The widespread decline and failure of subterranean clover forages to persist due to soilborne disease, leads to severe reductions in both the capacity to carry livestock and the overall whole-farm profitability across southern Australia. This leads to decreased desirable legume component, and an increased weedy content. Even without soilborne disease, producers have to cope with a naturally occurring feed scarcity across autumn–winter. That this coincides with the severe attack by oomycete soilborne pathogens like *A. trifolii* exasperates this shortage in autumn–winter biomass production in se-regenerating stands after the hot dry summer period. These MLA project studies are the first to highlight how fluctuating temperature and moisture conditions, and soil types, so common under the southern Australian annual subterranean clover forages, determine the severity and impact of *Aphanomyces* damping-off and root disease epidemics and it is the first to apply these modelling approaches to understanding complex interactions between environmental factors with soilborne forage legume disease. It also highlights how warming temperatures and drying climate across the southern Australian forage zones from climate change will likely reduce the future impact and importance of *A. trifolii*, a pathogen favoured by cold temperatures and wet and waterlogged conditions. In the interim, cultivation can be used to reduce both damping-off and root disease (Barbetti and MacNish 1978, You et al. 2017b), particularly if done in association with reseeding with varieties having some resistance to *A. trifolii*. Fortunately, at least one relatively recently released variety, Urana, has useful resistance against a mixture of all known *A. trifolii* pathotypes, making it variety of choice for farmers in higher rainfall areas where *A. trifolii* is prevalent. While locating and developing further improved host resistance offers the best long-term and most cost-effective way to curtail losses where severe *Aphanomyces* damping-off and root disease occurs in subterranean clover, this critical benefit for meat and other livestock producers will only occur if there is new funding support for such an approach.

5.4 DNA testing for soilborne pasture diseases

5.4.1 Development of DNA tests for *Aphanomyces*

The development and confirmation of a successful DNA test for *A. trifolii* is a very significant outcome of this MLA project. It will allow, for the first time, meaningful interpretation of DNA test results for the full range of soilborne pathogens in pastures. Previously, in contrast to pasture DNA tests, DNA tests available for crop soilborne pathogens were able to be well interpreted in terms of just what the test results mean in terms of the expected expression of soilborne crop diseases, and also allowing molecular DNA assays of soil-borne pathogens to be used as effective research monitoring tools.

5.4.2 Critical evaluation of DNA tests

Importantly, the DNA validations not only show that *A. trifolii* now clearly constitutes a much more widespread and serious threat to subterranean clover productivity than previously believed, DNA tests showed a relationship between amount of DNA detected and level of disease severity by *A. trifolii*. Definition of relationships between the expression of disease on plants with the associations of particular soil-borne pathogens and how environmental influences determine the outcomes of these interactions and relationships is essential both to define both the different symptom

expressions on roots and the relative impacts of different individual pathogens and pathogen complexes in different regions and across different seasons; and as the only sound basis for interpreting molecular DNA tests for producers.

Such critical evaluation of DNA tests was made in these MLA project studies in relation to the impact of pasture composition on soil-borne *Pythium* and *Rhizoctonia* root rot diseases and quantity of pathogen in the soil (expressed as DNA weight) when using subterranean clover composition at 20% and 100% were tested for pathogens *Pythium* and *Rhizoctonia* DNA (pg/g soil) weight by "Predicta B". These studies highlighted that DNA weights significantly differed in pasture composition, pathogen, variety, and also their interactions. For example, tap and lateral root disease indices and DNA weight were lower in 100% than 20% subterranean clover composition for all tested varieties. These relationships were also variety dependent, for example, Seaton Park and Riverina suffered least *Pythium* root rot and contained least *Pythium* DNA weight for 10% subterranean clover in soil in 100 % composition. However, it was encouraging that both *Pythium* and *Rhizoctonia* DNA weights were positively correlated with tap and lateral root disease. Further, while it was particularly encouraging that 'Predicta B' tests gave about 70 to 80 % indication for *Pythium* root disease, it was clear that this did not apply across all soilborne pathogens, for example, tests gave only about 40% for *Rhizoctonia*. The percent subterranean clover also strongly influenced the level of relevance of the 'Predicta B' tests. For example, for 10% subterranean clover, the root rot disease indices were only about 10 to 15 % when DNA weight was around 500 pg /g soil for *Pythium* but were 60 to 80% disease indices when DNA weight was only about 100pg /g soil for *Rhizoctonia*. Therefore, when use 'Predicta B' for soil testing, it is clear not only that for the level of root disease from *Rhizoctonia* is effected by other factors rather than just it's DNA weight indicated by 'Predicta B', but that even a relatively low 100pg DNA weight is sufficient to cause severe root rot on subterranean clover.

5.5 Identification of effective host resistance to *Aphanomyces*

A series of studies were undertaken on this aspect. In the first experiment high level host resistance to *A. trifolii* was identified in Bacchus Marsh, Dalkeith, Riverina and Yarloop with PDI ≤ 10 for both tap and lateral roots, suggesting these varieties will perform well under field conditions conducive to *A. trifolii*. Other subterranean clover varieties with slightly less resistance included Dalkeith, Enfield, Esperance, Leura, Nangeela, Northam and York, all with PDI <20 for both tap and lateral roots. Of the varieties retested against an individual isolate, Yarloop again had high levels of resistance compared to the other varieties tested, but had higher PDI than in the first experiment; and overall mean PDI in the second experiment was higher than in the first experiment. While this could possibly be due to the isolate in second experiment being more virulent than in the first experiment, the fact that some varieties (e.g., Yarloop) showed relatively similar responses against both isolates was an indication that resistance in that instance was effective against at least two different isolates and/or strains/pathotypes. However, Yarloop is no longer a viable commercial variety as it has high oestrogenic content (Little and Beale 1988) and is highly susceptible to northern anthracnose disease (*Kabatiella caulivora*), and to *Pythium* and *Phytophthora* root rots (Wong et al. 1986; Barbetti, 1989; Barbetti et al. 2007; Barbetti and You 2014). While Yarloop commercially generally remains at low incidence in most pastures across southern Australia due to its susceptibility to this range of other diseases, it could still be utilized as a source of resistance against *A. trifolii* in subterranean clover breeding programs to develop resistance against some individual prevailing *A. trifolii* isolate strains/pathotypes. Further, the potential of Yarloop's resistance is demonstrated by its persistence in some specific high rainfall locations in south-west Western Australia where *A. trifolii* is present (MJ Barbetti unpubl.). Relatively, the most resistant varieties against the mixture of 23 isolates were Antas, Uniwager and Leura with PDI <34, 49 for tap and lateral roots, respectively. It is noteworthy that Antas and Leura both are from Sardinia and are mid- to late-maturing varieties suitable to high rainfall (>700 mm) areas across southern Australia that are highly conducive to *A.*

trifolii. In the MLA project studies, screening varieties against a mixture of isolates resulted in a greater level of root disease on both tap and lateral roots compared with situations involving only a single isolate. The advantage of using a mixture of isolates was that any such resistance identified would likely be effective across different locations even where the pathotype populations of *A. trifolii* are different.

Virulence clearly varied across different isolates of *A. trifolii*. For example, some varieties showed distinctly different relative host responses (e.g., Dalkeith, Bacchus Marsh) against the different isolates. Notably, Bacchus Marsh was one of the most resistant varieties in the first experiment but the most susceptible variety in the second experiment where the severity of root disease was greater. Conversely, Clare was one of the more susceptible varieties in the first experiment but the second-most resistant in the second experiment. For Clare, the disease levels were very similar in both experiments, in contrast to all other varieties having higher levels of root disease in the second experiment. However, such pathotype or strain-independent host resistances can fail with changes in strain/pathotype or where deployed in areas where multiple strains/pathotypes already co-exist. Such challenging scenarios already occur across southern Australia in relation to races of another oomycete pathogen, *P. clandestina* (Barbetti et al. 2006b, 2007; Nichols et al. 2014) and there is no reason to suggest it would be otherwise for *A. trifolii*. There also was significant variation in terms of virulence among the 23 isolates of *A. trifolii*, with the most virulent isolates causing significantly higher levels (PDI >40) of lateral root disease than less pathogenic isolates (PDI <10). Taken together, the findings from the current study are strongly indicative of physiological specialisation. While developing current resistances and locating further improved host resistances offers the best long-term and most cost-effective way to curtail losses where severe *Aphanomyces* damping-off and root disease occurs in subterranean clover, this critical benefit for meat and other livestock producers will only occur if there is new funding support for such an approach.

5.6 Australia-wide national field trials 2015 and 2016

From the Australia-wide MLA project national field trials 2015 and 2016, it was found that the main pathogens were *Phytophthora, Pythium*, *Rhizoctonia* and *Aphanomyces* generally occurred as complexes of 2-4 different pathogens. In terms of overall losses, soilborne pathogens reduce germination by up to 70%; reduce root systems up to 90%; reduce shoot systems up to 85% and were the overriding reason for why subterranean clover in severely affected pastures does not persist. Individually, *Pythium* reduced germination by up to 60% and up to a 50% loss in plant productivity; *Phytophthora* reduced germination by up to 25% and caused a 4.5 fold loss in plant productivity; *Rhizoctonia* reduced germination by up to 90% and caused up to 75-80% loss in shoot and root production; *Aphanomyces* reduced germination by up to 14% and caused up to 50-55% loss in shoot and root systems; and when there were two or more pathogens, losses up to 100% germination failure and total loss of root and shoot production occurred. Fungicide seed treatments increased germination by up to 30%; fungicide spray treatments reduces root disease up to 30%; addition of a complete fertilizer application increased shoot productivity up to 1.5 fold; cultivation increased productivity up to 75%; and most spectacularly, more disease-resistant or disease-tolerant varieties showed increased plant productivity of up to 4-5 fold. These Australia-wide national field trials 2015 and 2016 have provided producers with effective, practical and flexible chemical and cultural options to reduce the losses during autumn-winter from soil-borne root disease. Further, these same and associated trials have ensured that producers have meaningful Predicta-B test to predict pathogens and their incidence; that they know true losses from soilborne pathogens; and also that they now understand influence of environmental factors on root disease epidemics.

5.7 Publications to date arising from this project

You, M. P., O'Rourke, T. A., Foster, K., Snowball, R., and Barbetti, M. J. (2016). Host resistances to *Aphanomyces trifolii* root rot of subterranean clover: first opportunity to successfully manage this severe pasture disease. *Plant Pathology* 65: 901–913.

You, M.P. and Barbetti, M.J. (2017). Severity of Phytophthora root rot and pre-emergence damping-off in subterranean clover is driven by moisture, temperature, nutrition, soil type, cultivar and their interactions. *Plant Pathology* 66: 1162–1181.

You, M. P. and Barbetti, M. J. (2017). Environmental factors determine severity of Rhizoctonia damping-off and root rot in subterranean clover. *Australasian Plant Pathology* 46: 357–368.

You, M.P., Guo, K.M., Nichol, D., Kidd, D., Ryan, M., Foster, K., and Barbetti, M.J. (2017). Cultivation offers effective management of subterranean clover damping-off and root disease. *Grass and Forage Science* 72: (Available online at Doi: 10.1111/gfs.12282).

6 Conclusions/recommendations

6.1 Conclusions

Tests across south-west, southern and south-east Australia – home to 29 million hectares of sub-clover pasture – found almost all samples suffered root disease. More than 80% of samples suffered extreme levels of disease. After three years of glasshouse and plot trials, during 2015 and 2016 research moved to large-scale field trials carried out at 16 field trial sites (two in each state each year — WA, SA, Vic, NSW). While no silver bullet was discovered to cure soil-borne diseases, the research reinforced that long-term success will rely on identifying resistant and field-tolerant subterranean clover varieties – which is likely to boost productivity up to 4–5 fold. In the interim, focussing on pasture health first and foremost is the best approach — healthy plants best tolerate disease. To this end, this project has identified a range of flexible management options producers can employ to minimise the impact while they wait for resistant varieties to be delivered to market and as outlined below.

6.2 Recommendations – a multi-pronged approach

The most cost-effective on-farm chemical treatments and cultural practices for control of root disease in subterranean clover pastures, developed from the research, include:

- cultivating soil to reduce pathogens and subsequent root disease impact on productivity for several years.
- applying a registered fungicide seed coating prior to replanting, or fungicide sprays on regenerated pastures.
- ensuring adequate soil and plant nutrition, through strategic fertiliser management, to enable better root and shoot growth even when disease is severe.
- choosing varieties that perform best in your area (e.g., two varieties performing well in South Australia and Victoria, showing resistance to root disease, were Clare and Trikala). Sow a mixture of clovers as an insurance policy.
- using a rotational grazing system that allows more plant growth and in turn improves root development, even where disease is severe.

6.3 Future R&D needed

Sub-clover breeding programs are currently running blind as the disease resistances/susceptibilities of parental lines, crosses and potential new cultivars are not being defined. Unfortunately, the PVTN

currently does not incorporate disease screening, although there is in-crop testing — a situation needing urgent remedy for pasture legumes in particular. Future priority needs to focus on selecting varieties that perform best against the pathogen populations under local conditions (i.e. have the best disease resistance and field tolerance), yet there is almost zero varietal screening in association with development of new subterranean clover varieties, a situation that commenced in 1999 and sadly continues today, making cultivar replacements by producers a 'lottery' where all risk is born by producers, often to their loss.

6.4 Adoption activities

Producers who suspect soil disease may be impacting on subterranean clover productivity now have access to rapid diagnostic testing through the 'Predicta B' test. While it can be cost-prohibitive in the short-term, 'Predicta B' results can help producers establish the main causes of root disease on their property and support strategic management decisions in relation to those pathogen challenges.

7 Key messages

An important message from the research is that management practices that allow faster root growth through the profile are most likely to lower the incidence of the disease. What is clear is the factors that slow root growth also increase root disease (e.g. poor nutrition, soil compaction).

Cultivation is currently achieving the most widespread success and with spectacular results where successful (e.g. cultivation can increase control by up to 75% and effects can persist for up to four years). Cultivation breaks up pathogen hyphae, reducing tap root disease and increasing legume root nodulation. Carrying out shallow cultivation (up to 10cm) following break-of-season rain prior to germination is best. Avoiding cultivating after seedling germination will maximise seedling survival. Disc seeders do not provide sufficient disturbance to create an impact on disease. However, cultivation is not a realistic option in established permanent pasture systems unless undertaking renovation and/or resowing of badly deteriorated pastures.

Effective pasture nutrition can increase shoot productivity by 1.5 times. Farmers need to carry out regular soil tests an address nutritional deficiencies through strategic fertiliser applications, to optimise growth. Applying fertiliser as test strips in a paddock will indicate a potential deficiency.

Fungicide sprays can reduce root disease by up to 30% and seed treatments have increased germination by up to 30% at some sites. Best to try test strips before embarking on paddock-scale operation and applying fertiliser as test strips in a paddock will indicate a potential deficiency.

Swards where subterranean clover content is high also demonstrated significantly better disease tolerance than grass-dominated pastures (although this is contrary to common thinking).

While grazing, particularly continuous grazing, leads to more severe tap and lateral root disease, poorer nodulation and smaller plants in terms of both roots and shoots, reducing grazing pressure offers potential to significantly increase sub-clover pasture productivity during the autumn feed-gap period from decreased root disease and increased nodulation associated with less intensive grazing of root rot-affected sub-clover pastures.

The only cost-effective and long-term option relates to an urgent need to develop and deploy new subterranean clover varieties with resistance and/or field tolerance to soilborne diseases. However, there is limited or no disease data on new subterranean clover varieties being developed, or in fact

the vast majority of those developed since 1999. However, in the interim, best to select varieties which are known to perform well in your region until data on resistance levels in newer sub-clover varieties becomes available. It is critical to stress, that while locating and developing further improved host resistance offers the best long-term and most cost-effective way to curtail losses from damping-off and root disease occurring in subterranean clover, this critical benefit for meat and other livestock producers will only occur if there is new funding support for such an approach.

Pathology skills and expertise have been greatly enhanced and consolidated, particularly in relation to the technical and knowledge capacity on pasture plant pathology - soil biology. This skills have now been secured for current and, providing there is ongoing industry support, future pathology requirements of the livestock industry.

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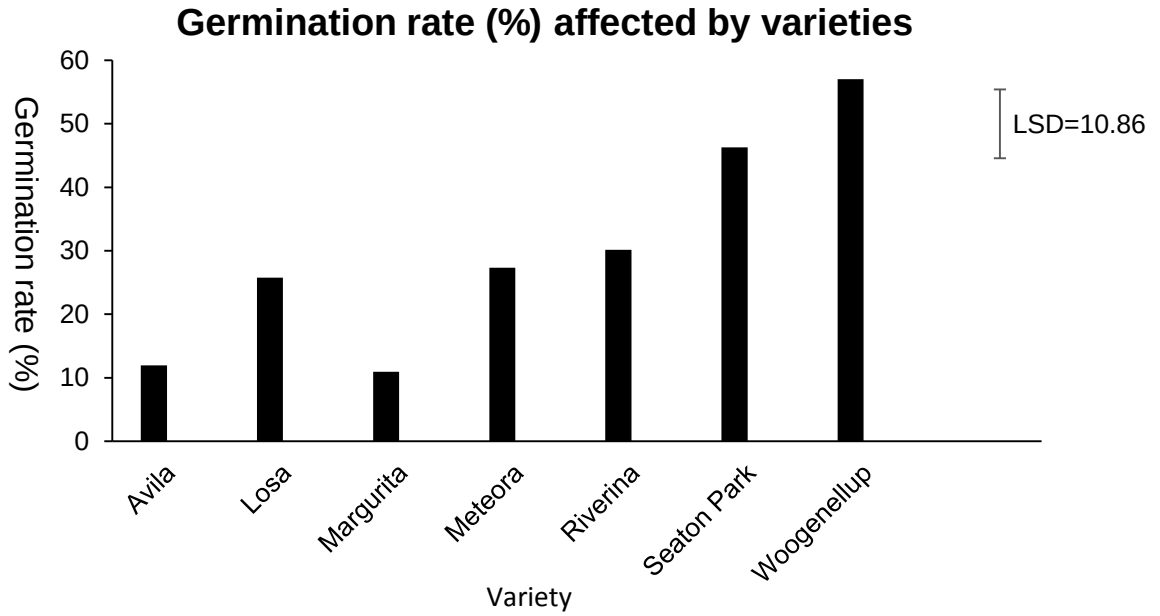
9 APPENDICES 1-8 FOR FIELD TRIALS 2015 AND APPENDICES 9-16 FOR 2016

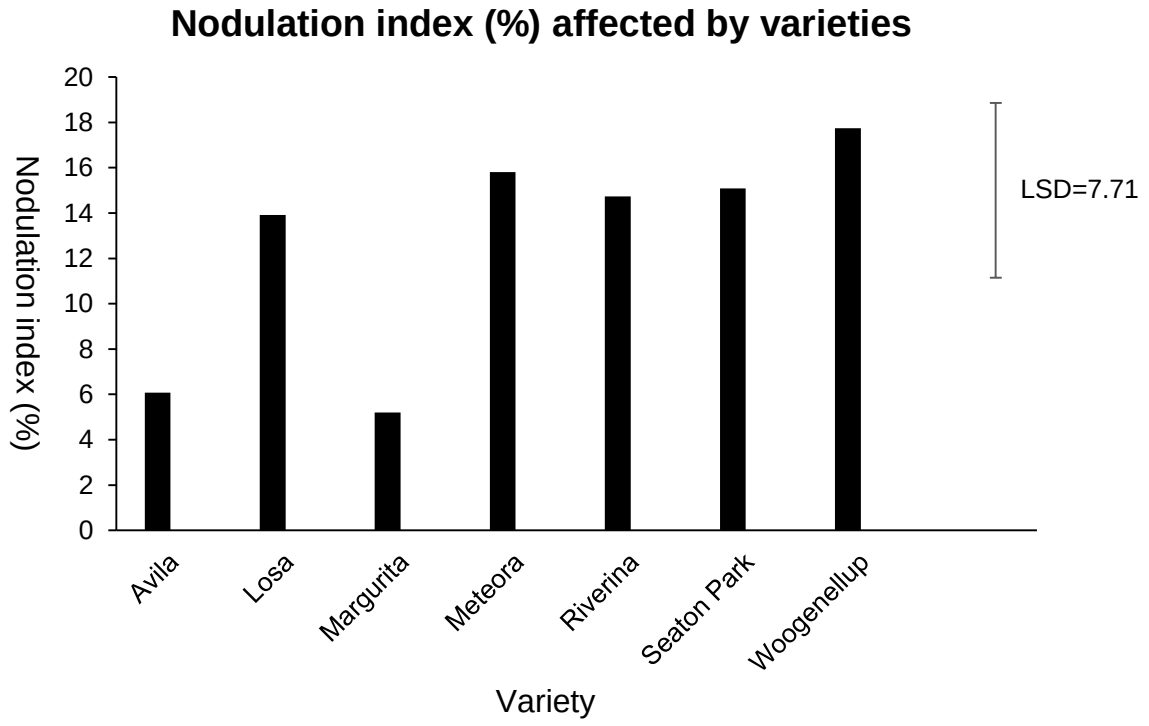
APPENDIX 1 – 2015 South eastern SA: First and second sampling results

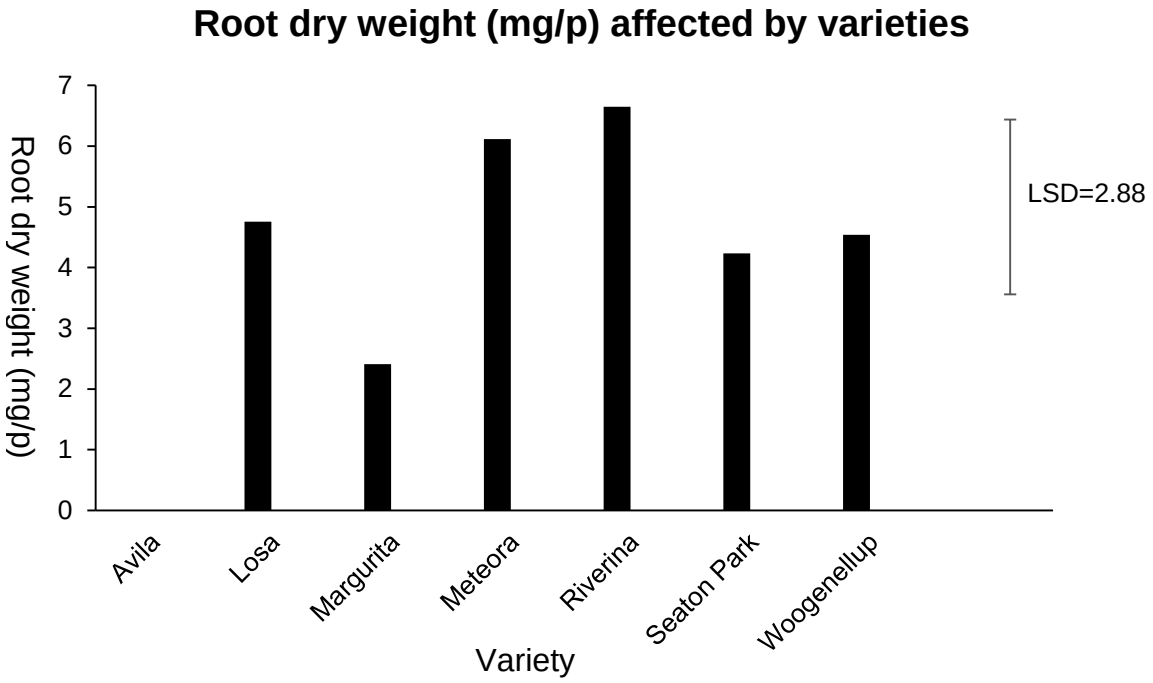
First sampling results:

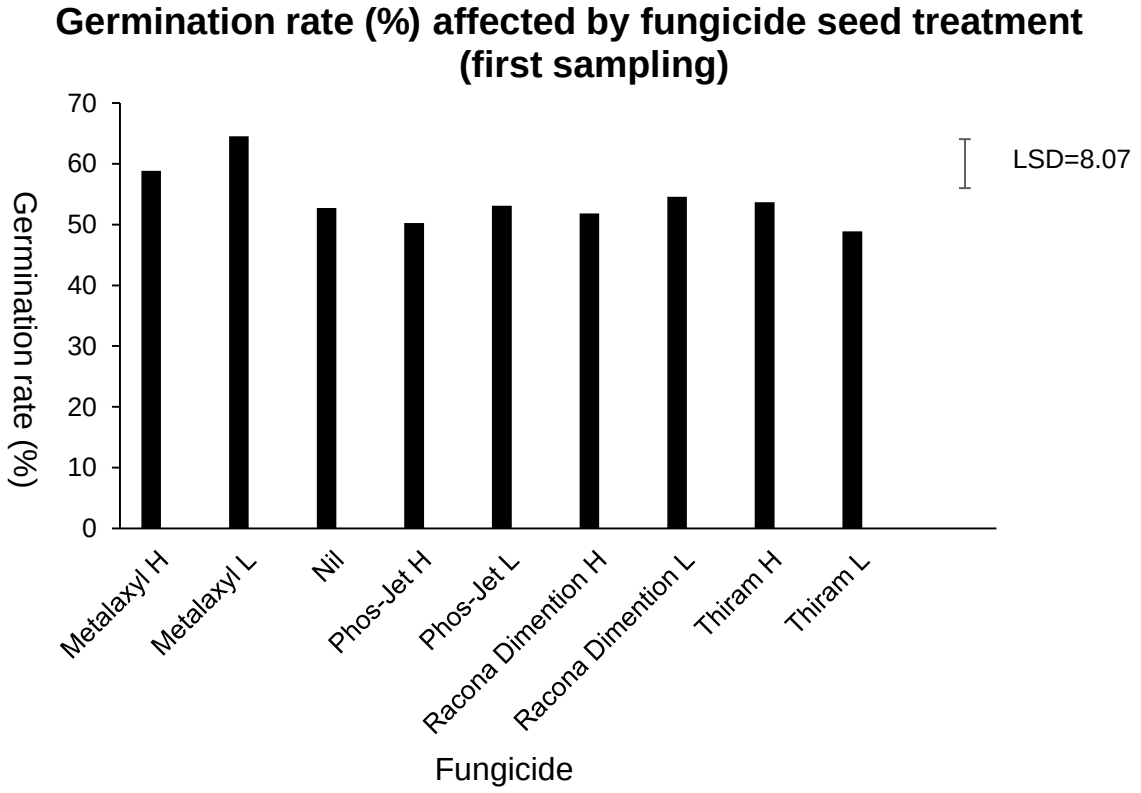
Statistical main effects fertilizer, seed treatment and variety on subterranean clover tap and lateral roots and nodulation indices, germination rate (TI%, LI%, NI% and GR %) and root and shoot dry weights (RDW and SDW) (mg/p)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		GR%	
	$p_{0.05}$	LSD $_{0.05}$	$p_{0.05}$	LSD $_{0.05}$	$p_{0.05}$	LSD $_{0.05}$	$p_{0.05}$	LSD $_{0.05}$	$p_{0.05}$	LSD $_{0.05}$	$p_{0.05}$	LSD $_{0.05}$
Variety	NS	*	NS	*	0.023	7.71	0.001	2.877	NS	*	< 0.001	10.86
Fertilizer treatment	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	0.009	8.069


Germination rate (%) affected by varieties
Germination rate (%)
60
50
40
30
20
10
0
LSD=10.86
Avila
Losa
Margurita
Meteora
Riverina
Seaton Park
Woogenellup
Variety


Nodulation index (%) affected by varieties
Nodulation index (%)
20
18
16
14
12
10
8
6
4
2
0
LSD=7.71
Avila
Losa
Margurita
Meteora
Riverina
Seaton Park
Woogenellup
Variety

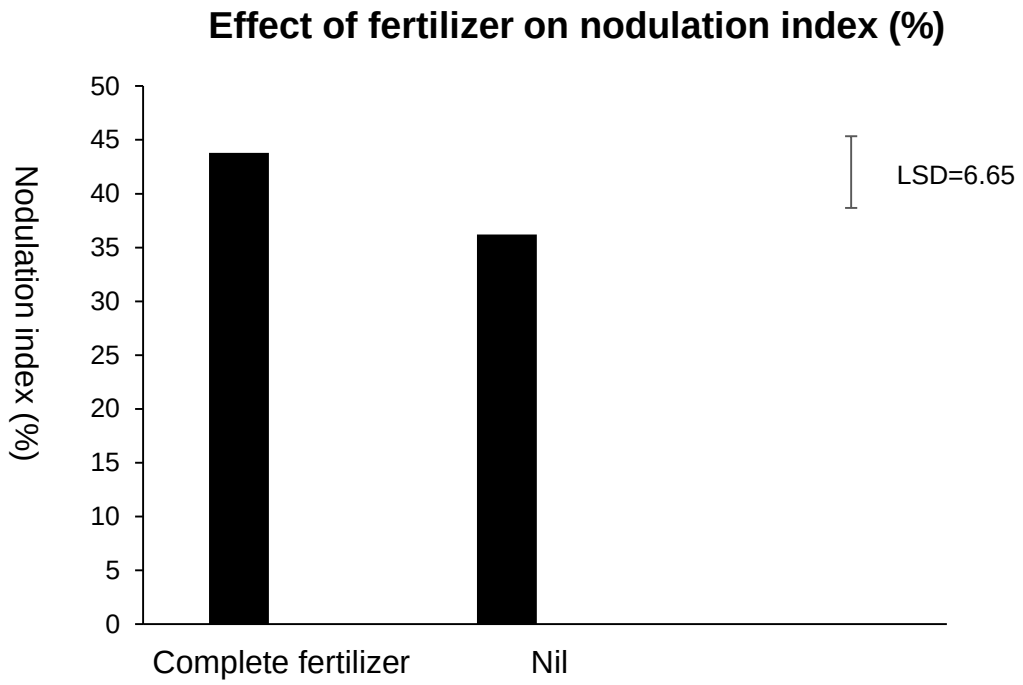

Root dry weight (mg/p) affected by varieties
Root dry weight (mg/p)
0
1
2
3
4
5
6
7
LSD=2.88
Avila
Losa
Margurita
Meteora
Riverina
Seaton Park
Woogenellup
Variety

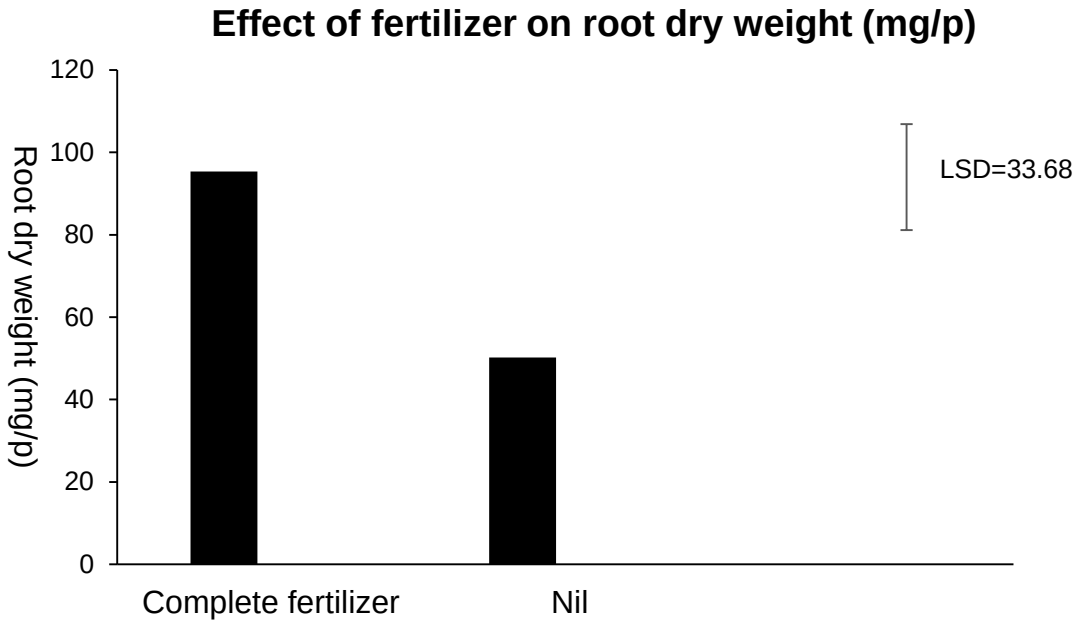

Germination rate (%) affected by fungicide seed treatment (first sampling)
Germination rate (%)
0
10
20
30
40
50
60
70
LSD=8.07
Metalaxyl H
Metalaxyl L
Nil
Phos-Jet H
Phos-Jet L
Racona Dimention H
Racona Dimention L
Thiram H
Thiram L
Fungicide

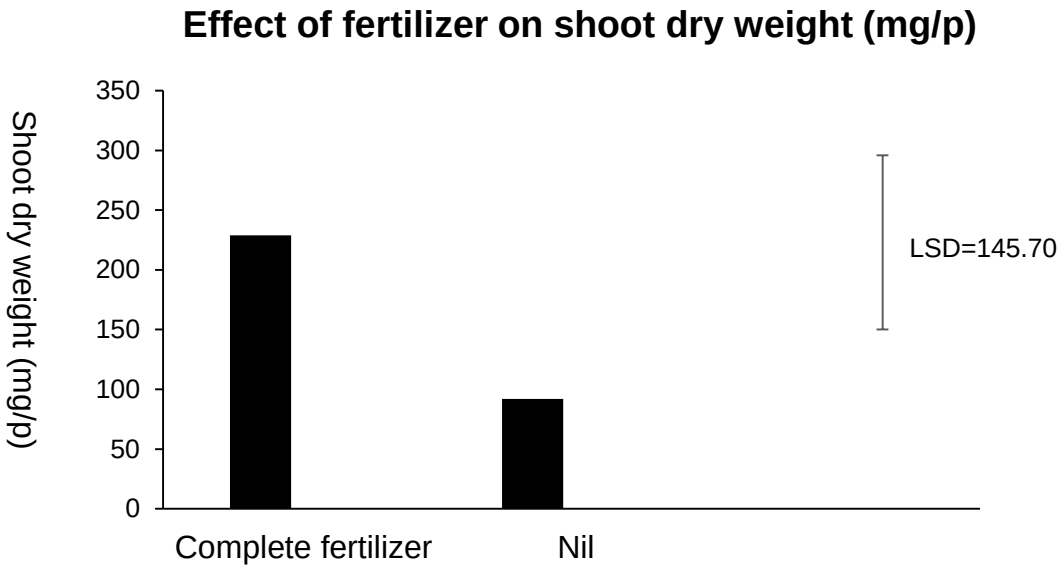
Second sampling results:

Statistical main effects fungicide spray (treatment and field), cultivation, fertilizer, seed treatment and variety on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%) and root and shoot dry weights (RDW and SDW) (mg/p)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Spray treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Field spray	NS	*	0.02	16.35	NS	*	0.02	45.8		
Cultivation	NS	*	NS	*	NS	*	0.02	26.59	NS	*
Fertilizer treatment	NS	*	NS	*	0.029	6.65	0.01	33.68	0.062	145.7
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Variety	NS	*	NS	*	NS	*	NS	*	NS	*




Effect of fertilizer on root dry weight (mg/p)
Root dry weight (mg/p)
120
100
80
60
40
20
0
LSD=33.68
Complete fertilizer
Nil

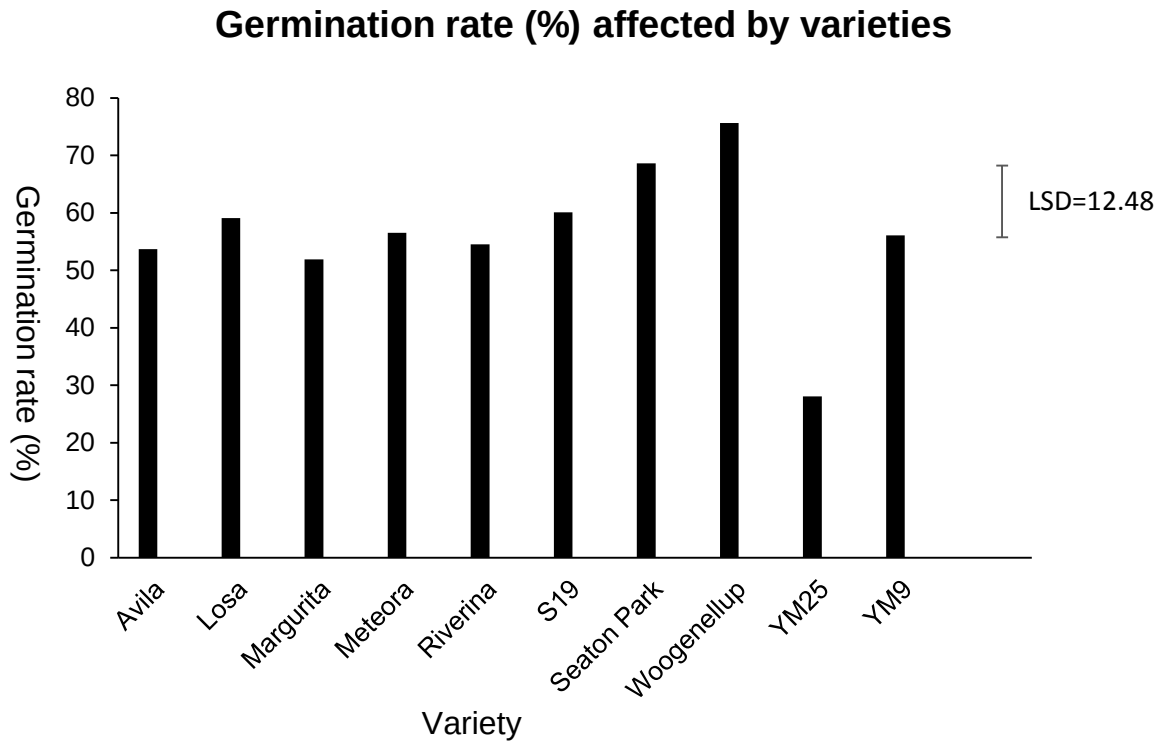

Effect of fertilizer on shoot dry weight (mg/p)
Shoot dry weight (mg/p)
350
300
250
200
150
100
50
0
LSD=145.70
Complete fertilizer
Nil

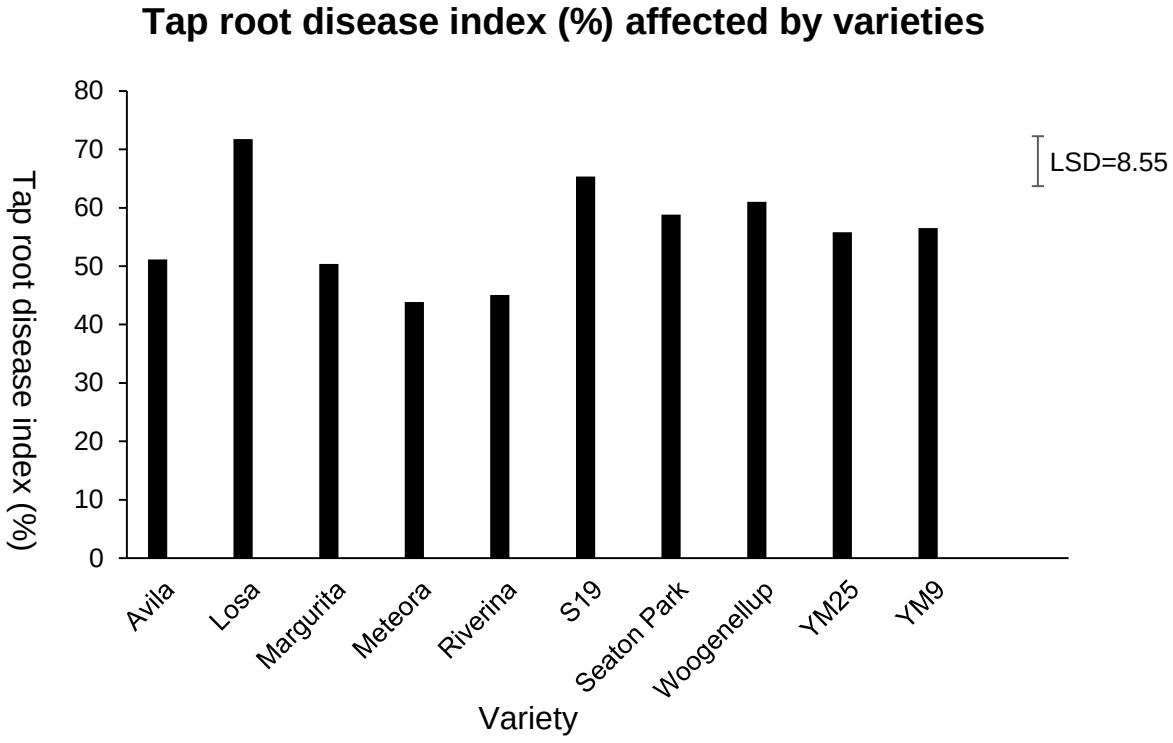
APPENDIX 2 – 2015 Barossa SA: First and second sampling results

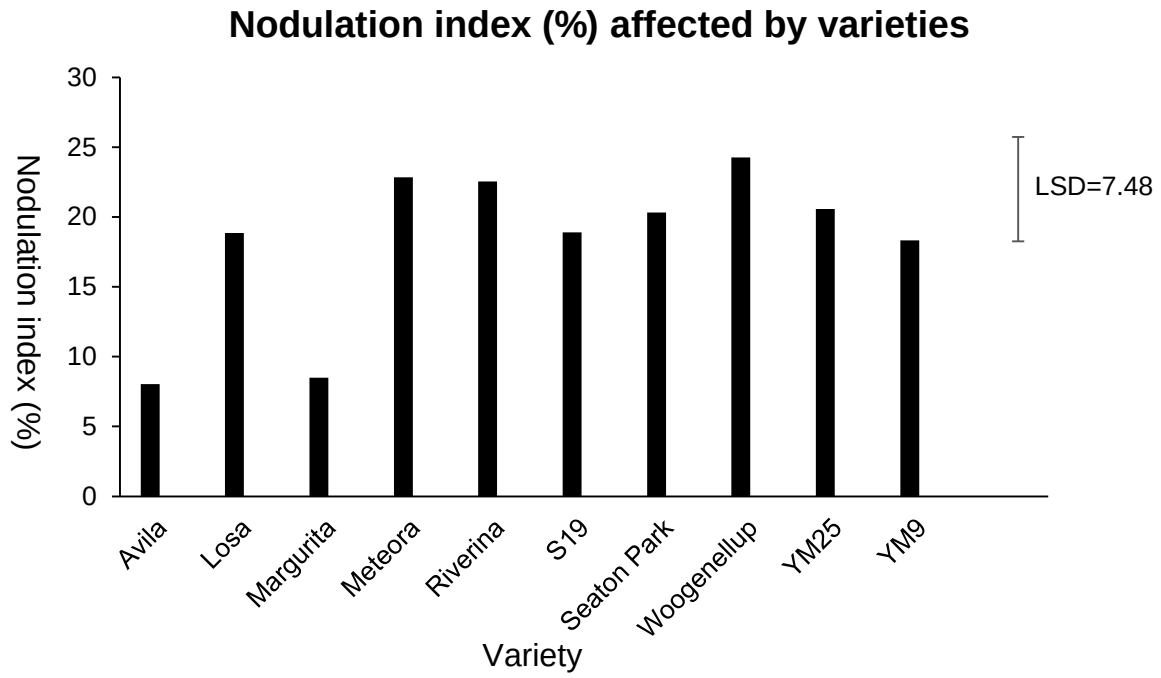
First sampling results:

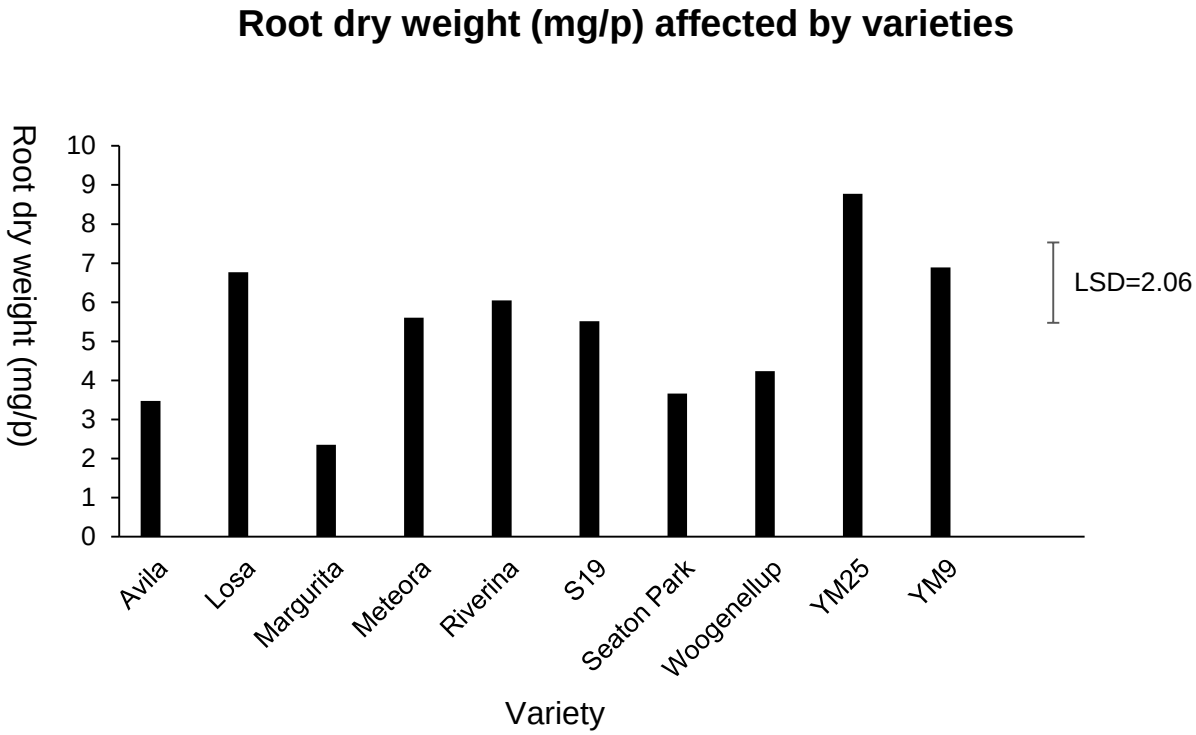
First sampling statistical main effect of variety, fertilizer treatment and seed treatment on subterranean clover tap and lateral roots and nodulation indices, germination rate % (TI%, LI%, NI % and GR%) and root and shoot dry weights (RDW and SDW) (mg/p).

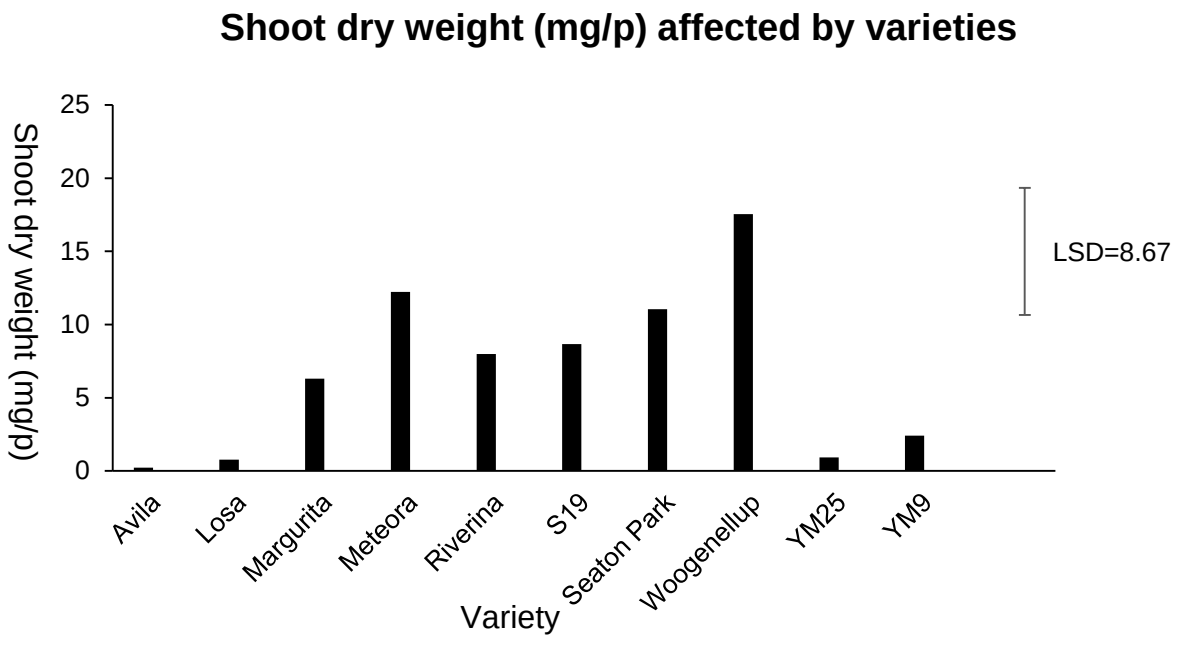
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		GR%	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	8.552	NS	*	0.002	7.475	< 0.001	2.057	0.001	8.671	< 0.001	12.48
Fertilizer treatment	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*
Seed treatment	NS	*	NS	*	0.02	3.93	NS	*	NS	*	NS	*

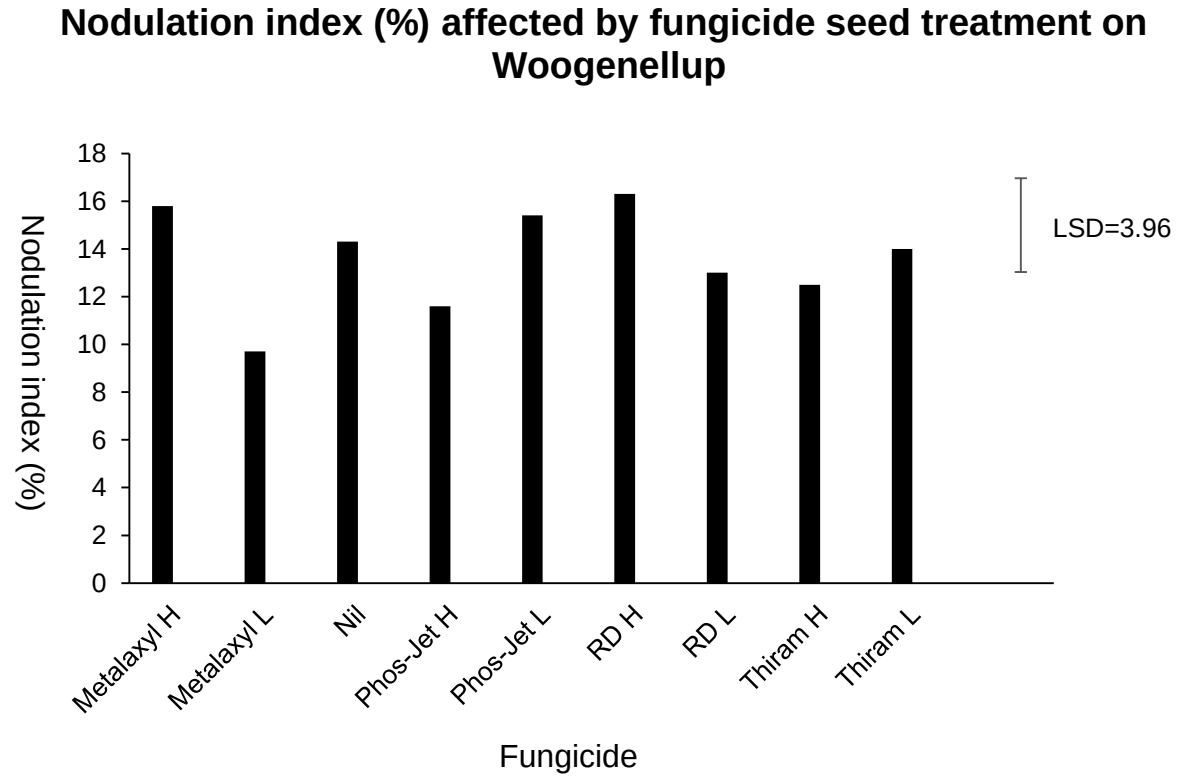

Germination rate (%) affected by varieties
Germination rate (%)
80
70
60
50
40
30
20
10
0
LSD=12.48
Avila
Losa
Margurita
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Tap root disease index (%) affected by varieties
Tap root disease index (%)
80
70
60
50
40
30
20
10
0
LSD=8.55
Avila
Losa
Margurita
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Nodulation index (%) affected by varieties
Nodulation index (%)
30
25
20
15
10
5
0
LSD=7.48
Avila
Losa
Margurita
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Root dry weight (mg/p) affected by varieties
Root dry weight (mg/p)
10
9
8
7
6
5
4
3
2
1
0
LSD=2.06
Avila
Losa
Margurita
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

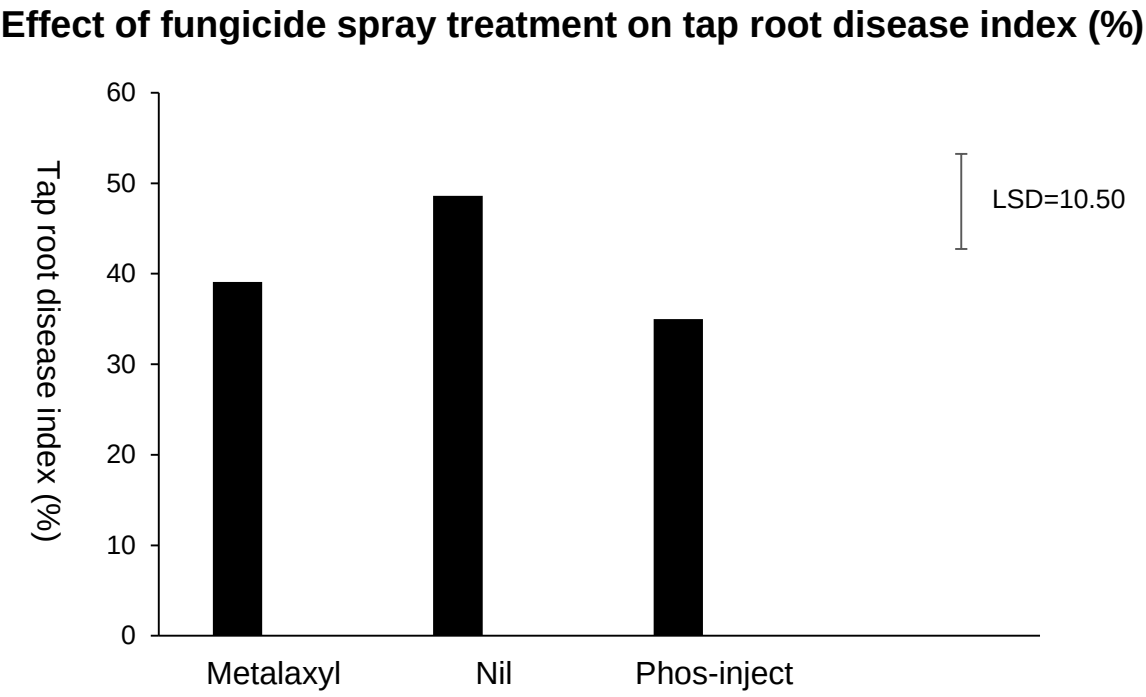

Shoot dry weight (mg/p) affected by varieties
Shoot dry weight (mg/p)
25
20
15
10
5
0
LSD=8.67
Avila
Losa
Margurita
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

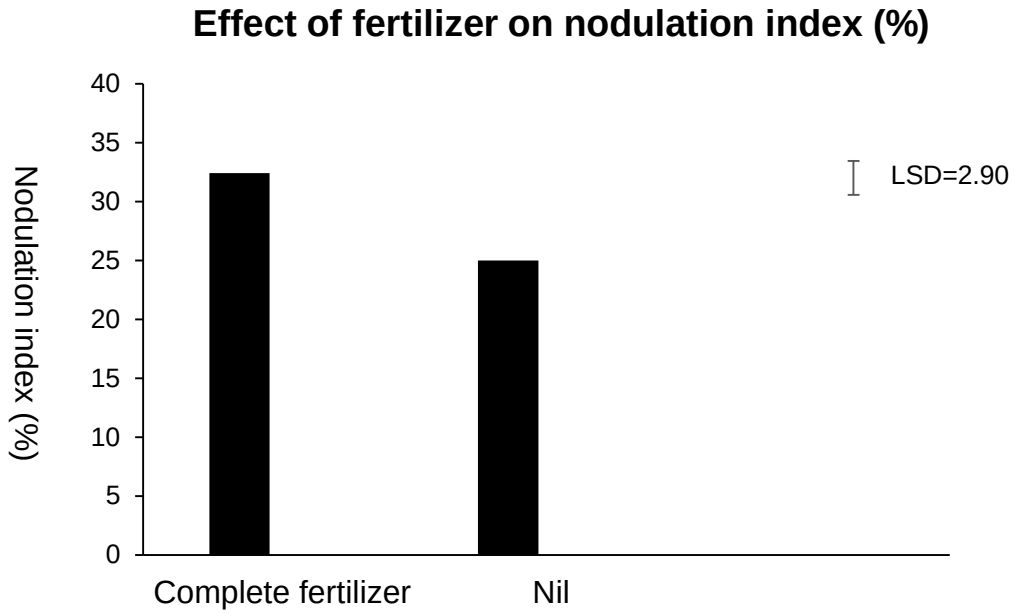

Nodulation index (%) affected by fungicide seed treatment on Woogenellup
Nodulation index (%)
18
16
14
12
10
8
6
4
2
0
LSD=3.96
Metalaxyl H
Metalaxyl L
Nil
Phos-Jet H
Phos-Jet L
RD H
RD L
Thiram H
Thiram L
Fungicide

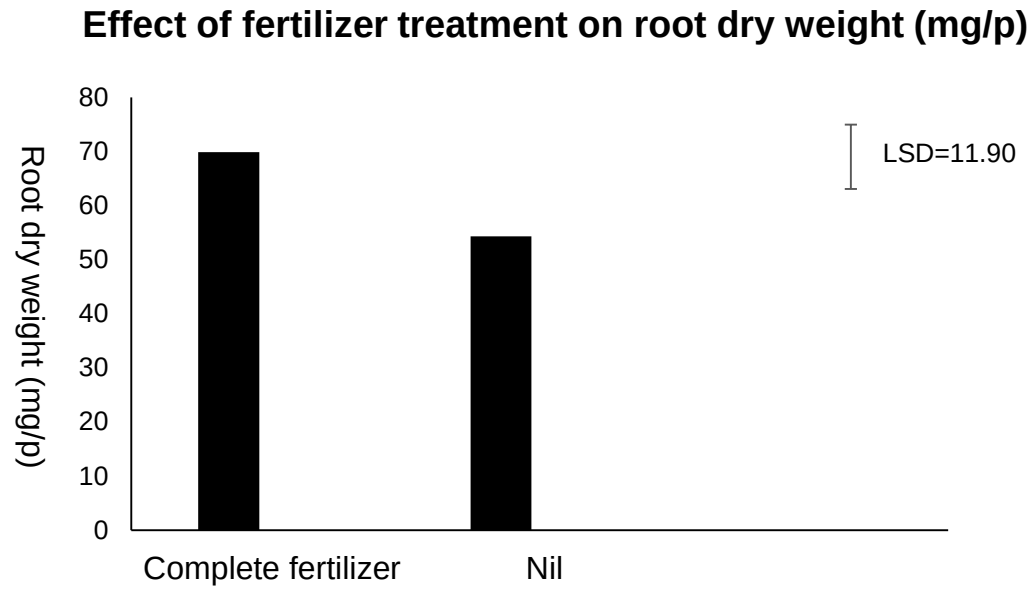
Second sampling results:

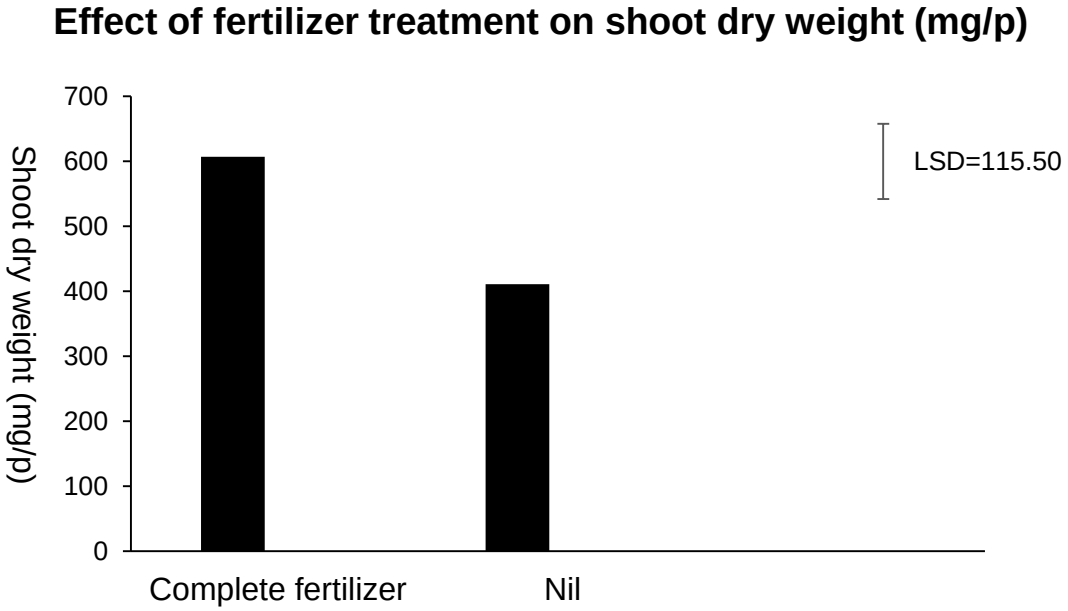
Second sampling statistical main effects of grazing, fungicide sprays (treatment and field), fertilizer (treatment and field), cultivation, seed treatment and variety on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI %) and root and shoot dry weights (RDW and SDW) (mg/p)

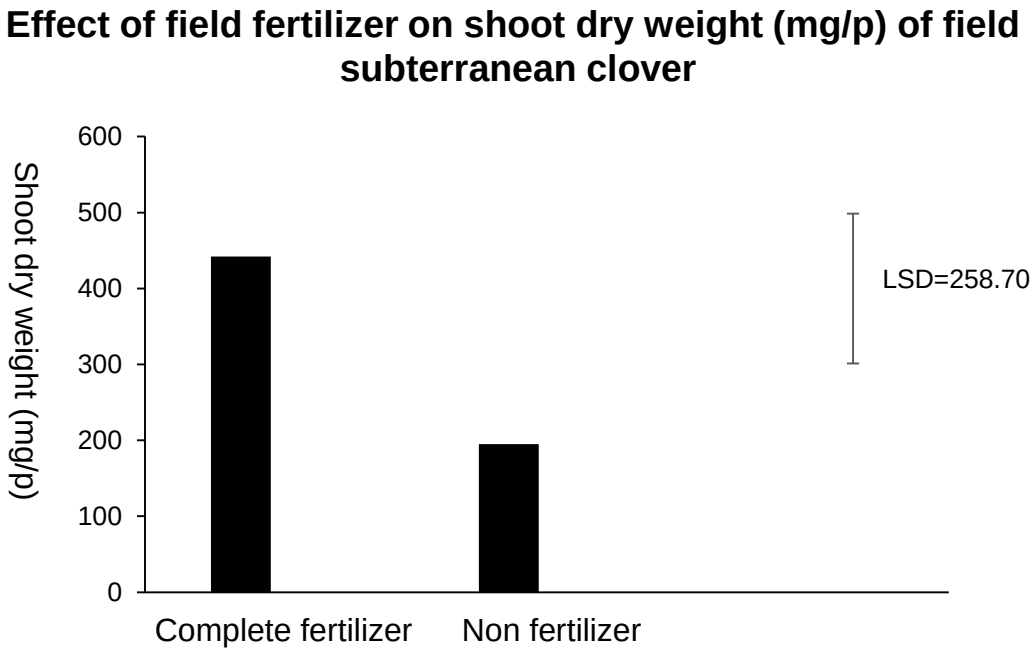
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Grazing	NS	*	NS	*	NS	*	NS	*	NS	*
Spray Treatment	0.038	10.5	NS	*	NS	*	NS	*	NS	*
Field spray	0.036	7.29	NS	*	NS	*	NS	*	NS	*
Fertilizer treatment	NS	*	NS	*	<0.001	2.9	0.016	11.9	0.004	115.5
Field fertilizer	NS	*	NS	*	NS	*	NS	*	0.02	197.4
Cultivation	NS	*	0.01	8.68	0.003	5.76	<0.001	33.43	<0.001	506.9
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Variety	< 0.001	18.17	0.021	11.09	< 0.001	13.41	< 0.001	24.87	0.006	258.7

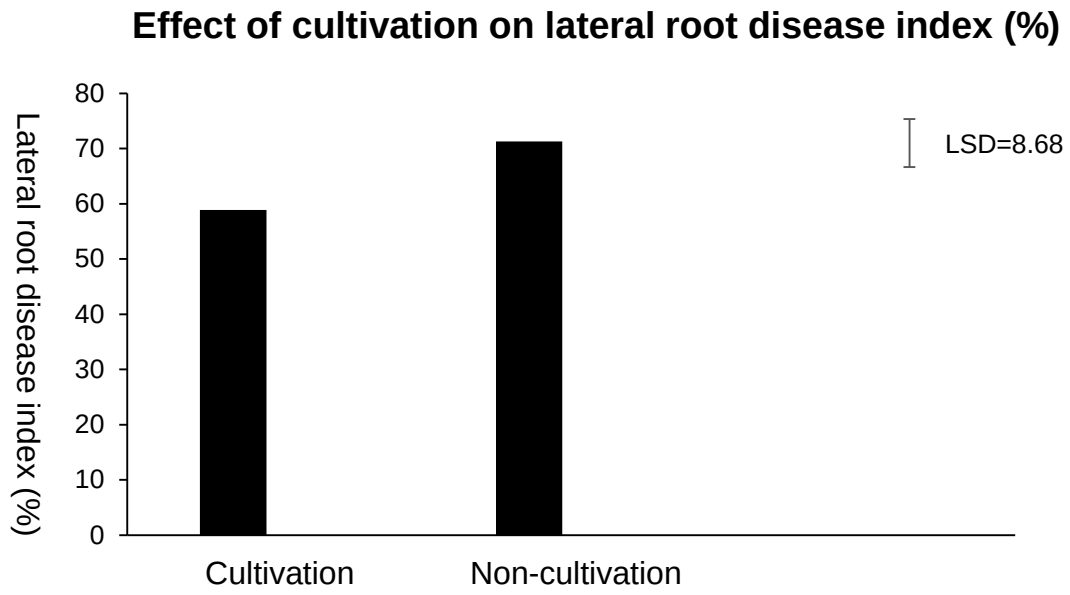


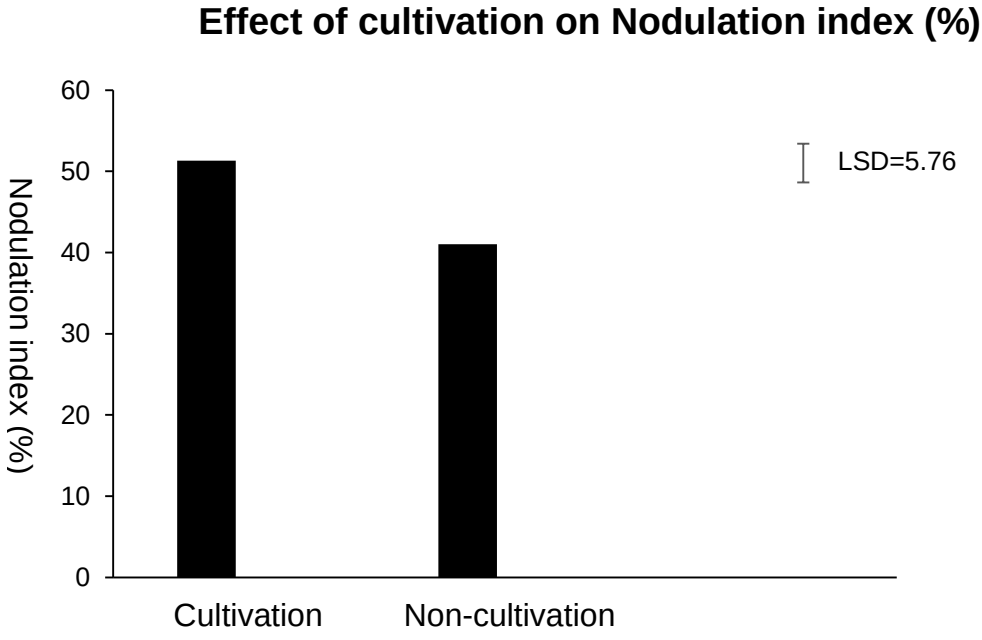

Effect of fertilizer on nodulation index (%)
Nodulation index (%)
40
35
30
25
20
15
10
5
0
LSD=2.90
Complete fertilizer
Nil

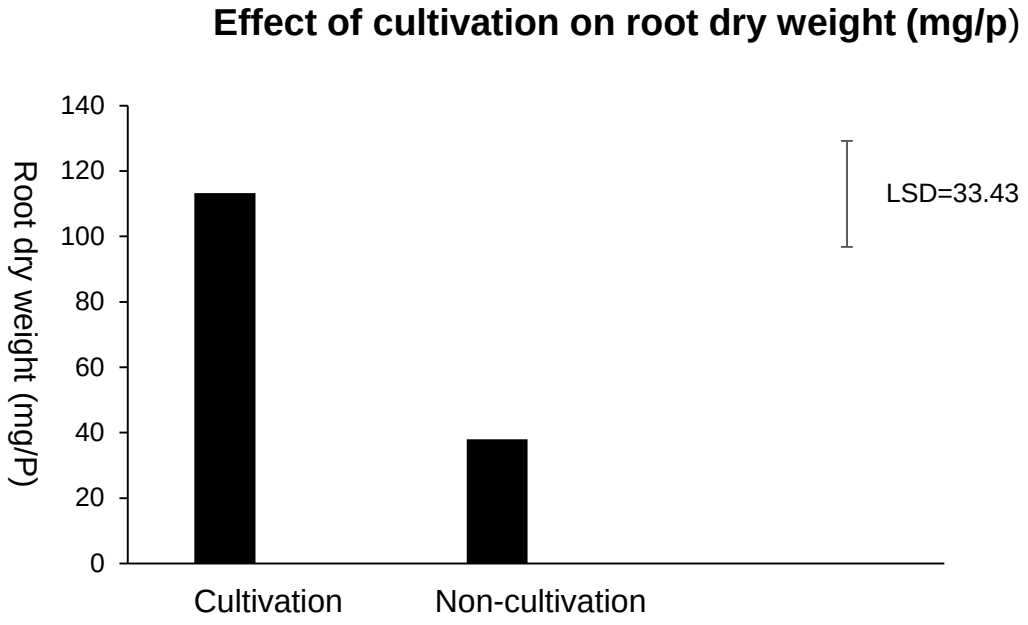

Effect of fertilizer treatment on root dry weight (mg/p)
Root dry weight (mg/p)
80
70
60
50
40
30
20
10
0
LSD=11.90
Complete fertilizer
Nil

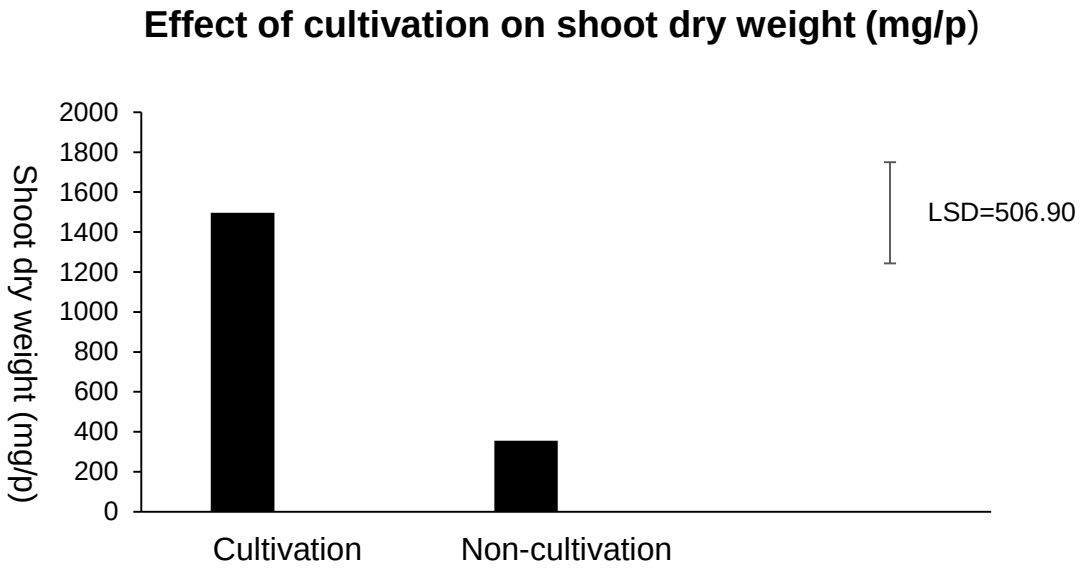

Effect of fertilizer treatment on shoot dry weight (mg/p)
Shoot dry weight (mg/p)
700
600
500
400
300
200
100
0
LSD=115.50
Complete fertilizer
Nil

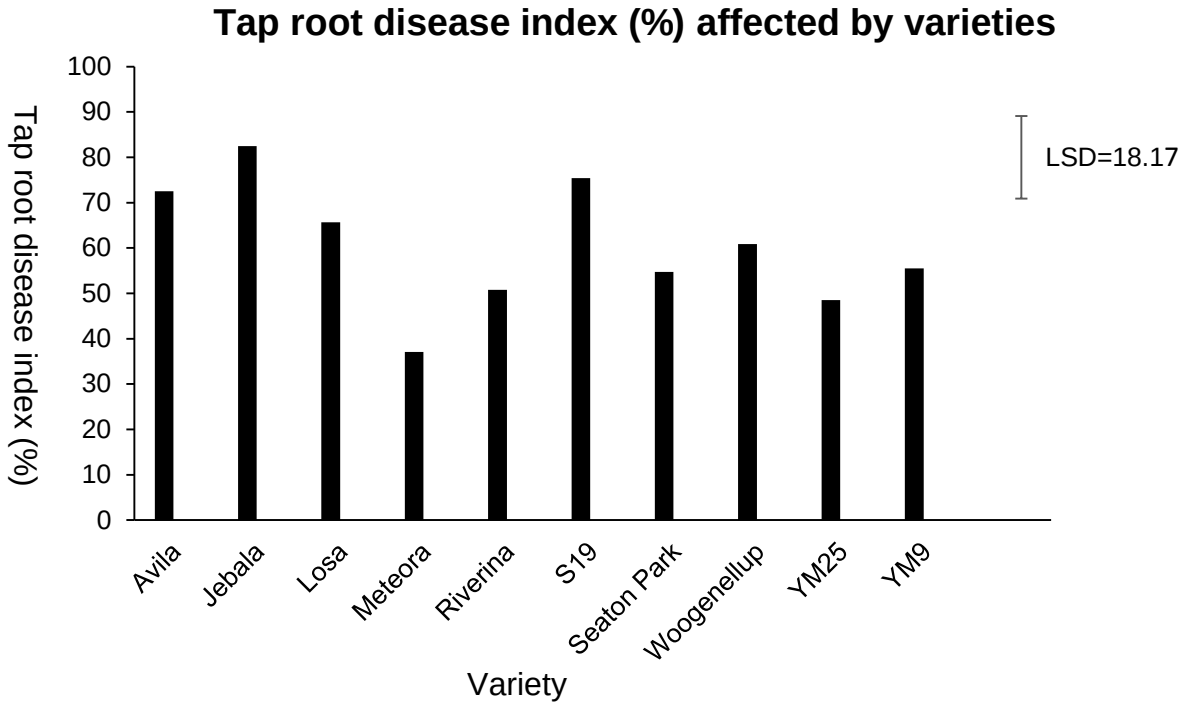

Effect of field fertilizer on shoot dry weight (mg/p) of field subterranean clover
Shoot dry weight (mg/p)
600
500
400
300
200
100
0
LSD=258.70
Complete fertilizer
Non fertilizer

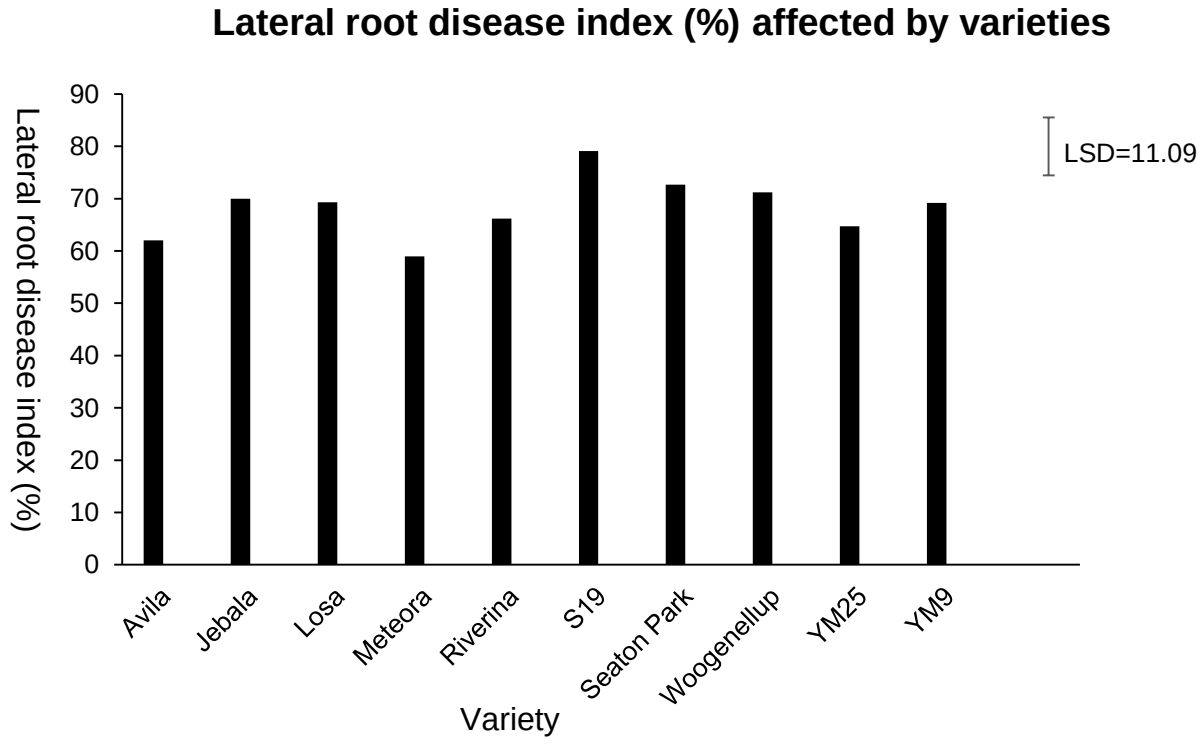

Effect of cultivation on lateral root disease index (%)
Lateral root disease index (%)
80
70
60
50
40
30
20
10
0
LSD=8.68
Cultivation
Non-cultivation

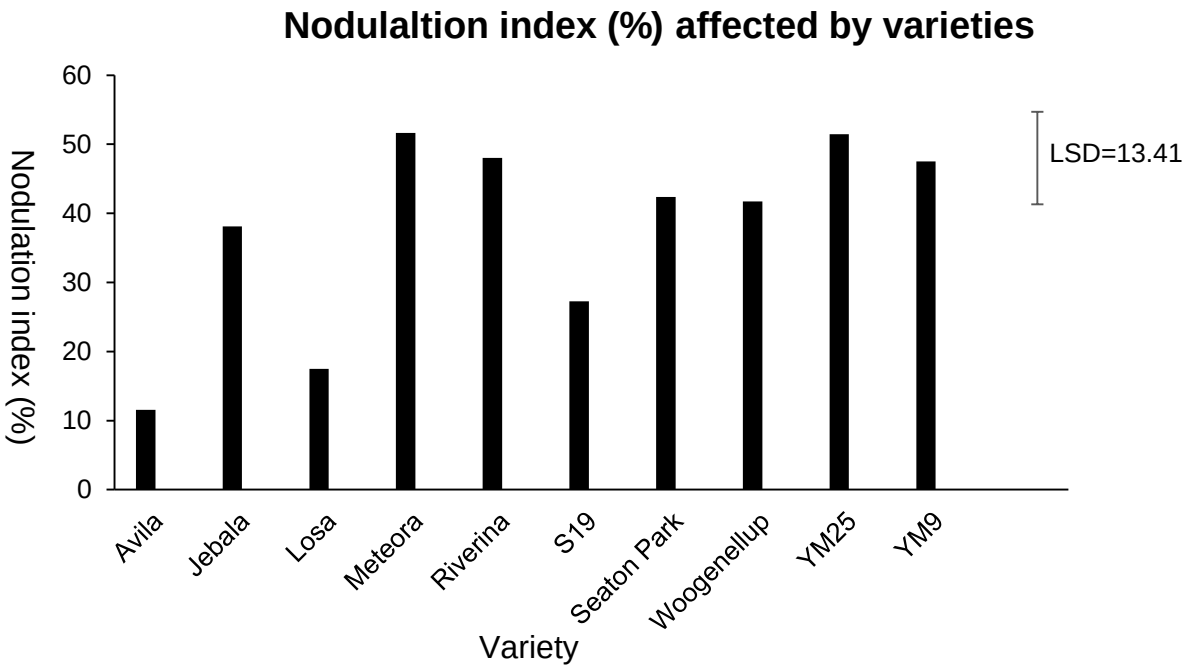

Effect of cultivation on Nodulation index (%)
Nodulation index (%)
60
50
40
30
20
10
0
LSD=5.76
Cultivation
Non-cultivation

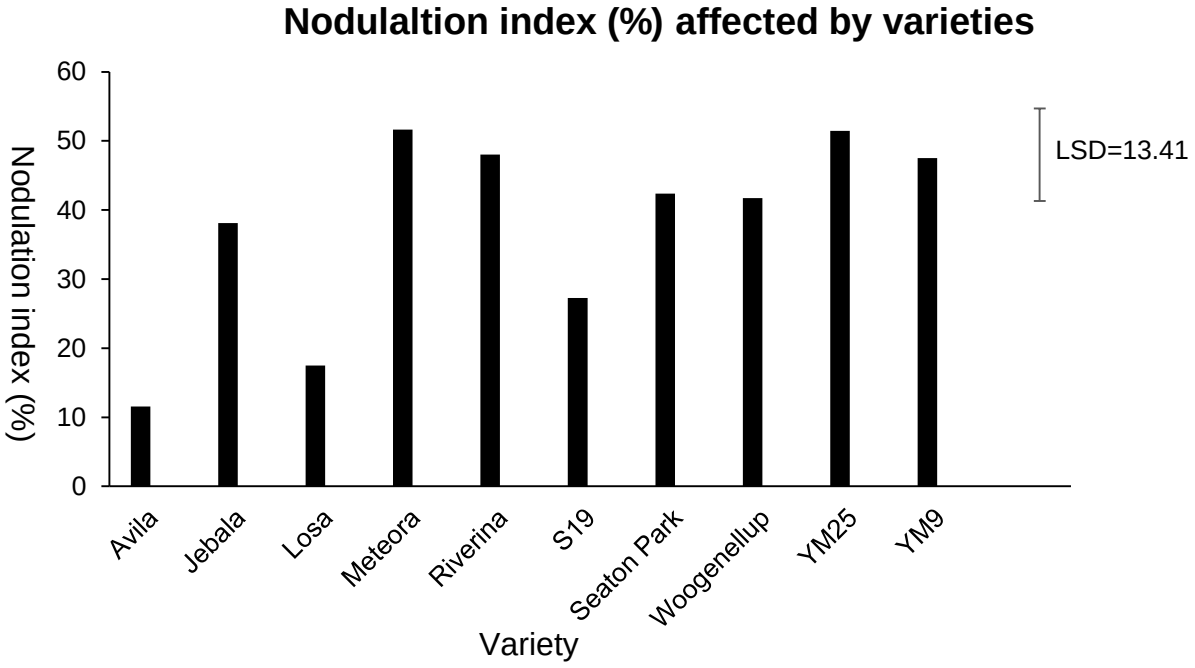

Effect of cultivation on root dry weight (mg/p)
Root dry weight (mg/P)
140
120
100
80
60
40
20
0
LSD=33.43
Cultivation
Non-cultivation

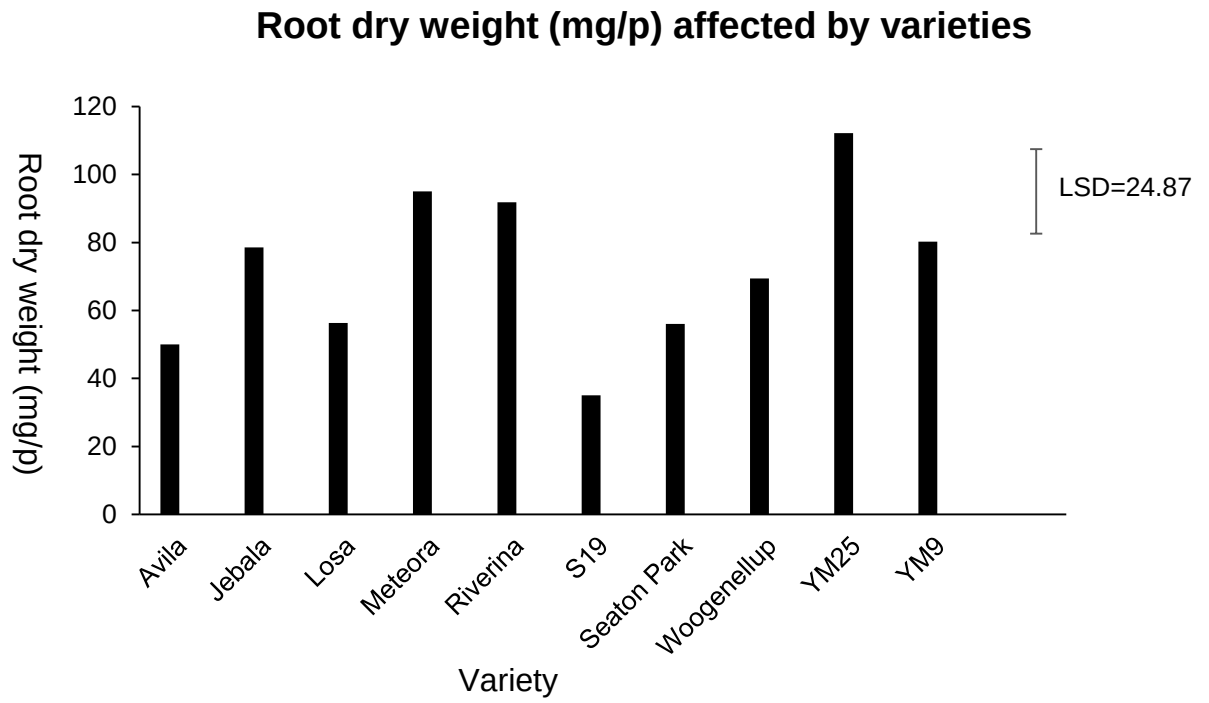

Effect of cultivation on shoot dry weight (mg/p)
Shoot dry weight (mg/p)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
LSD=506.90
Cultivation
Non-cultivation

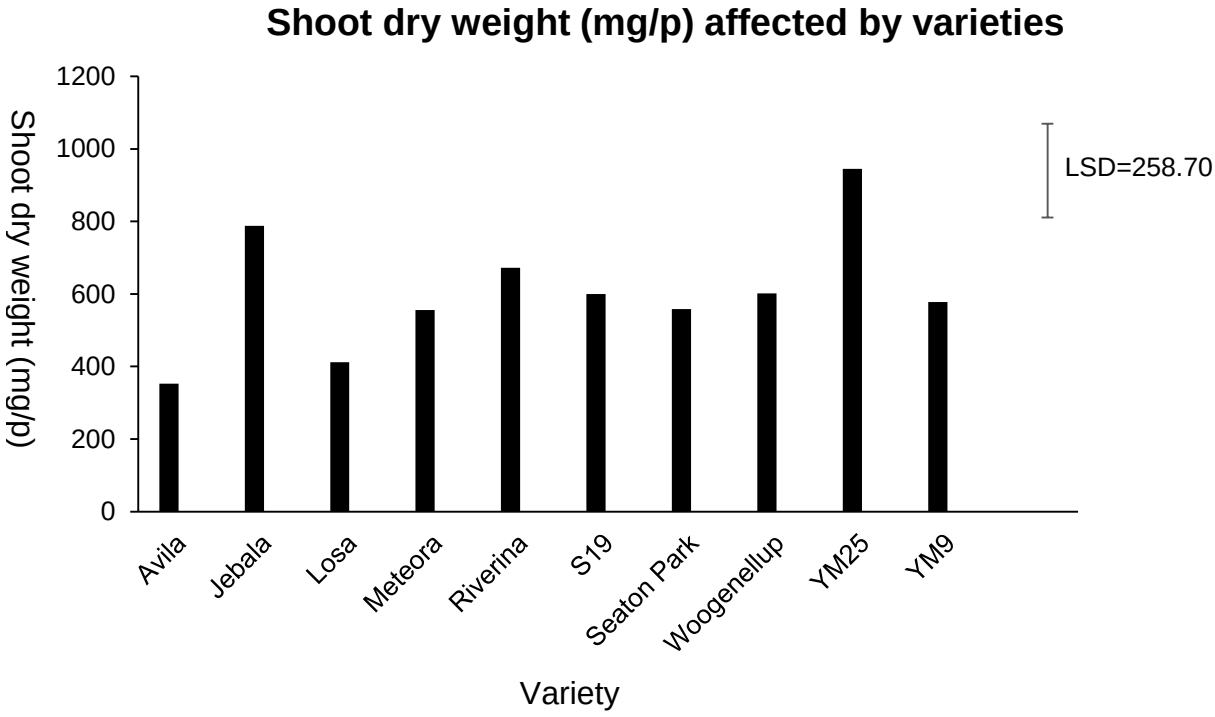

Tap root disease index (%) affected by varieties
Tap root disease index (%)
100
90
80
70
60
50
40
30
20
10
0
LSD=18.17
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Lateral root disease index (%) affected by varieties
Lateral root disease index (%)
90
80
70
60
50
40
30
20
10
0
LSD=11.09
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Nodulaltion index (%) affected by varieties
Nodulation index (%)
60
50
40
30
20
10
0
LSD=13.41
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Nodulaltion index (%) affected by varieties
Nodulation index (%)
60
50
40
30
20
10
0
LSD=13.41
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Root dry weight (mg/p) affected by varieties
Root dry weight (mg/p)
120
100
80
60
40
20
0
LSD=24.87
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

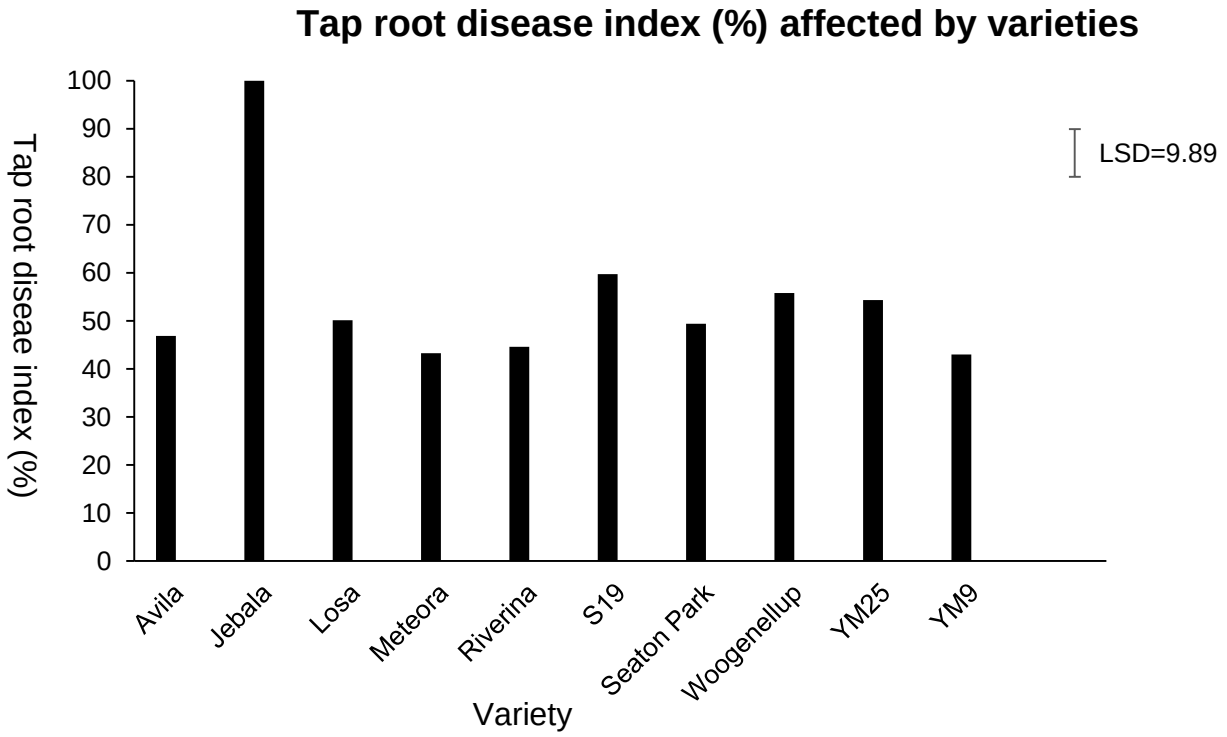

Shoot dry weight (mg/p) affected by varieties
Shoot dry weight (mg/p)
1200
1000
800
600
400
200
0
LSD=258.70
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

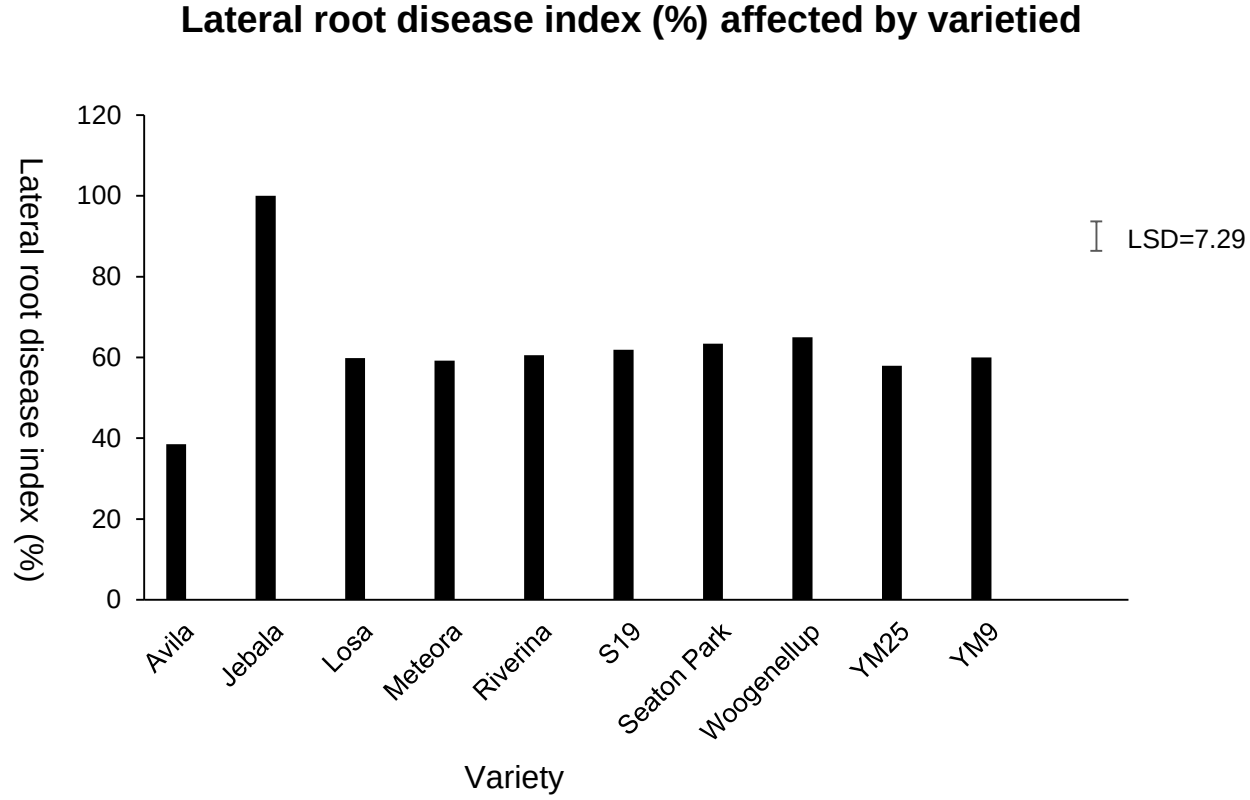
APPENDIX 3 – 2015 Western Victoria: First and second sampling results

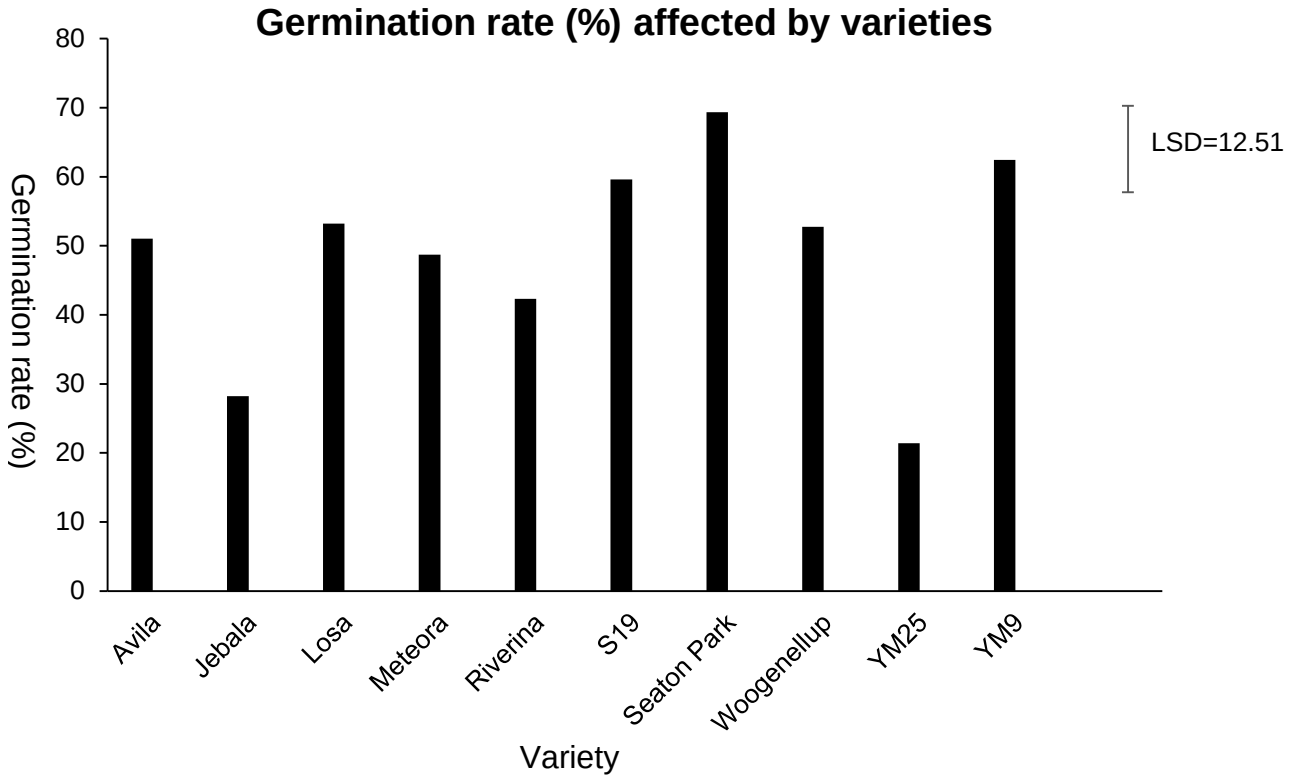
First sampling results:

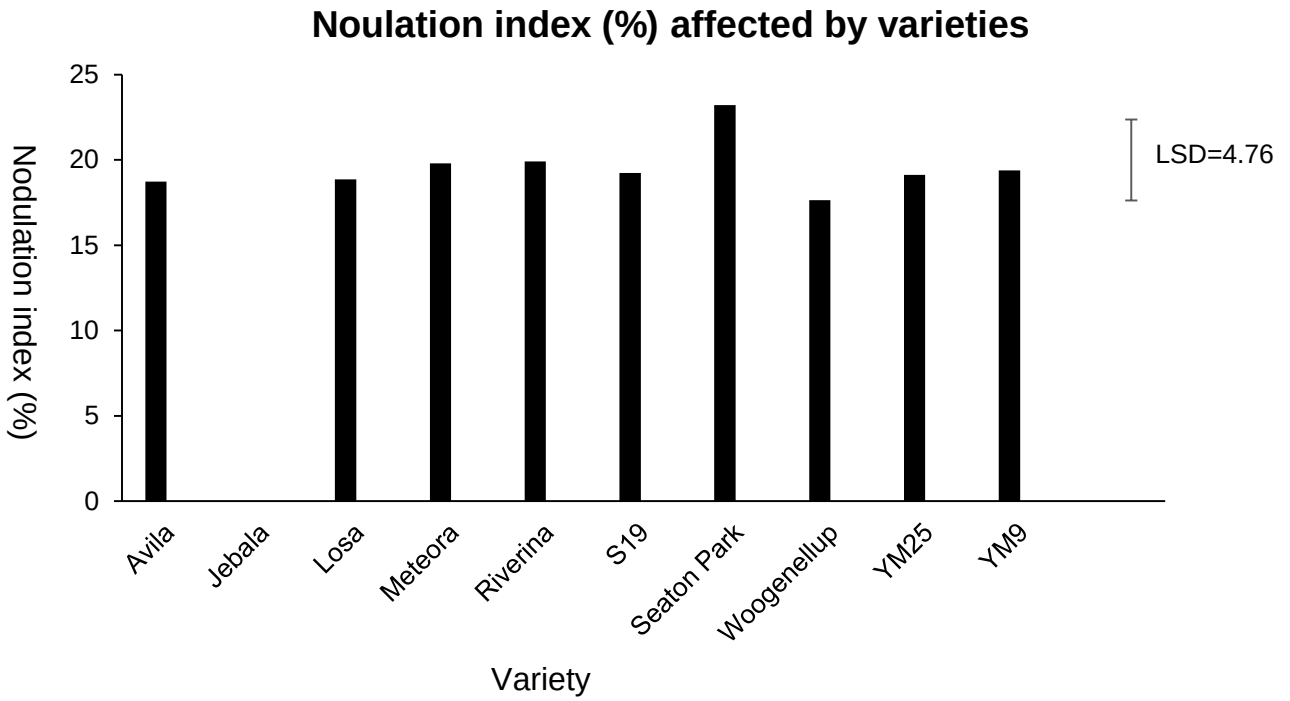
Statistical main effect of variety, seed treatment and fertilizer on tap and lateral root and nodulation indices (TI%, LI% and NI %), root and shoot dry weights (RDW and SDW) (mg/p) and germination rate (GR) (%)

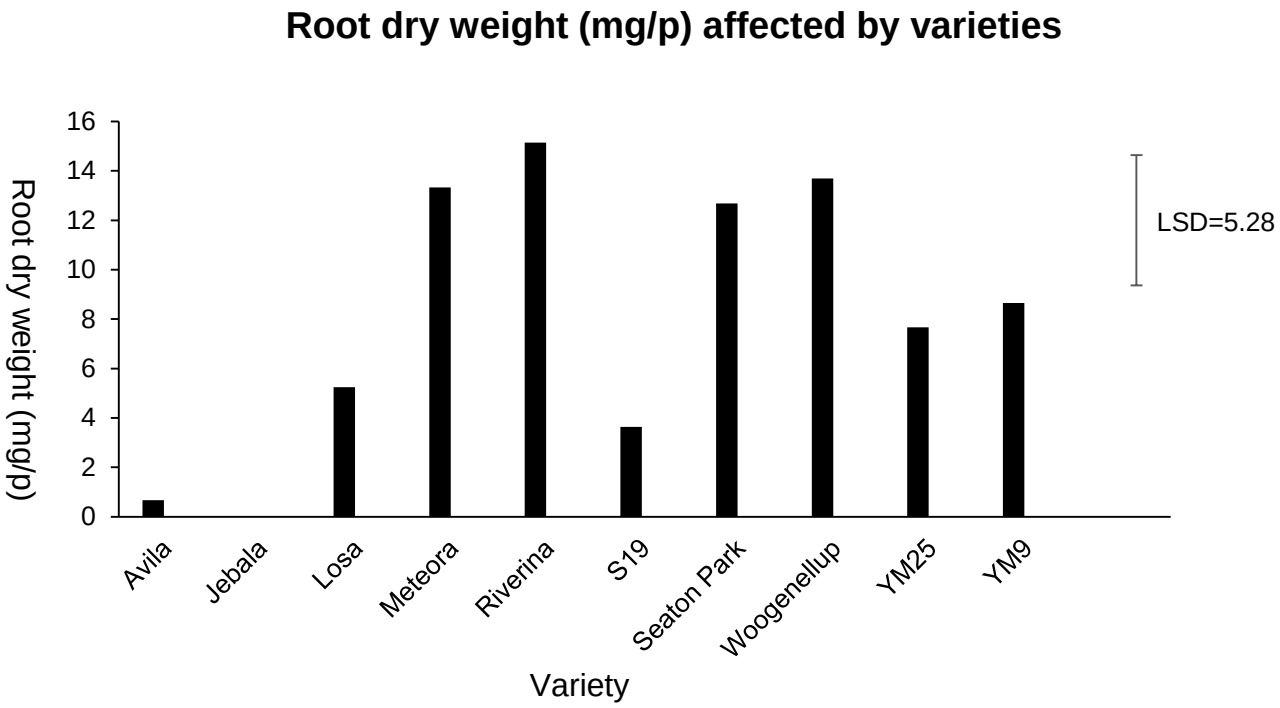
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		GR%	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	< 0.001	9.891	< 0.001	7.293	< 0.001	4.759	< 0.001	5.276	0.02	119.3	< 0.001	12.51
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*
Fertilizer	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*

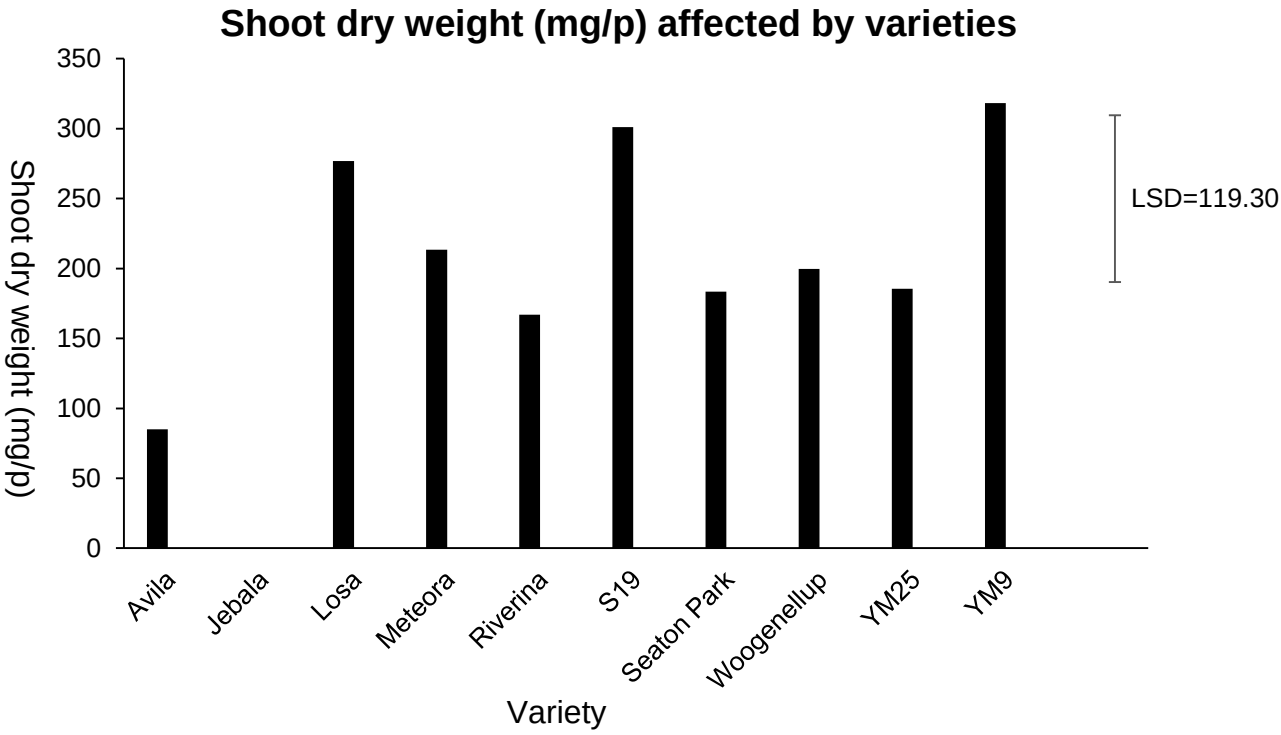

Tap root disease index (%) affected by varieties
Tap root diseae index (%)
100
90
80
70
60
50
40
30
20
10
0
LSD=9.89
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Lateral root disease index (%) affected by varietied
Lateral root disease index (%)
120
100
80
60
40
20
0
LSD=7.29
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Germination rate (%) affected by varieties
Germination rate (%)
80
70
60
50
40
30
20
10
0
LSD=12.51
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Noulation index (%) affected by varieties
Nodulation index (%)
25
20
15
10
5
0
LSD=4.76
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

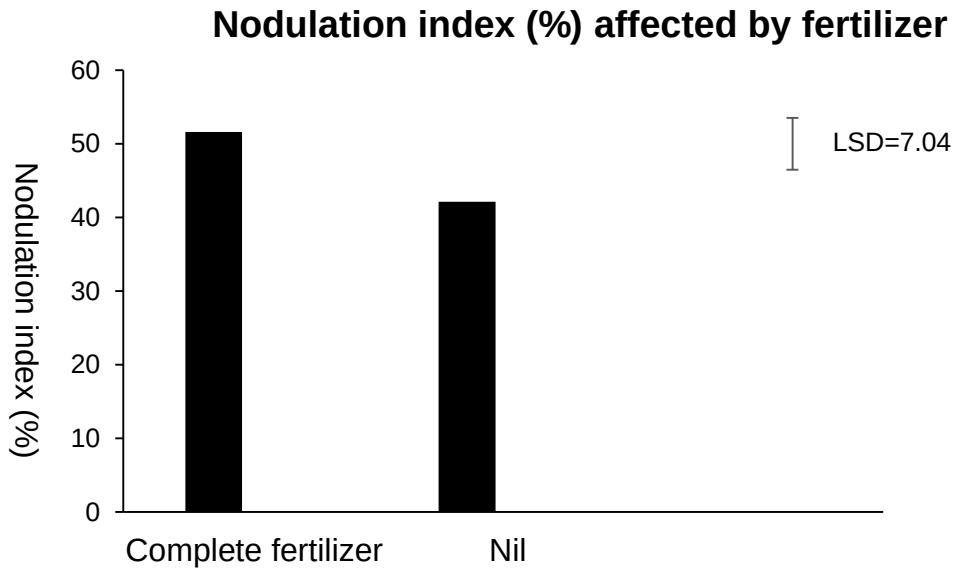

Root dry weight (mg/p) affected by varieties
Root dry weight (mg/p)
16
14
12
10
8
6
4
2
0
LSD=5.28
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

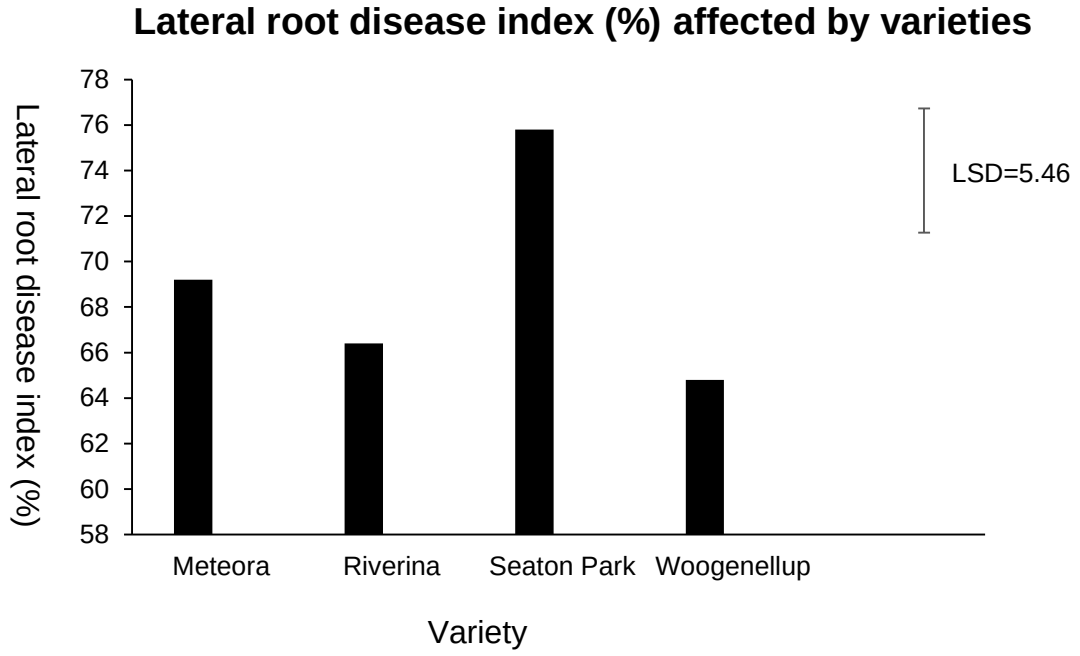

Shoot dry weight (mg/p) affected by varieties
Shoot dry weight (mg/p)
350
300
250
200
150
100
50
0
LSD=119.30
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

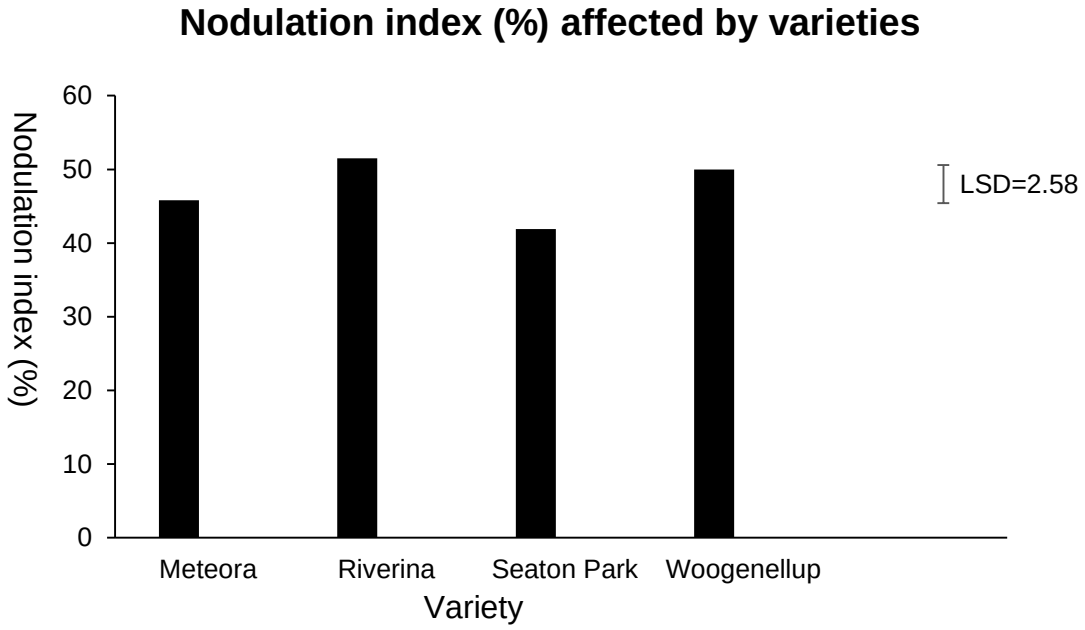
Second sampling results:

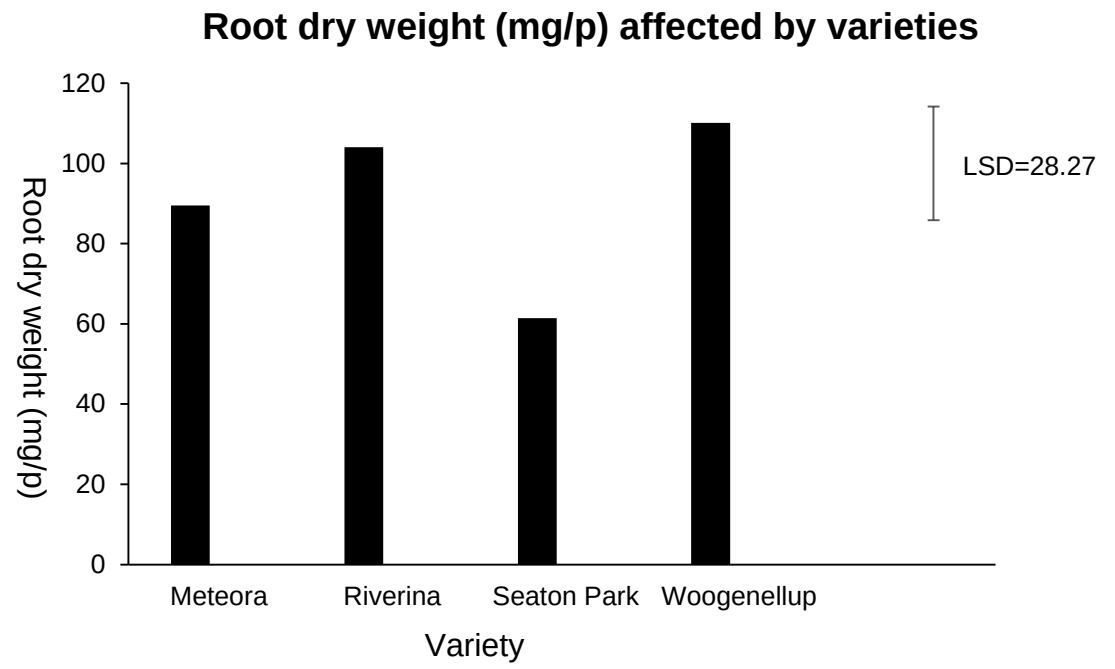
Second sampling statistical main effects of grazing, fungicide sprays, fertilizer, seed treatment and variety on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI %) and root and shoot dry weights (RDW and SDW) (mg/p)

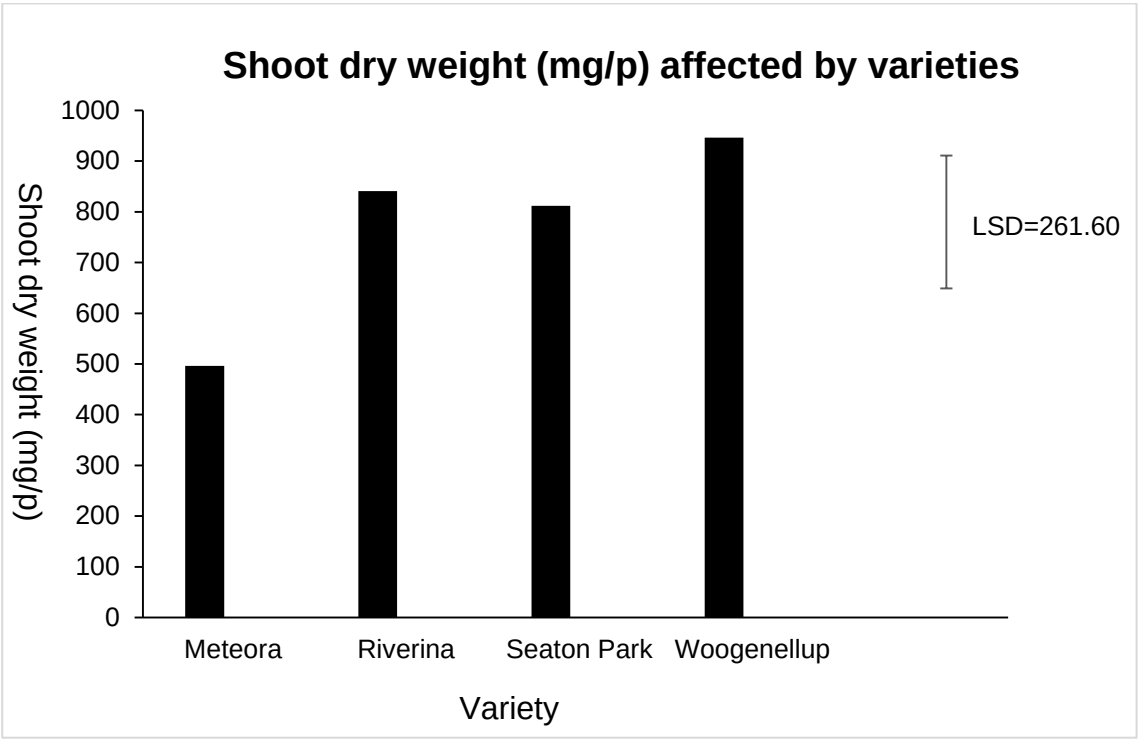
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Grazing	NS	*	NS	*	NS	*	NS	*	NS	*
Fertilizer	NS	*	NS	*	0.014	7.04	NS	*	NS	*
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Variety	NS	*	0.002	5.46	0.003	5.18	0.01	28.27	0.01	261.6




Lateral root disease index (%) affected by varieties
Lateral root disease index (%)
78
76
74
72
70
68
66
64
62
60
58
LSD=5.46
Meteora
Riverina
Seaton Park
Woogenellup
Variety


Nodulation index (%) affected by varieties
Nodulation index (%)
60
50
40
30
20
10
0
LSD=2.58
Meteora
Riverina
Seaton Park
Woogenellup
Variety


Root dry weight (mg/p) affected by varieties
120
100
80
60
40
20
0
Root dry weight (mg/p)
LSD=28.27
Meteora
Riverina
Seaton Park
Woogenellup
Variety

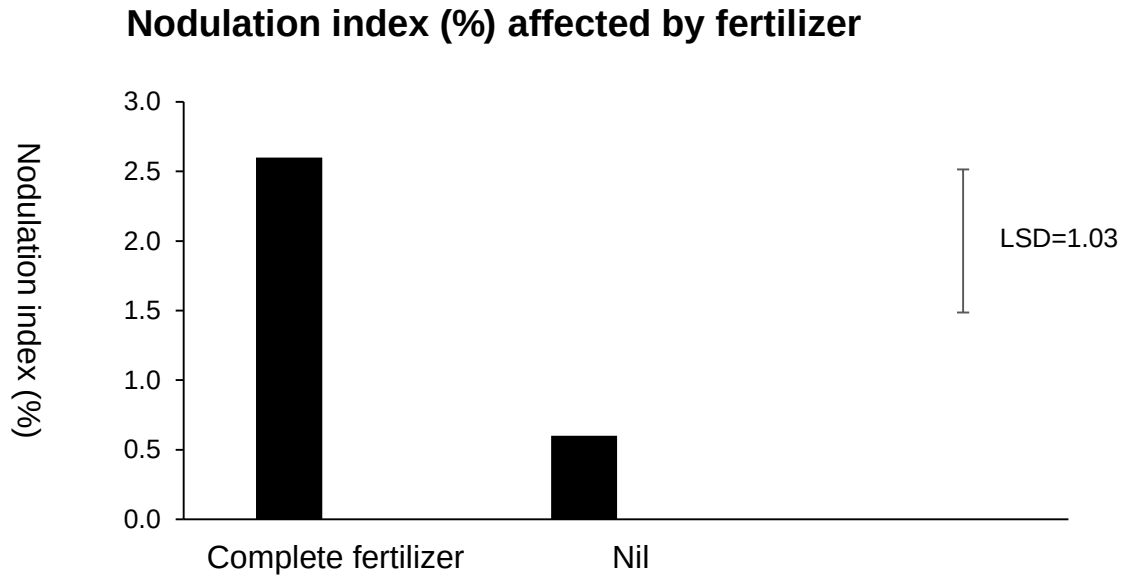

Shoot dry weight (mg/p) affected by varieties
1000
900
800
700
600
500
400
300
200
100
0
Shoot dry weight (mg/p)
LSD=261.60
Meteora
Riverina
Seaton Park
Woogenellup
Variety

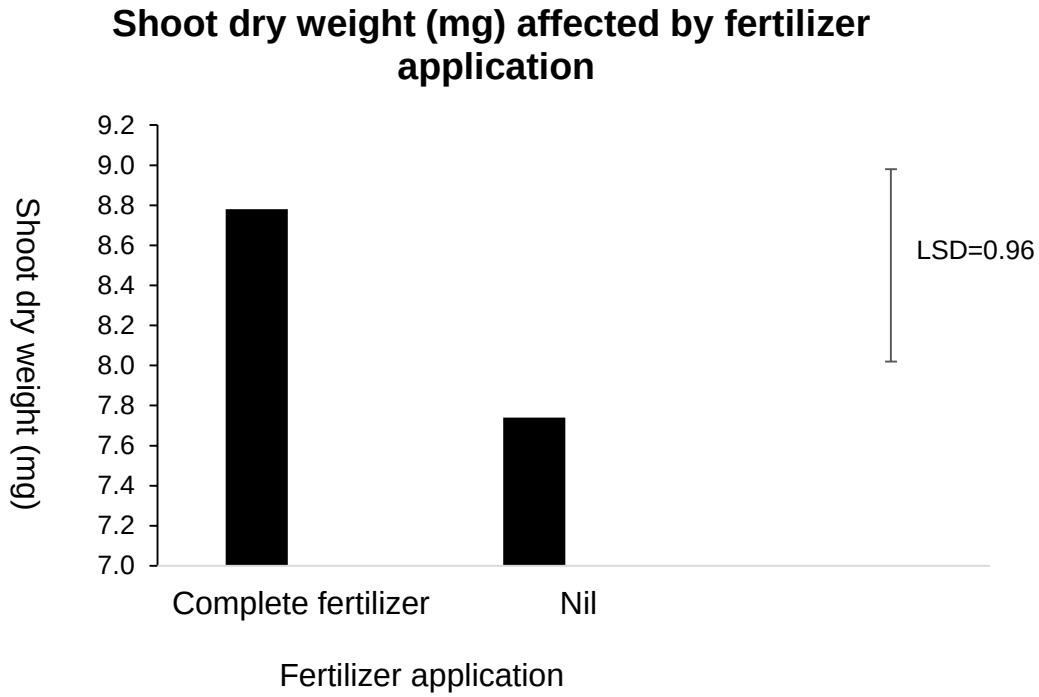
APPENDIX 4 – 2015 Bendigo region Victoria: First and second sampling results

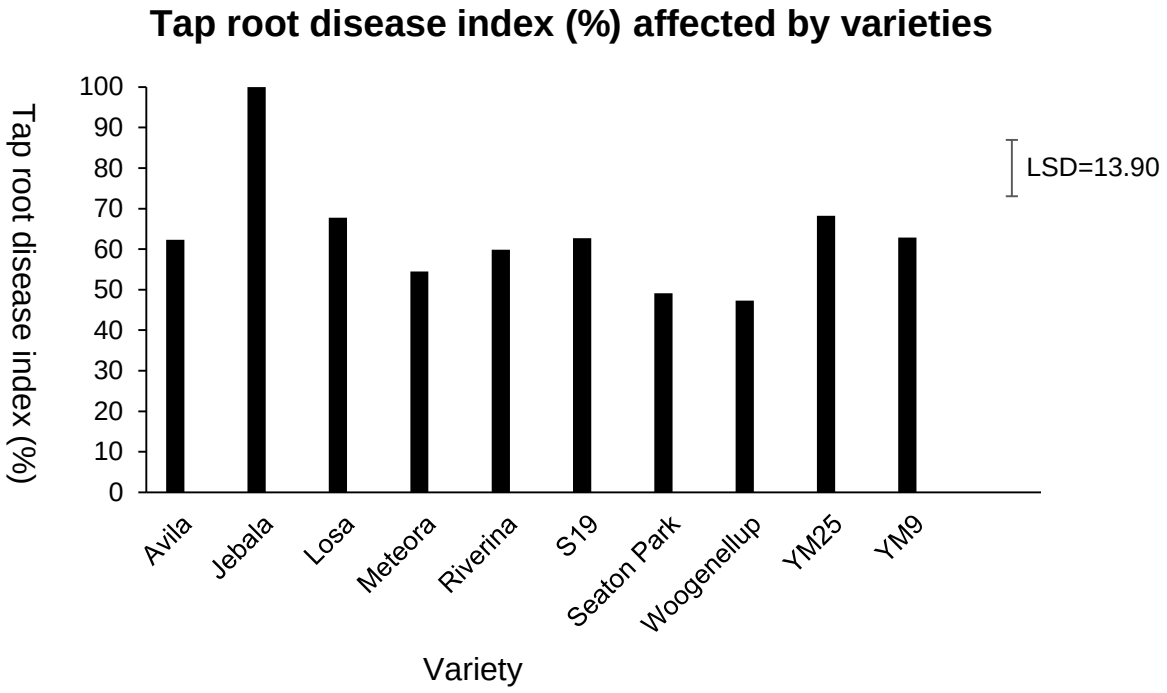
First sampling results:

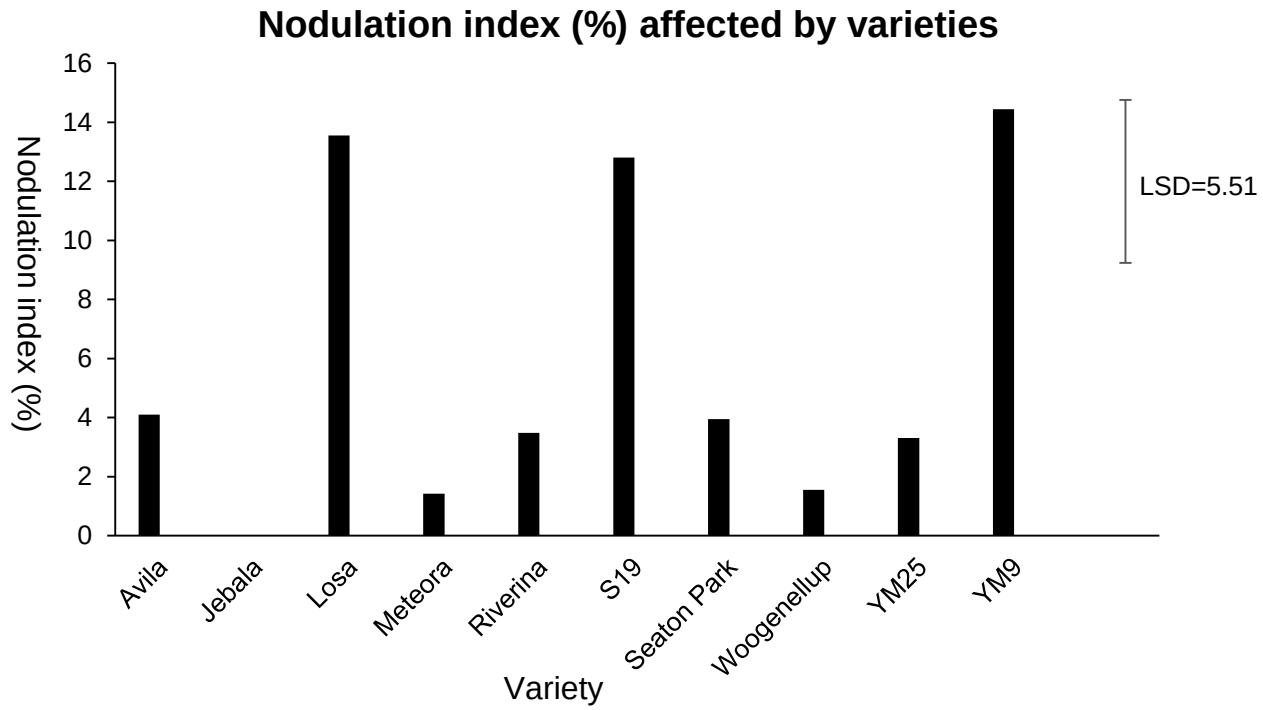
First sampling statistical main effects of fertilizer, variety and seed treatment on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI %), root and shoot dry weights (RDW and SDW) (mg/p) and germination rate (Gr%)

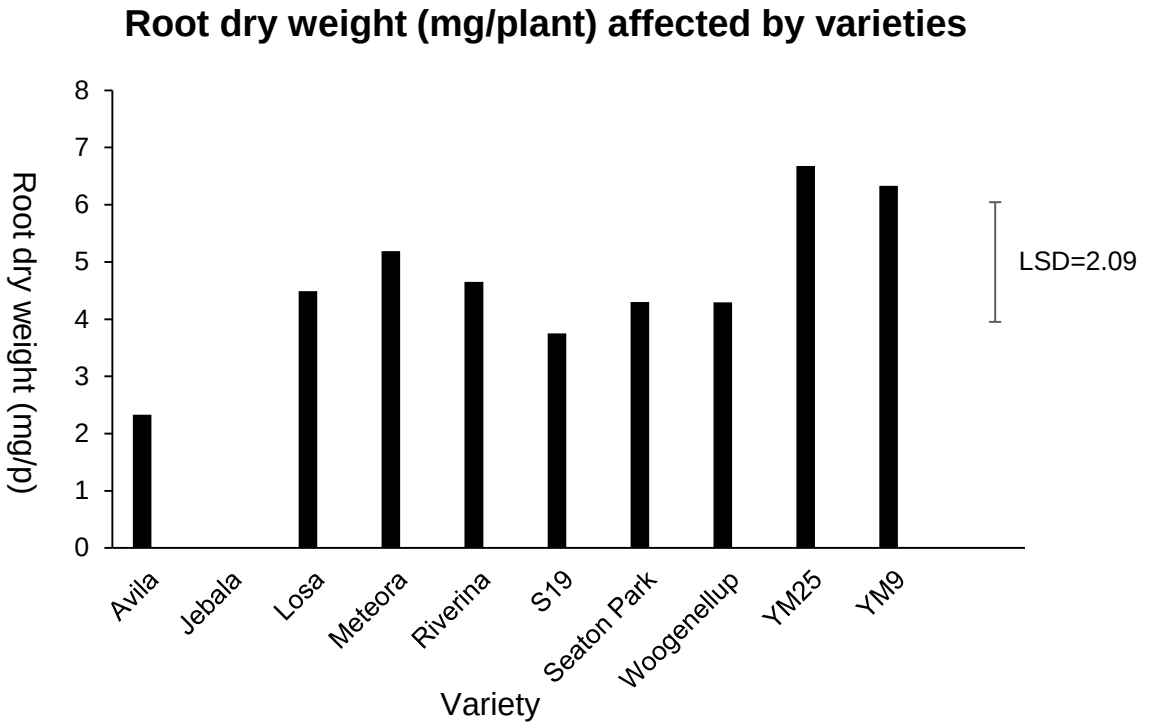
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr %	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.06}$	$LSD_{0.06}$
Fertilizer	NS	*	NS	*	0.002	1.03	NS	*	0.038	0.96	NS	*
Variety	0.035	13.9	NS	*	<0.001	5.513	0.018	2.094	< 0.001	2.329	0,0.001	8.459
Seed treatment	0.039	5.79	NS	*	NS	*	NS	*	NS	*	NS	*

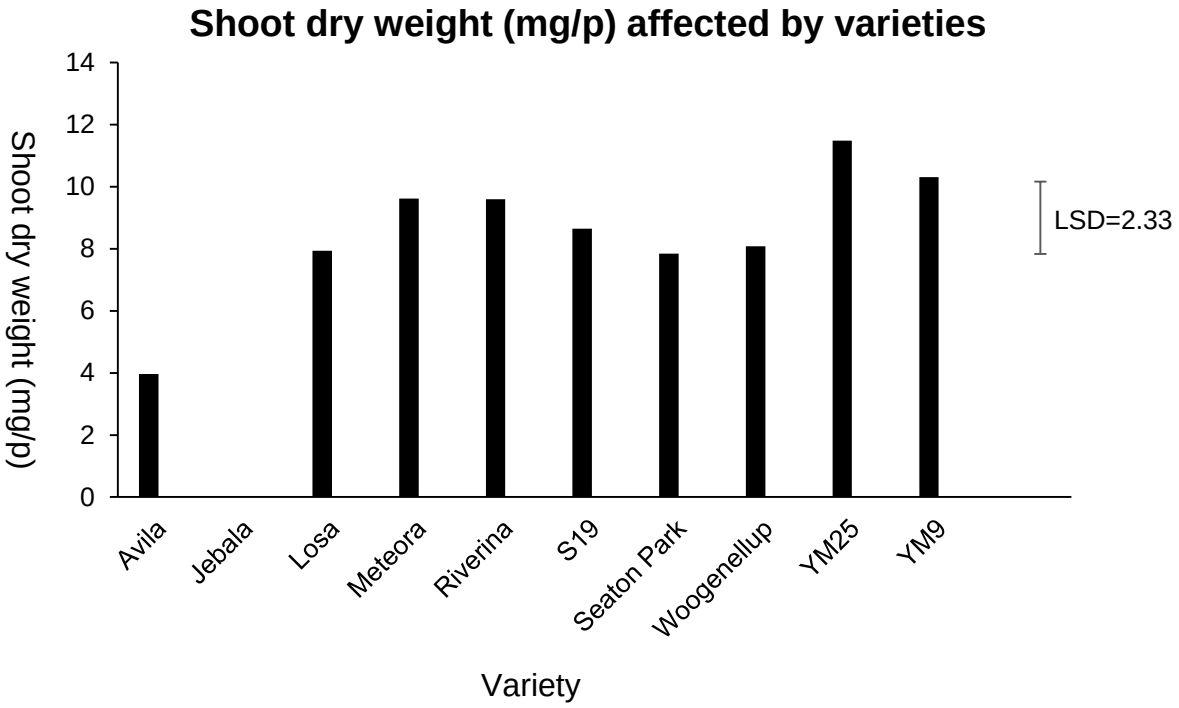

Nodulation index (%) affected by fertilizer
Nodulation index (%)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
LSD=1.03
Complete fertilizer
Nil

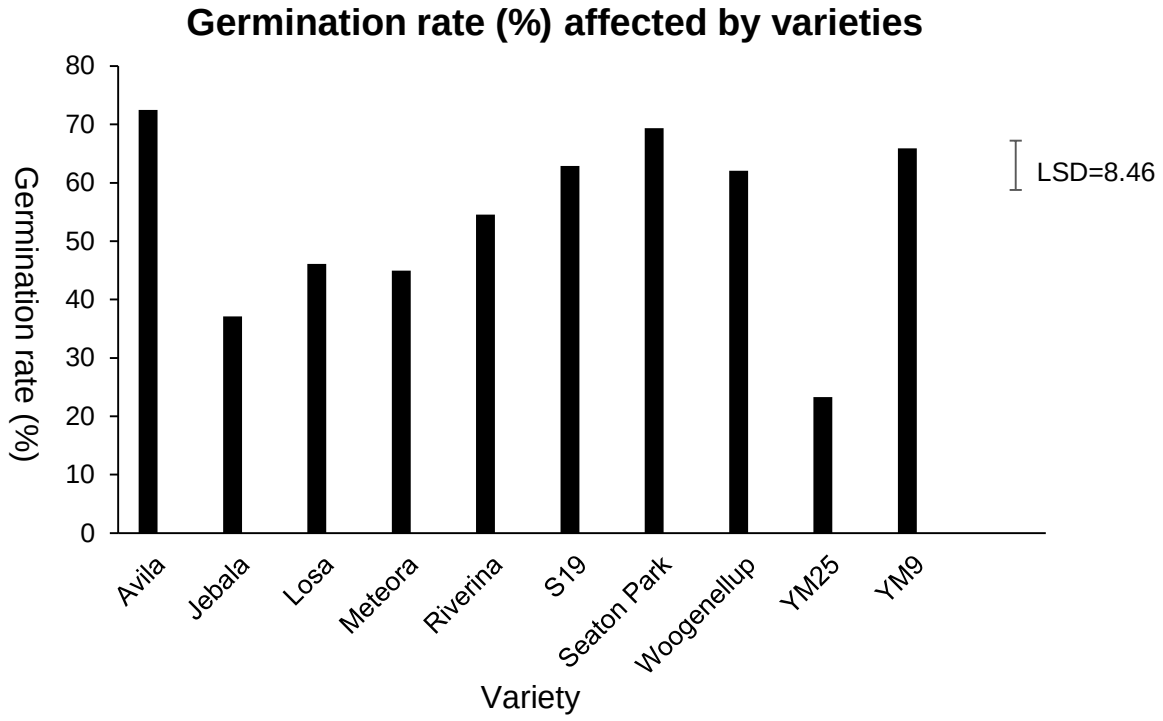

Shoot dry weight (mg) affected by fertilizer application
Shoot dry weight (mg)
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
LSD=0.96
Complete fertilizer
Nil
Fertilizer application

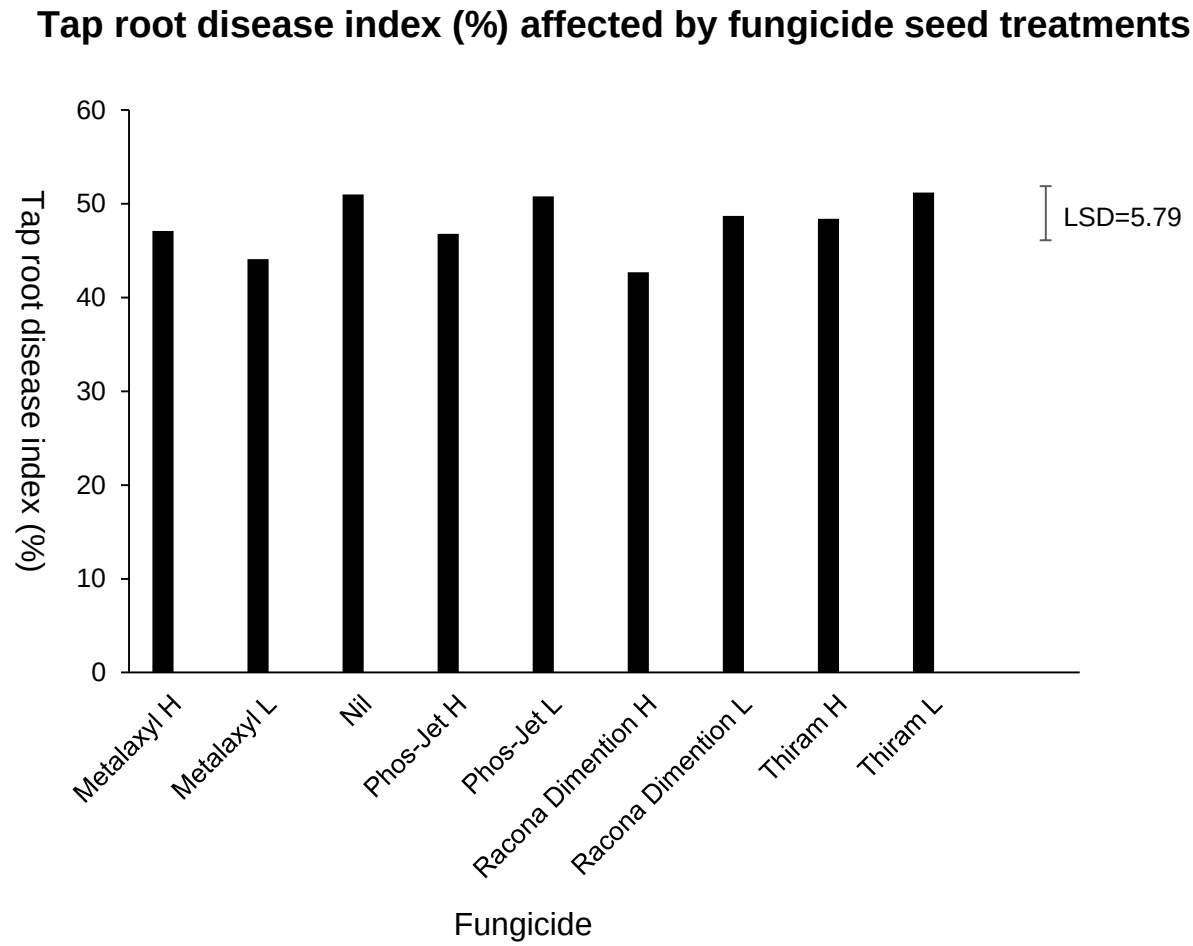

Tap root disease index (%) affected by varieties
Tap root disease index (%)
100
90
80
70
60
50
40
30
20
10
0
LSD=13.90
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Nodulation index (%) affected by varieties
Nodulation index (%)
16
14
12
10
8
6
4
2
0
LSD=5.51
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Root dry weight (mg/plant) affected by varieties
Root dry weight (mg/p)
0
1
2
3
4
5
6
7
8
LSD=2.09
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Shoot dry weight (mg/p) affected by varieties
Shoot dry weight (mg/p)
0
2
4
6
8
10
12
14
LSD=2.33
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

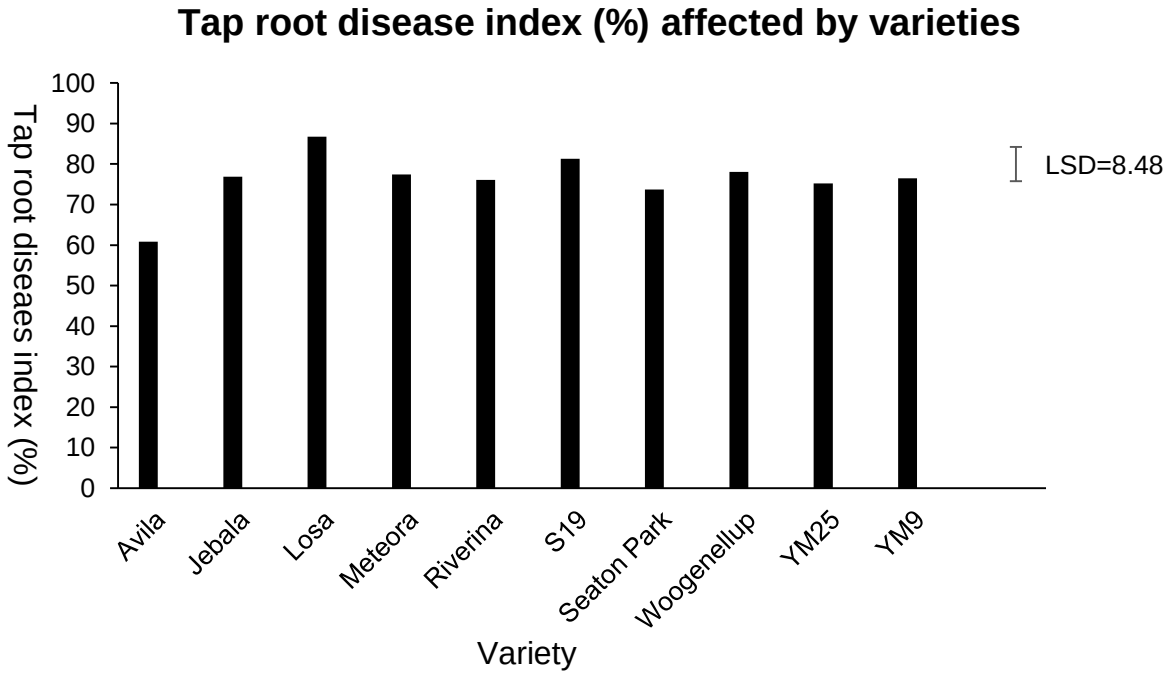

Germination rate (%) affected by varieties
Germination rate (%)
80
70
60
50
40
30
20
10
0
LSD=8.46
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

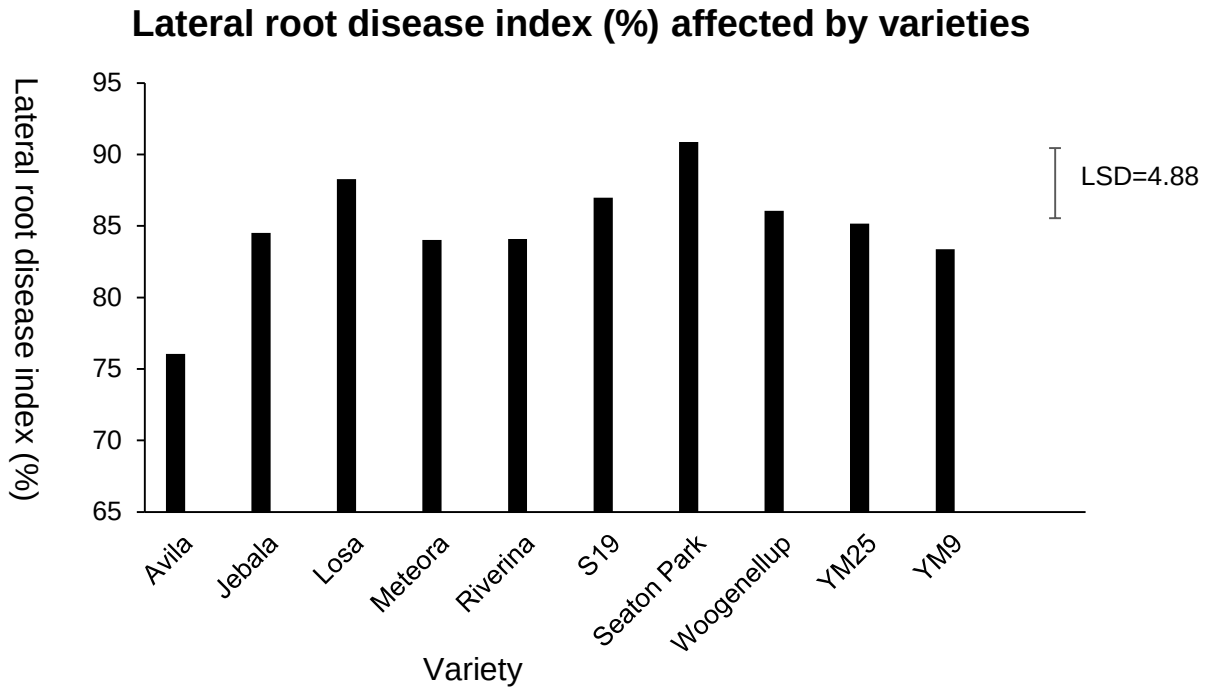

Tap root disease index (%) affected by fungicide seed treatments
Tap root disease index (%)
60
50
40
30
20
10
0
LSD=5.79
Metalaxyl H
Metalaxyl L
Nil
Phos-Jet H
Phos-Jet L
Racona Dimention H
Racona Dimention L
Thiram H
Thiram L
Fungicide

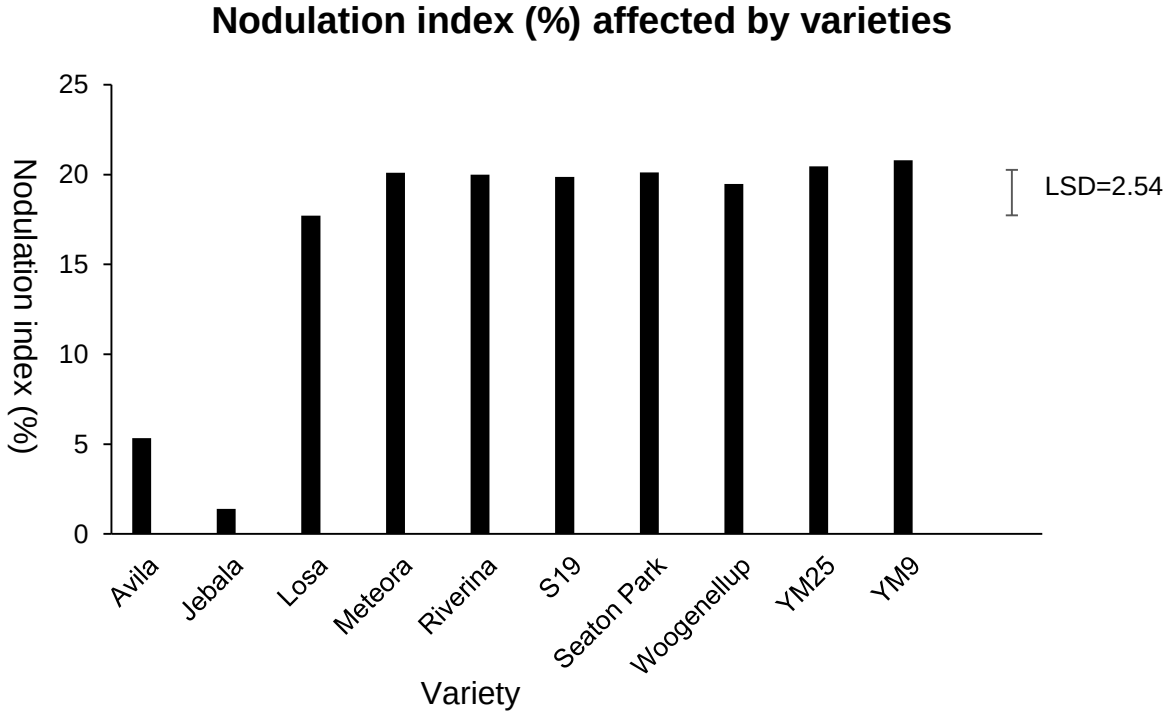
Second sampling results:

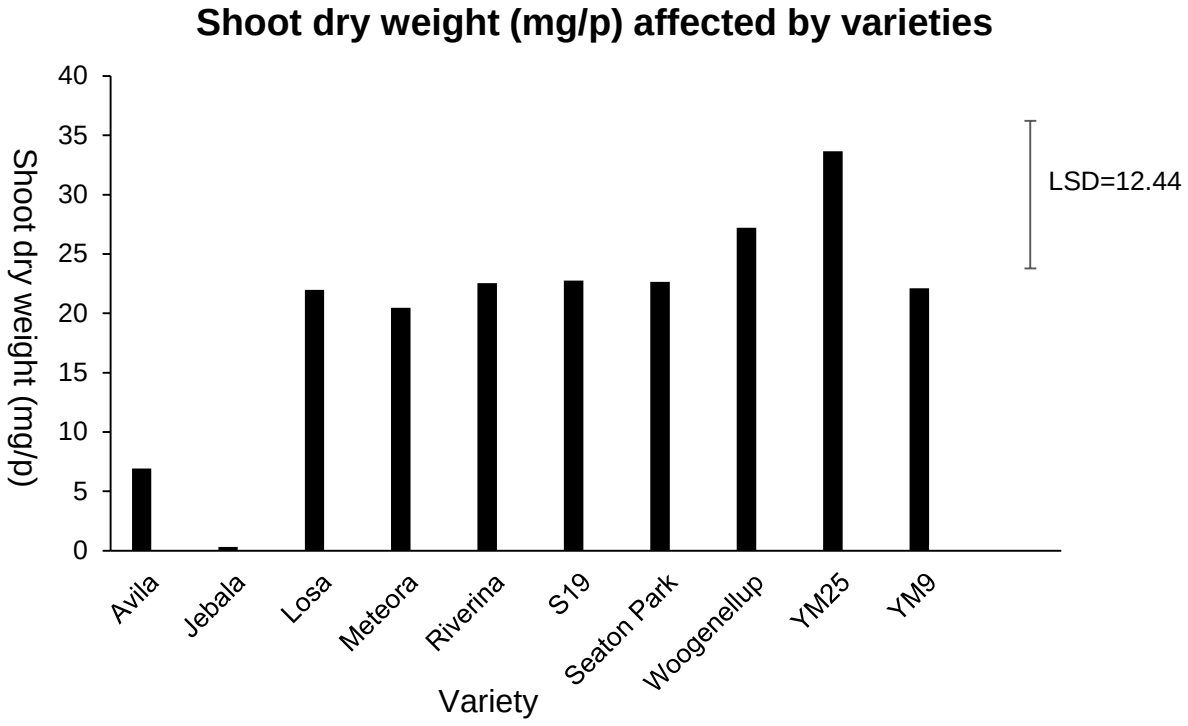
Second sampling statistical main effects of variety, seed treatment, sprays (treatment and field), fertilizer (treatment and field), grazing and cultivation on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI %), and root and shoot dry weights (RDW and SDW) (mg/p)

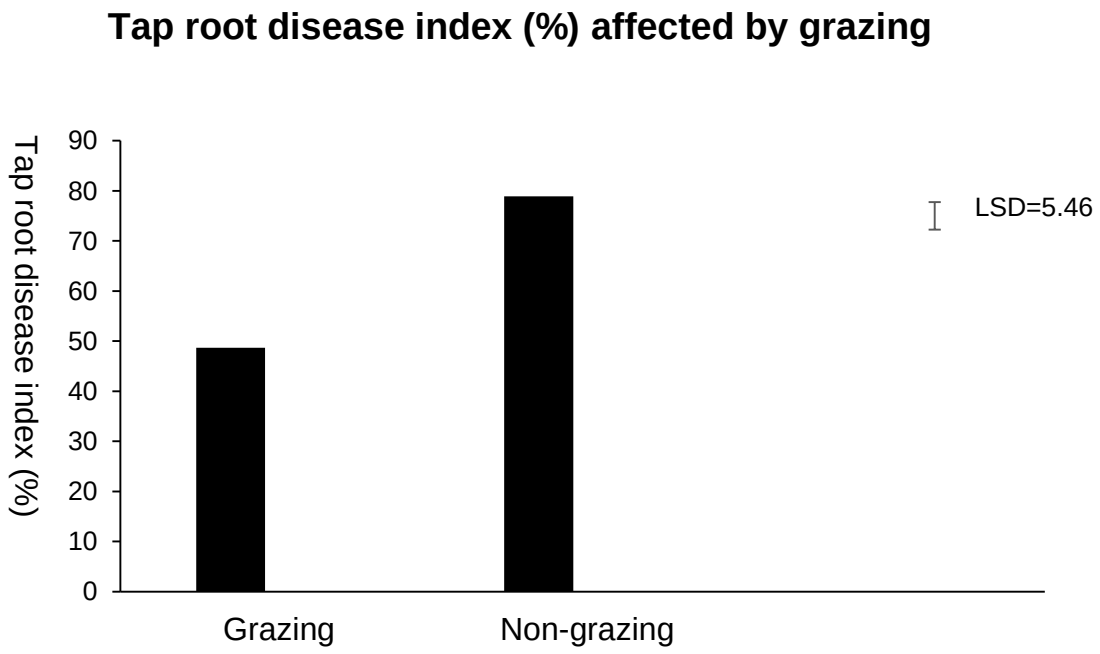
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	< 0.001	8.477	< 0.001	4.884	< 0.001	2.54	NS	*	< 0.001	12.44
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Spray treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Fertilizer	NS	*	NS	*	NS	*	NS	*	NS	*
Grazing	<0.001	5.46	0.002	6.54	NS	*	NS	*	NS	*
Field fertilizer	NS	*	0.006	12.41						
Field spray	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	0.043	10.14	0.023	8.48	NS	*	NS	*	NS	*

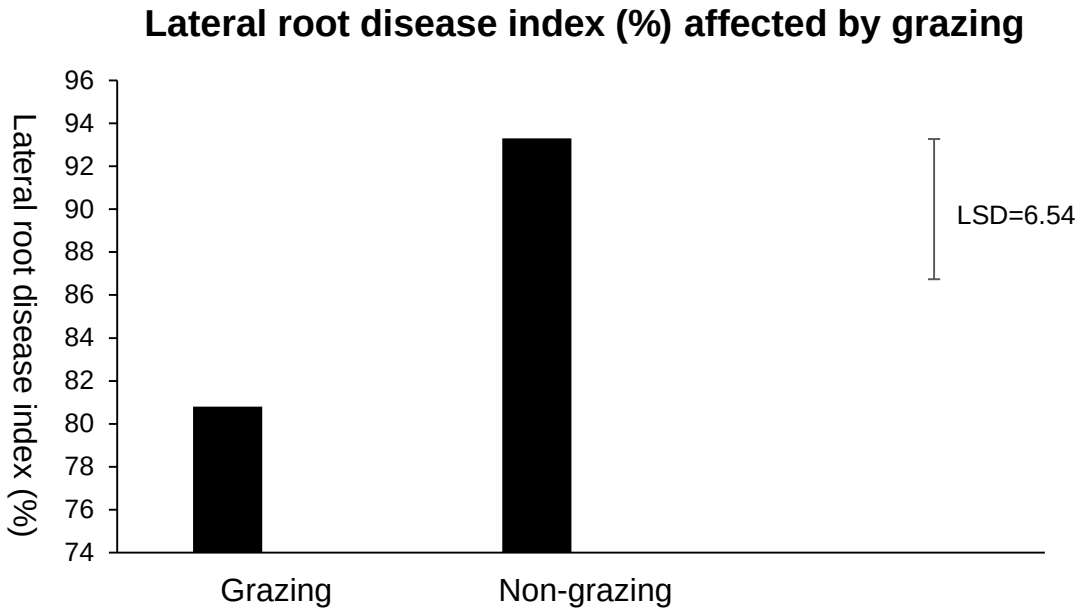


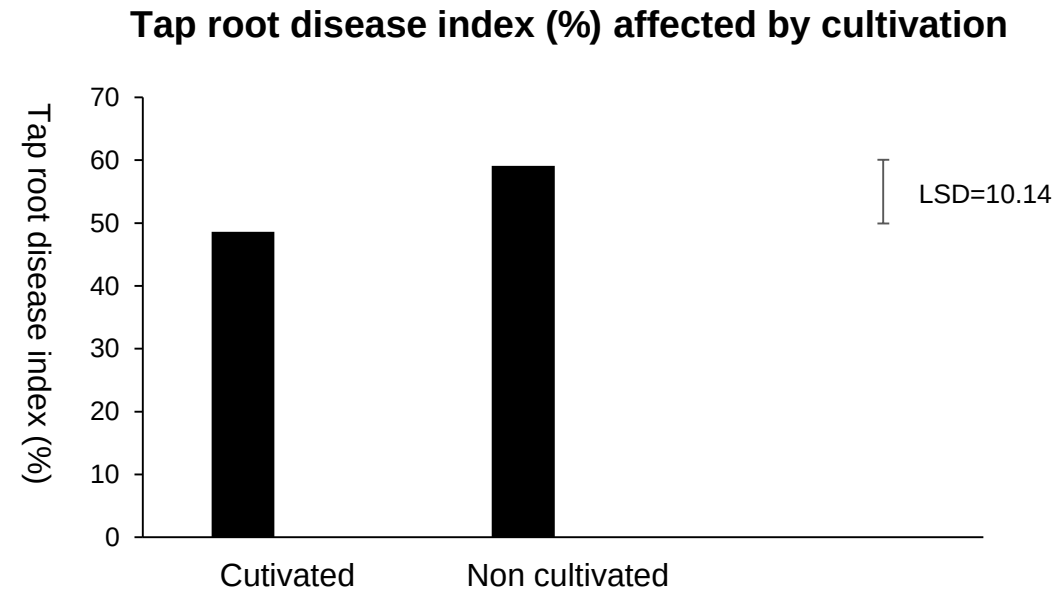

Lateral root disease index (%) affected by varieties
Lateral root disease index (%)
95
90
85
80
75
70
65
LSD=4.88
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

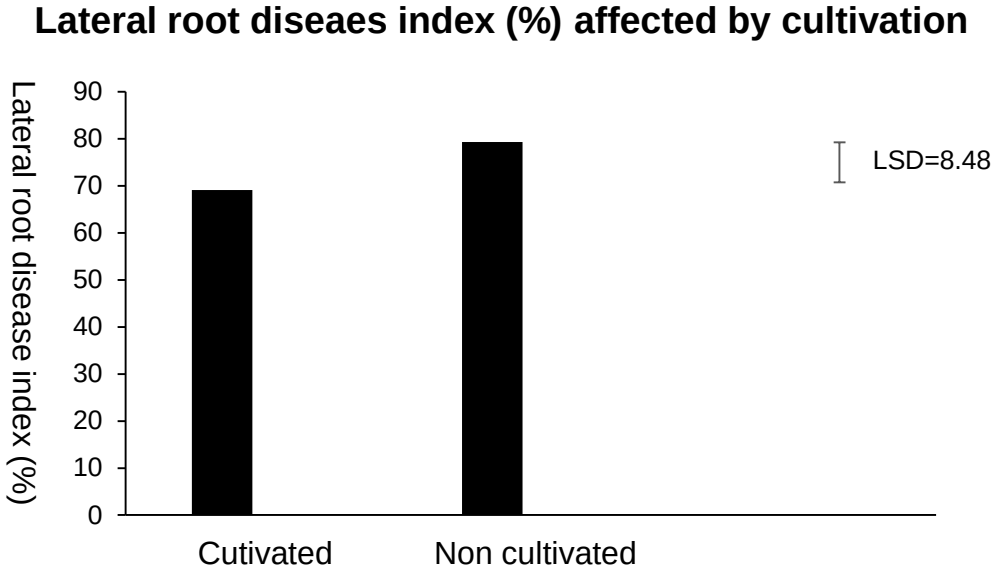

Nodulation index (%) affected by varieties
Nodulation index (%)
25
20
15
10
5
0
LSD=2.54
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Shoot dry weight (mg/p) affected by varieties
Shoot dry weight (mg/p)
40
35
30
25
20
15
10
5
0
LSD=12.44
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Tap root disease index (%) affected by grazing
Tap root disease index (%)
90
80
70
60
50
40
30
20
10
0
LSD=5.46
Grazing
Non-grazing


Lateral root disease index (%) affected by grazing
Lateral root disease index (%)
96
94
92
90
88
86
84
82
80
78
76
74
LSD=6.54
Grazing
Non-grazing


Tap root disease index (%) affected by cultivation
Tap root disease index (%)
70
60
50
40
30
20
10
0
LSD=10.14
Cutivated
Non cultivated

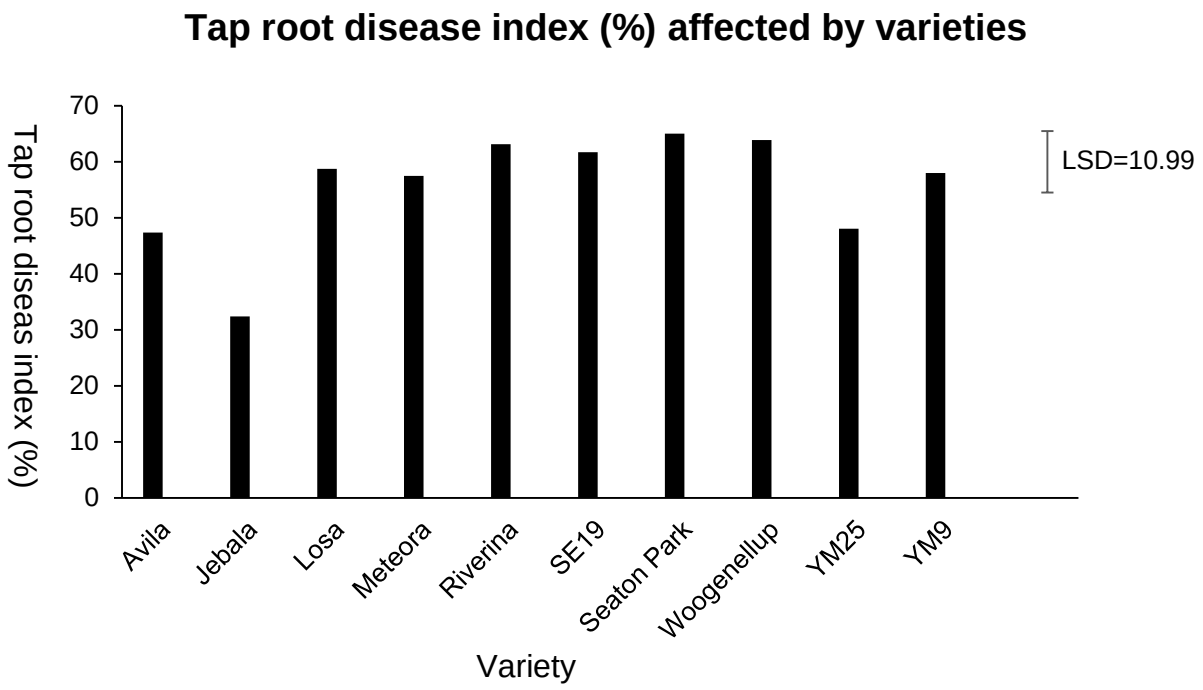

Lateral root diseaes index (%) affected by cultivation
Lateral root disease index (%)
90
80
70
60
50
40
30
20
10
0
LSD=8.48
Cutivated
Non cultivated

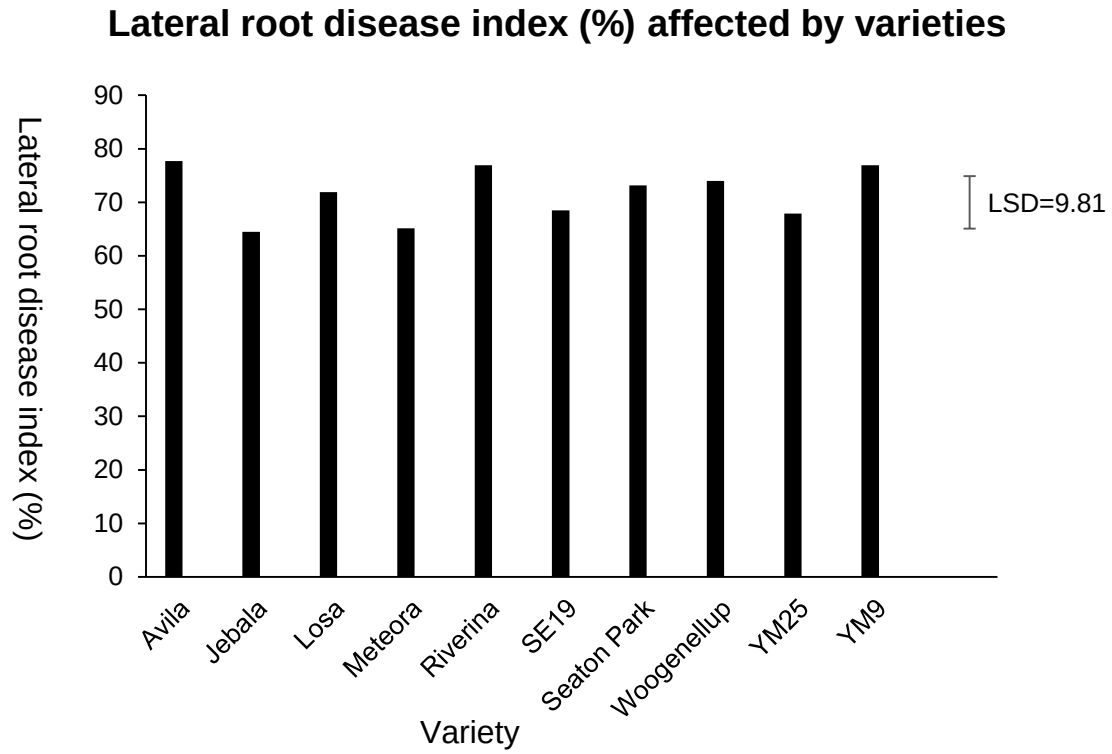
APPENDIX 5 – 2015 NSW/ACT: First and second sampling results

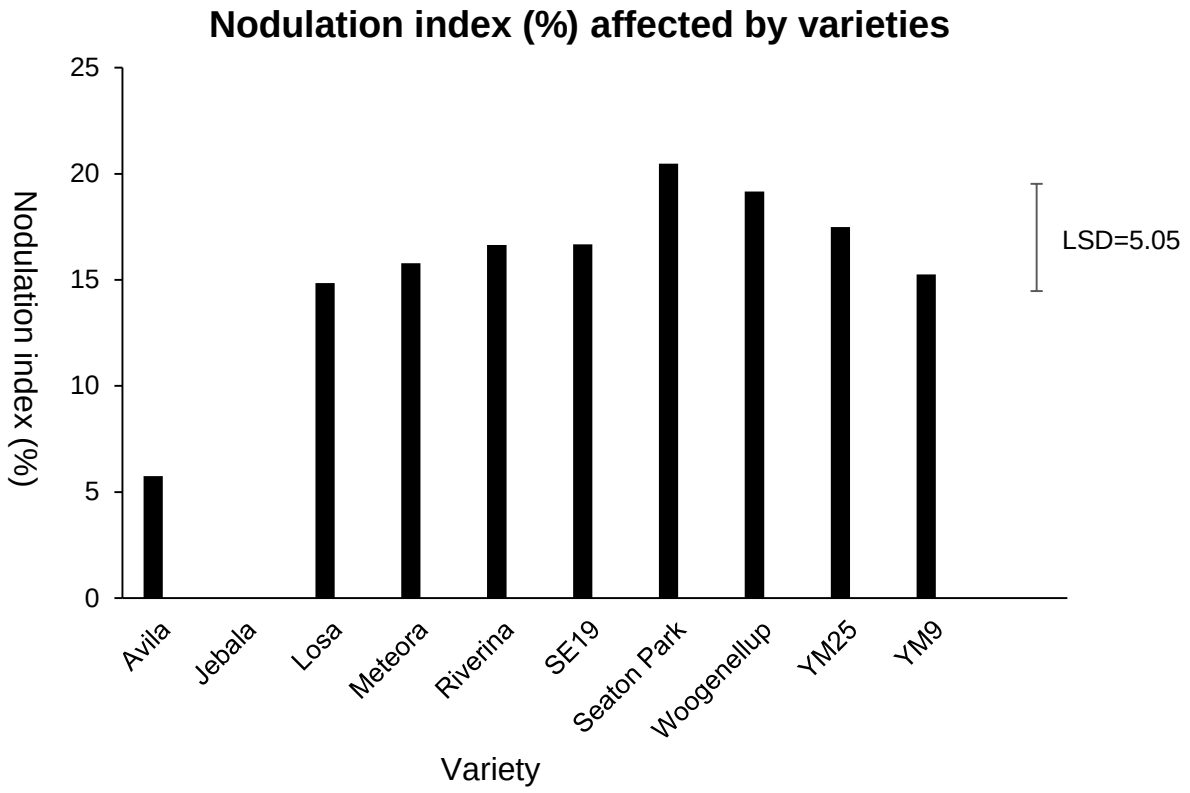
First sampling results:

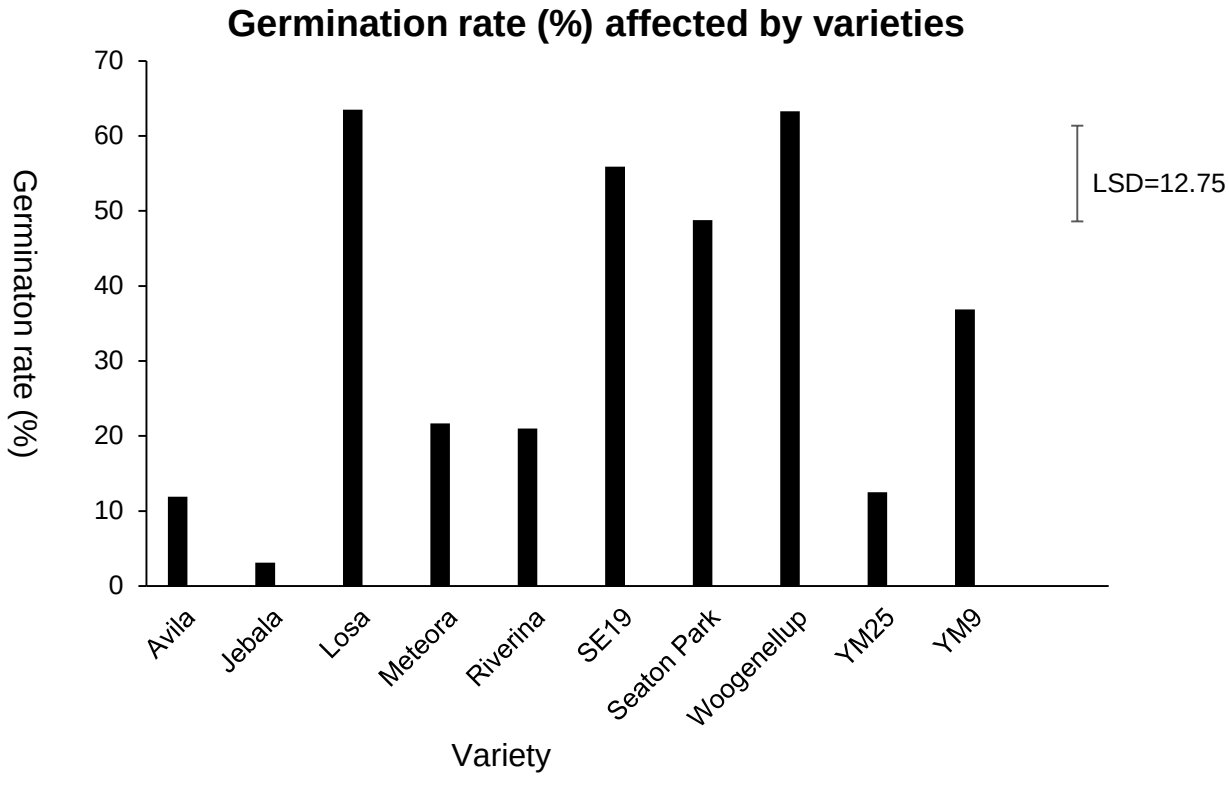
Statistical main effects of variety, seed treatment and fertilizer on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%) and root and shoot dry weights (RDW and SDW) (mg/p)

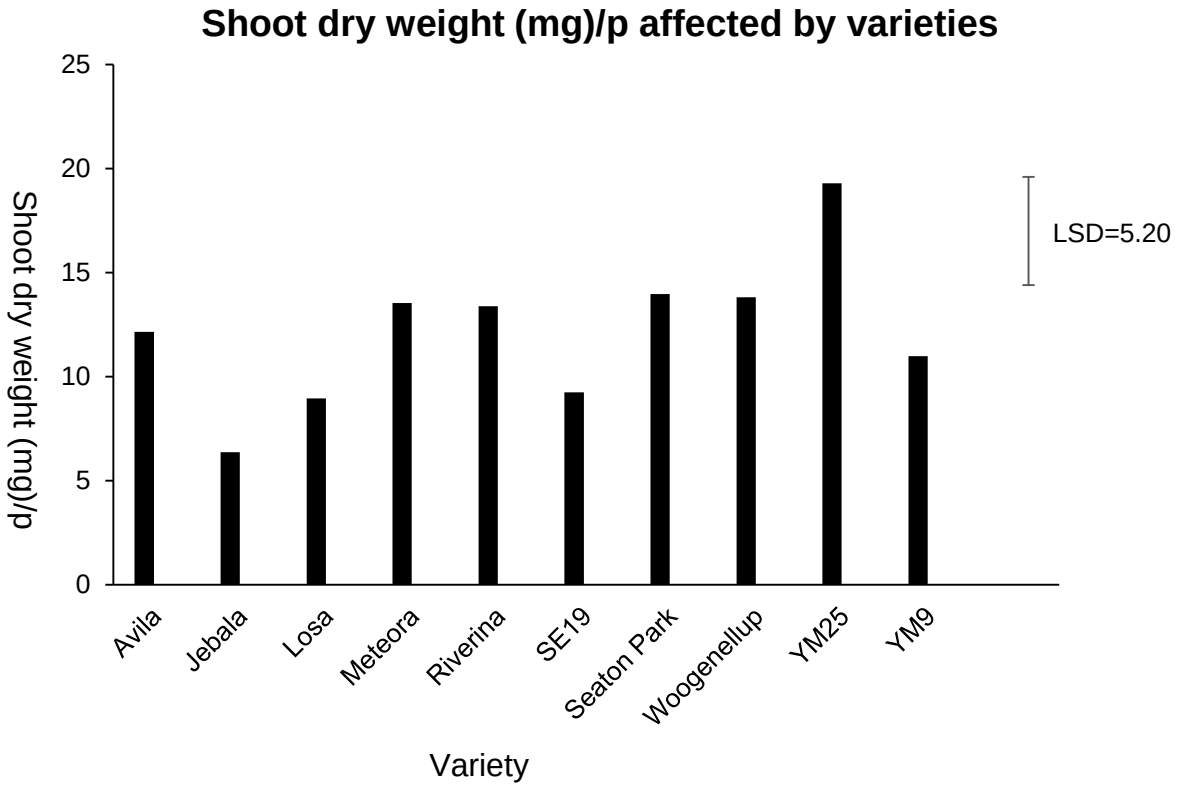
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		GR%	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	< 0.001	10.99	0.035	9.805	< 0.001	5.05	NS	*	0.006	5.203	< 0.001	12.75
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	0.009	7.566
Fertilizer	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*

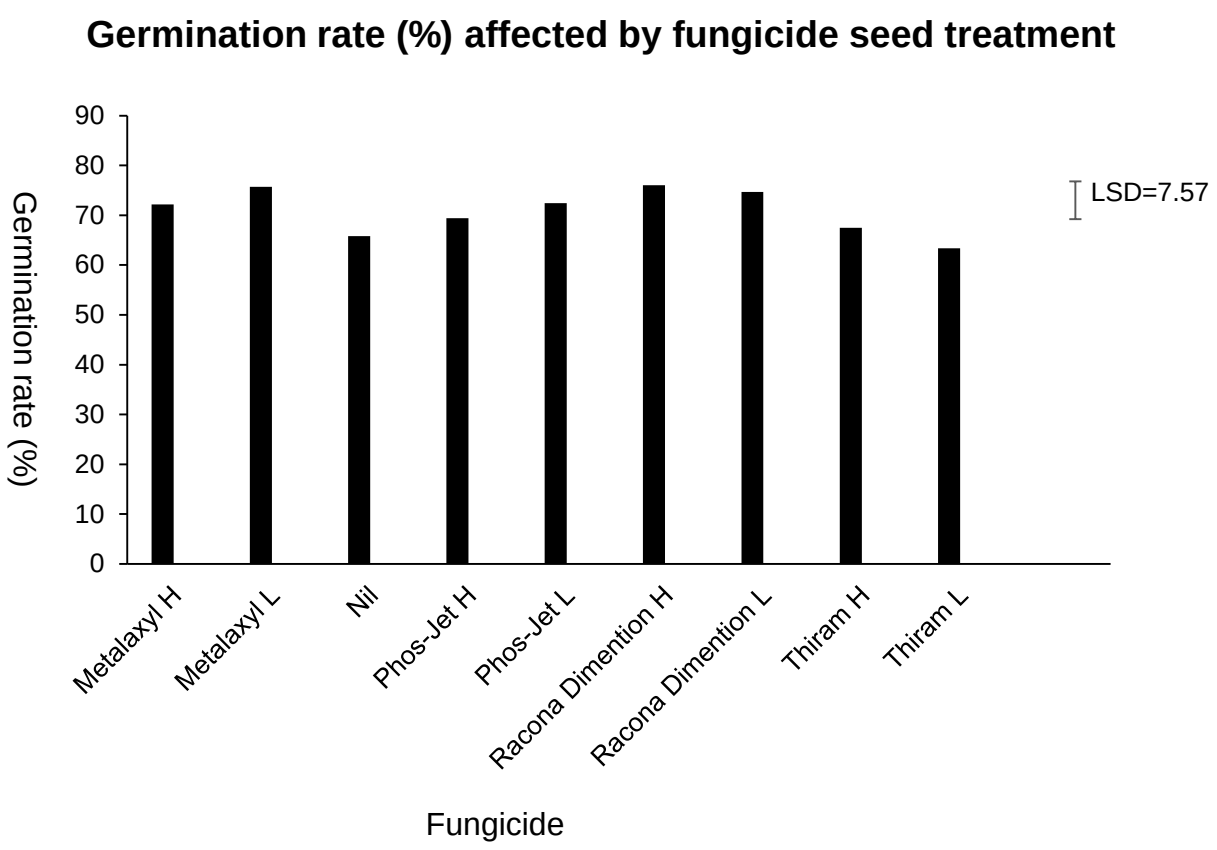

Tap root disease index (%) affected by varieties
Tap root diseas index (%)
70
60
50
40
30
20
10
0
LSD=10.99
Avila
Jebala
Losa
Meteora
Riverina
SE19
Seaton Park
Woogenellup
YM25
YM9
Variety


Lateral root disease index (%) affected by varieties
Lateral root disease index (%)
90
80
70
60
50
40
30
20
10
0
LSD=9.81
Avila
Jebala
Losa
Meteora
Riverina
SE19
Seaton Park
Woogenellup
YM25
YM9
Variety


Nodulation index (%) affected by varieties
Nodulation index (%)
25
20
15
10
5
0
LSD=5.05
Avila
Jebala
Losa
Meteora
Riverina
SE19
Seaton Park
Woogenellup
YM25
YM9
Variety


Germination rate (%) affected by varieties
Germinaton rate (%)
70
60
50
40
30
20
10
0
LSD=12.75
Avila
Jebala
Losa
Meteora
Riverina
SE19
Seaton Park
Woogenellup
YM25
YM9
Variety

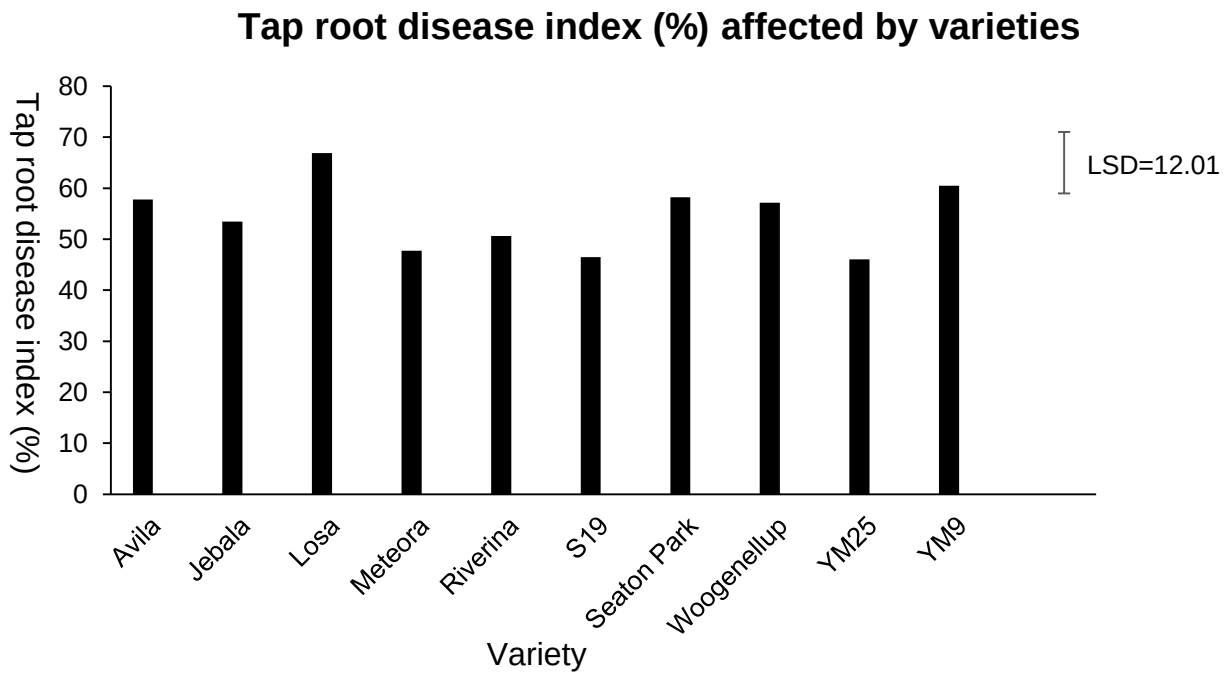

Shoot dry weight (mg)/p affected by varieties
Shoot dry weight (mg)/p
25
20
15
10
5
0
LSD=5.20
Avila
Jebala
Losa
Meteora
Riverina
SE19
Seaton Park
Woogenellup
YM25
YM9
Variety

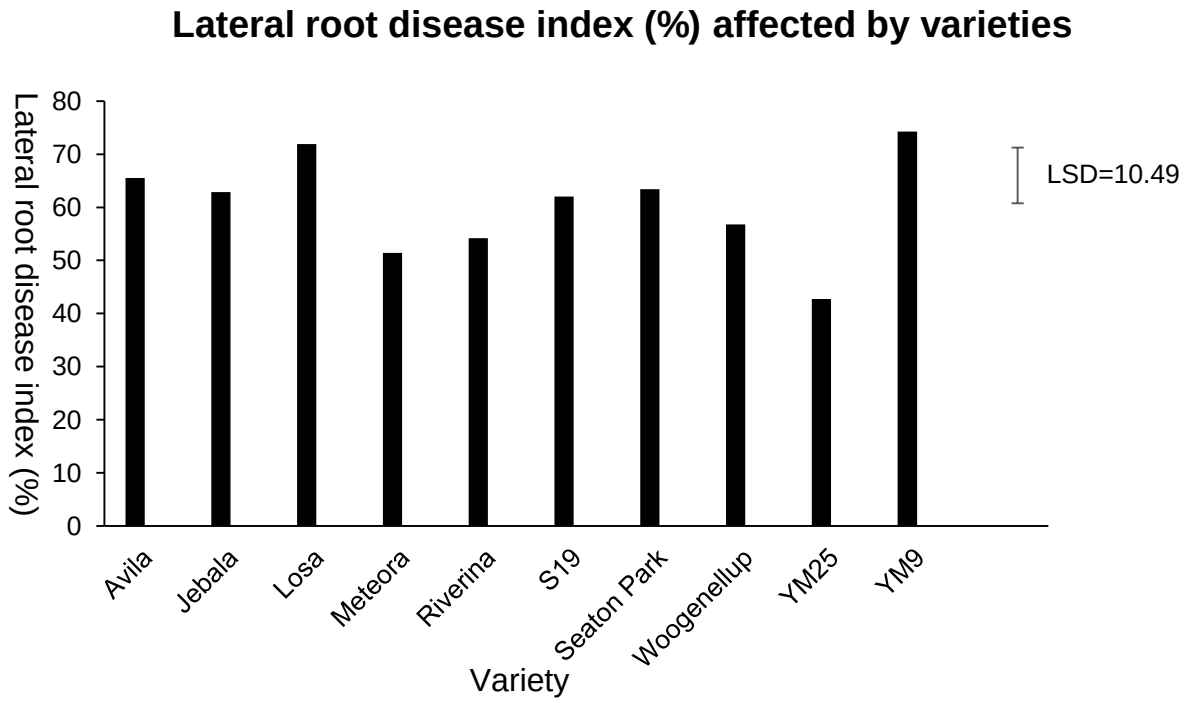

Germination rate (%) affected by fungicide seed treatment
Germination rate (%)
90
80
70
60
50
40
30
20
10
0
LSD=7.57
Metalaxyl H
Metalaxyl L
Nil
Phos-Jet H
Phos-Jet L
Racona Dimention H
Racona Dimention L
Thiram H
Thiram L
Fungicide

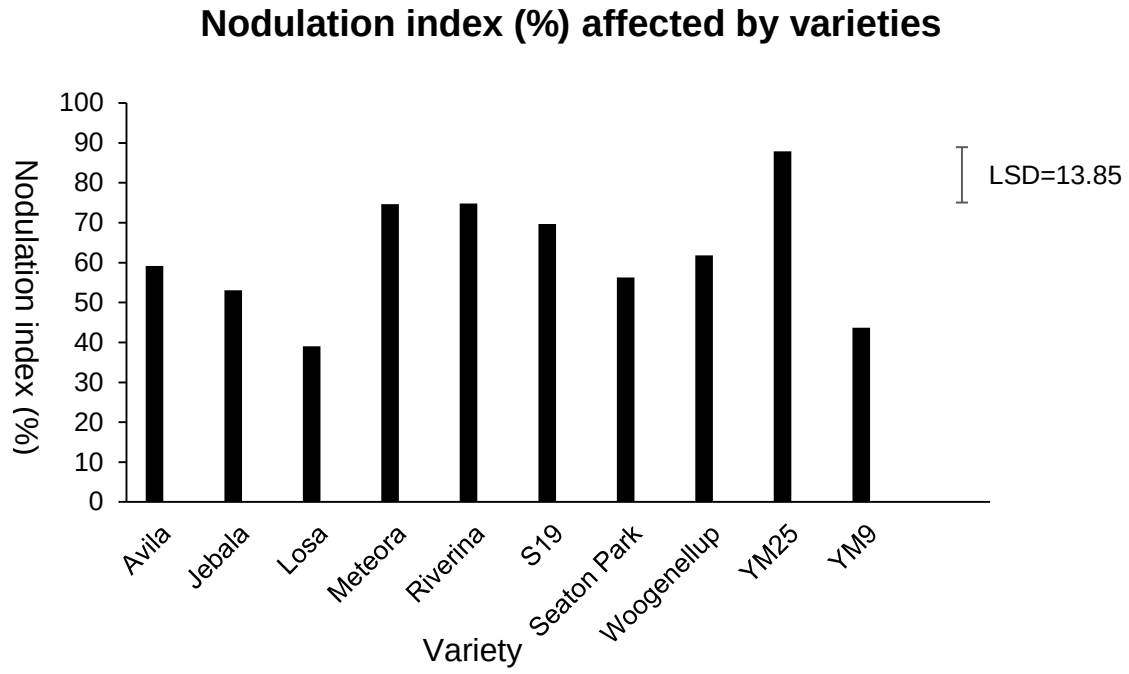
Second sampling results:

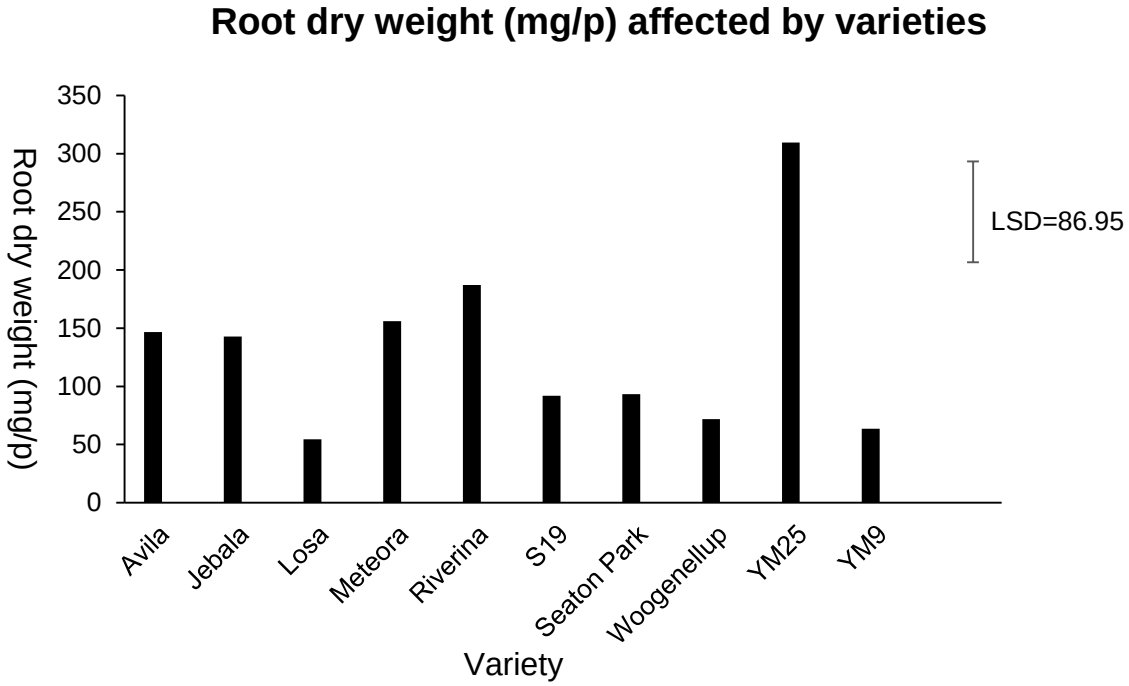
Statistical main effects Grazing, fertilizer, fungicide spray (field and treatment), seed treatment and variety on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%) and root and shoot dry weights (RDW and SDW) (mg/p)

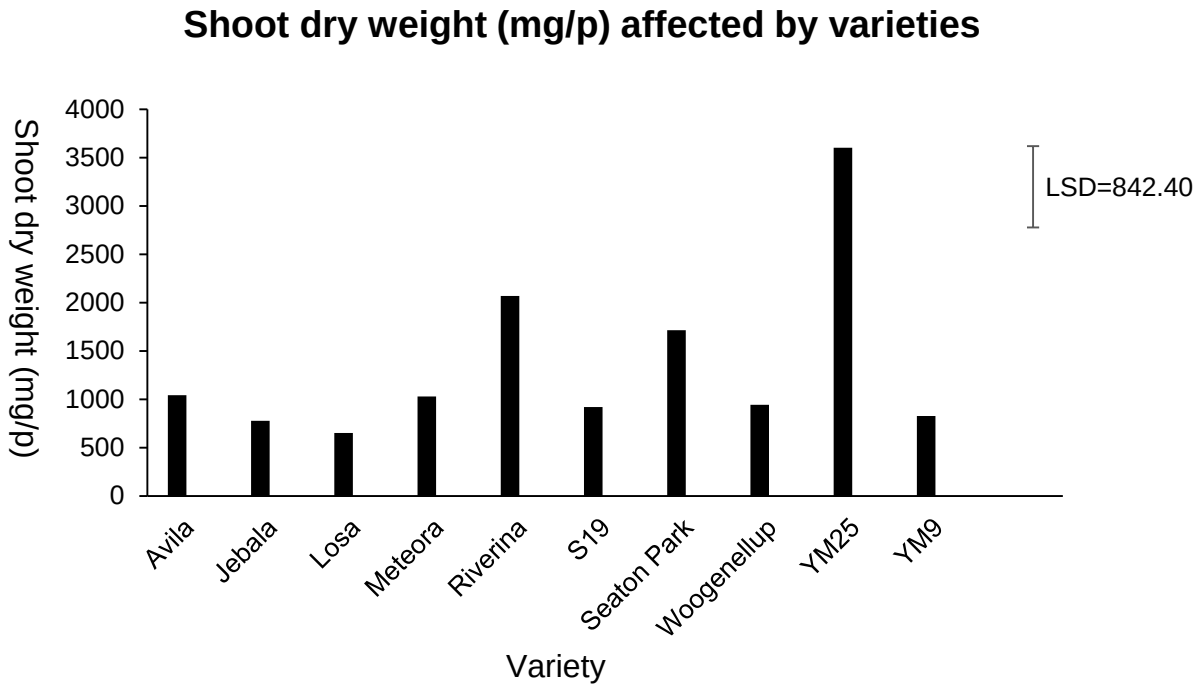
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Grazing	NS	*	NS	*	NS	*	NS	*	NS	*
Fertilizer	NS	*	NS	*	NS	*	NS	*	NS	*
Field spray	NS	*	NS	*	NS	*	NS	*	NS	*
Spray treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Variety	0.017	12.01	< 0.001	10.49	< 0.001	13.85	< 0.001	86.95	< 0.001	842.4


Tap root disease index (%) affected by varieties
Tap root disease index (%)
80
70
60
50
40
30
20
10
0
LSD=12.01
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Lateral root disease index (%) affected by varieties
Lateral root disease index (%)
80
70
60
50
40
30
20
10
0
LSD=10.49
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Nodulation index (%) affected by varieties
Nodulation index (%)
100
90
80
70
60
50
40
30
20
10
0
LSD=13.85
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety


Root dry weight (mg/p) affected by varieties
Root dry weight (mg/p)
350
300
250
200
150
100
50
0
LSD=86.95
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

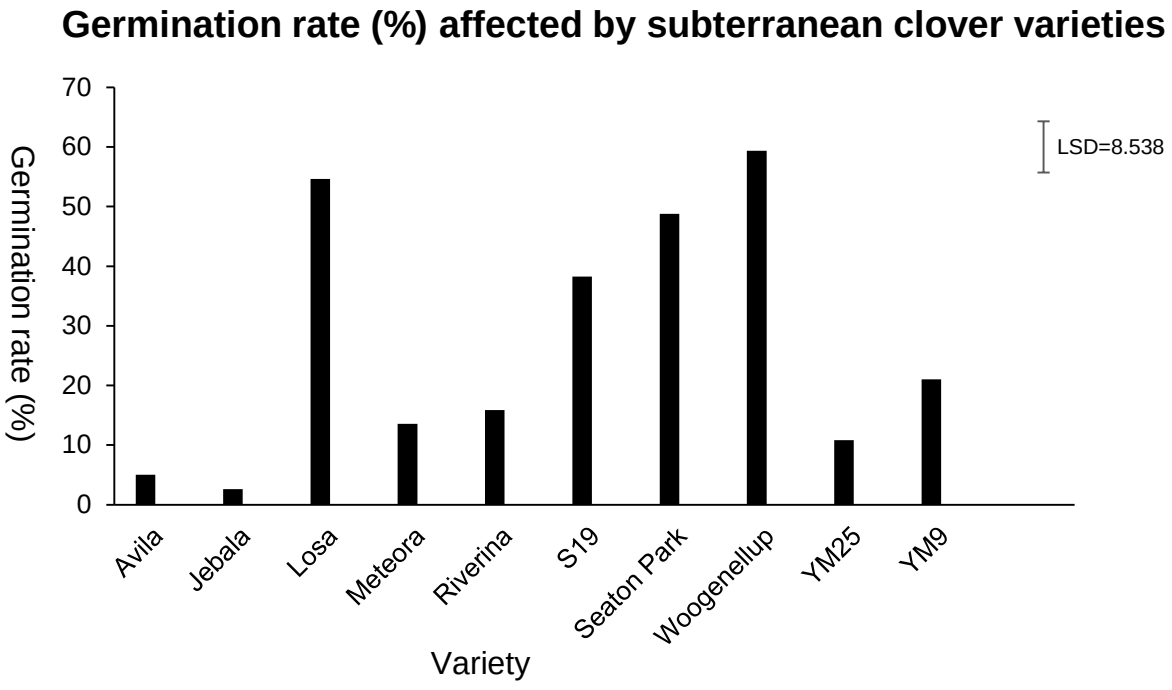

Shoot dry weight (mg/p) affected by varieties
Shoot dry weight (mg/p)
4000
3500
3000
2500
2000
1500
1000
500
0
LSD=842.40
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

APPENDIX 6 – 2015 NSW: First and second sampling results

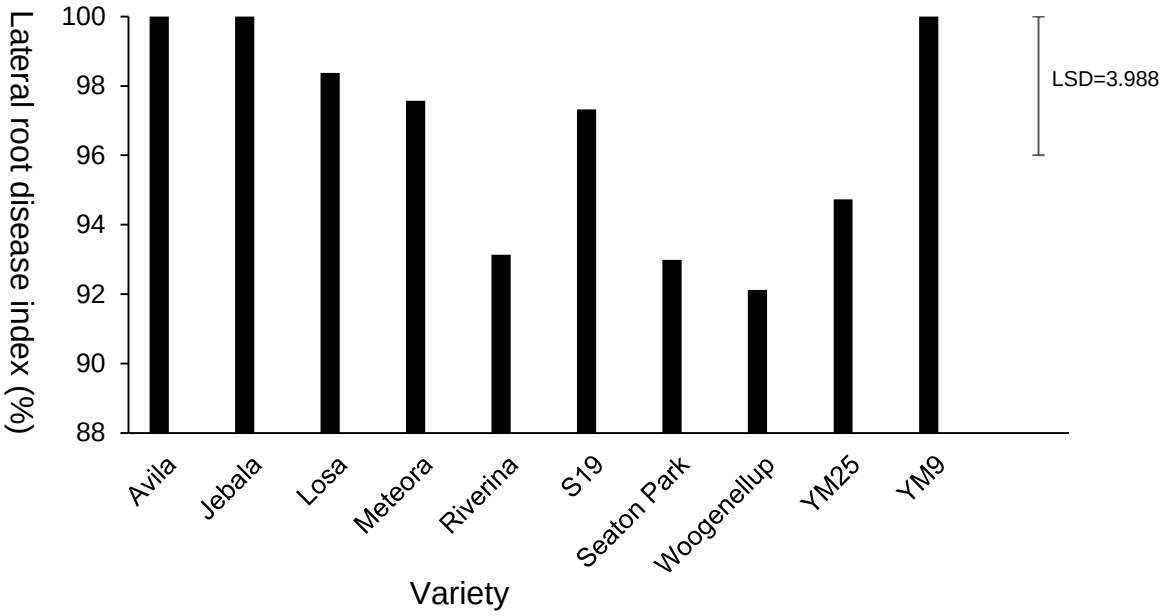
First sampling results:

Statistical main effects of fertilizer, variety and seed treatment on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%), germination rate (Ger%) and root and shoot dry weights (RDW and SDW) (mg/p)

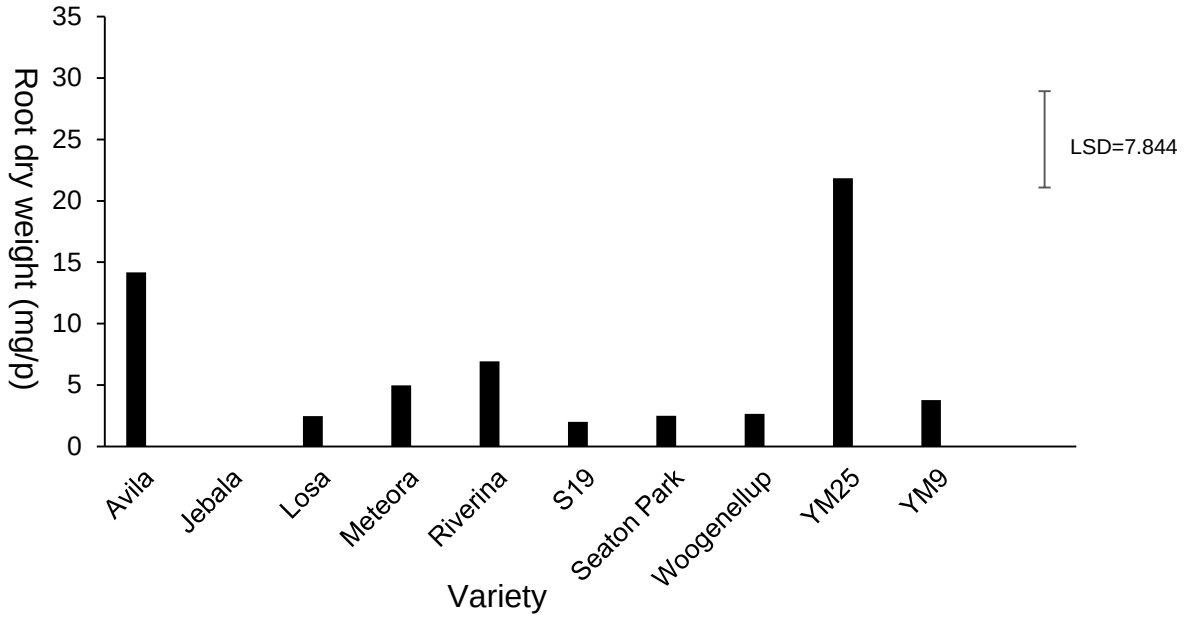
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Ger %	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Fertilizer	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*
Variety	NS	*	<0.001	3.988	NS	*	<0.001	7.844	<0.001	9.229	<0.001	8.538
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*

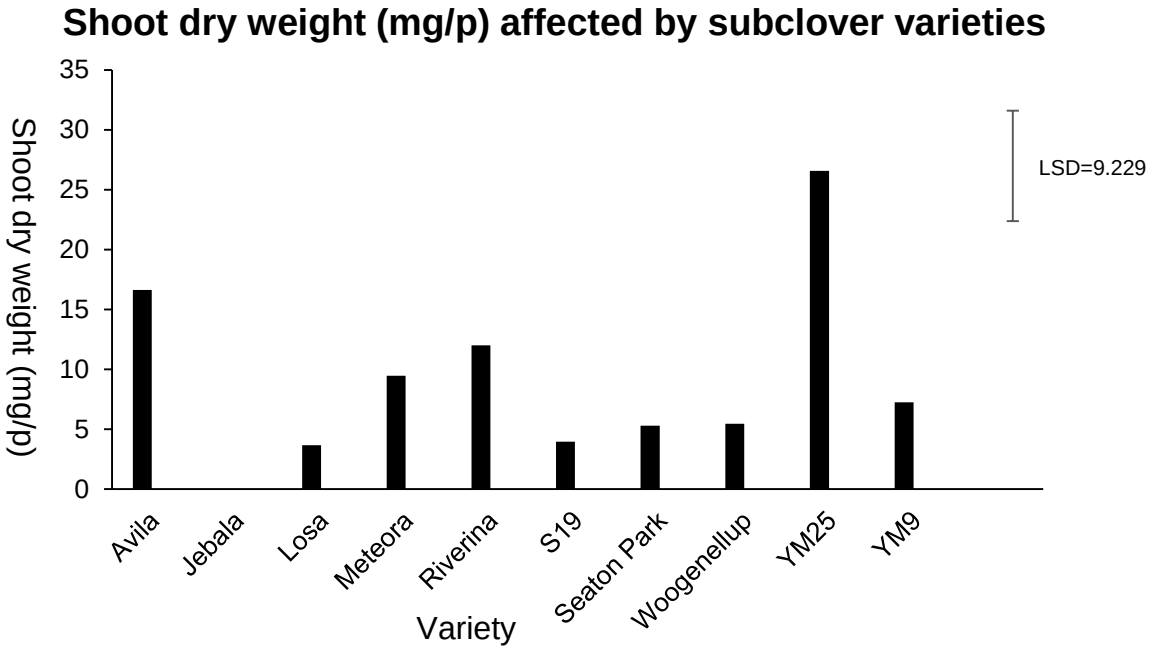


Lateral root disease index (%) affected by subclover varieties



Root dry weight (mg/p) affected by subterranean clover varieties

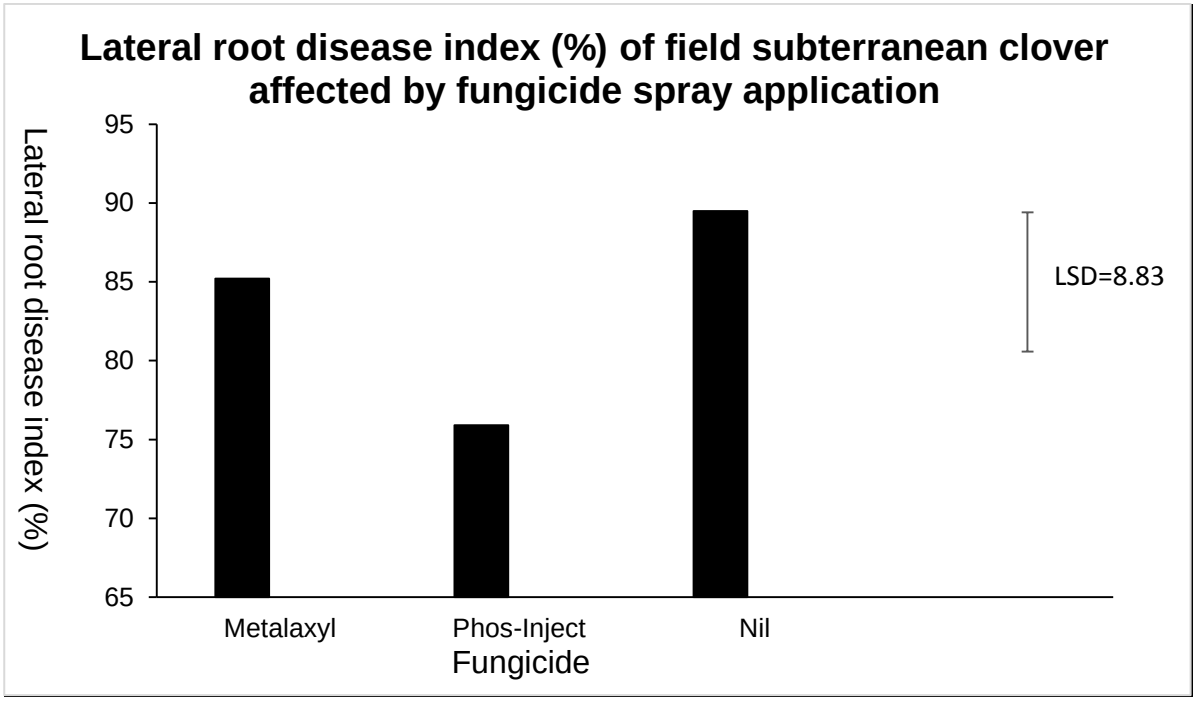


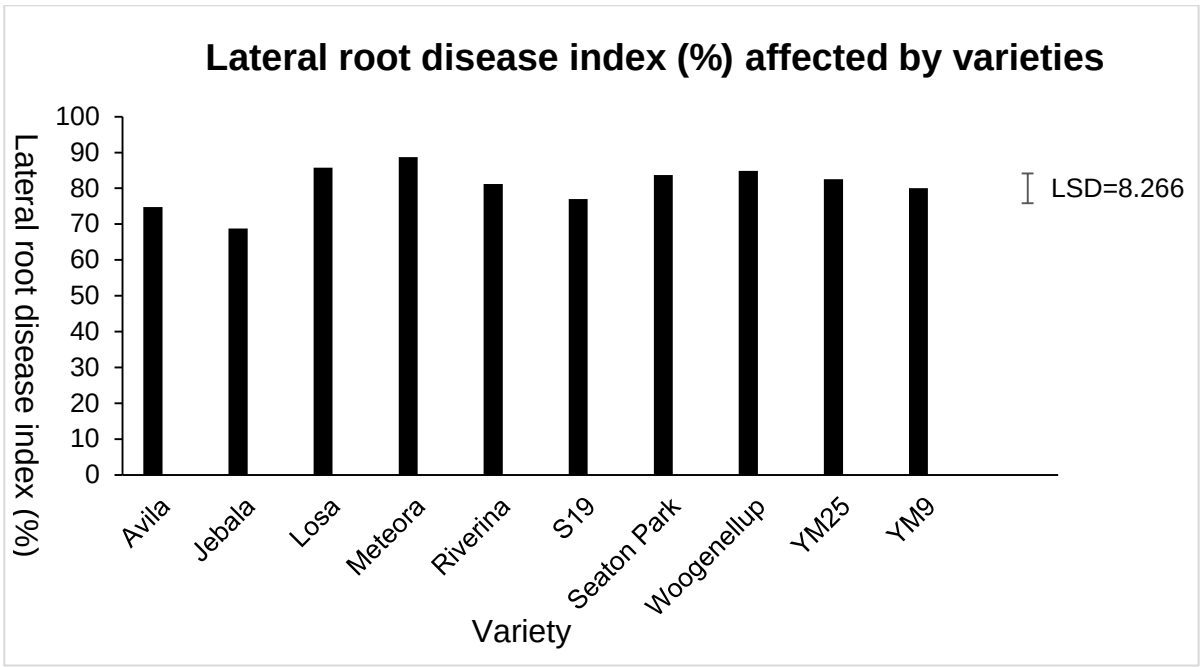


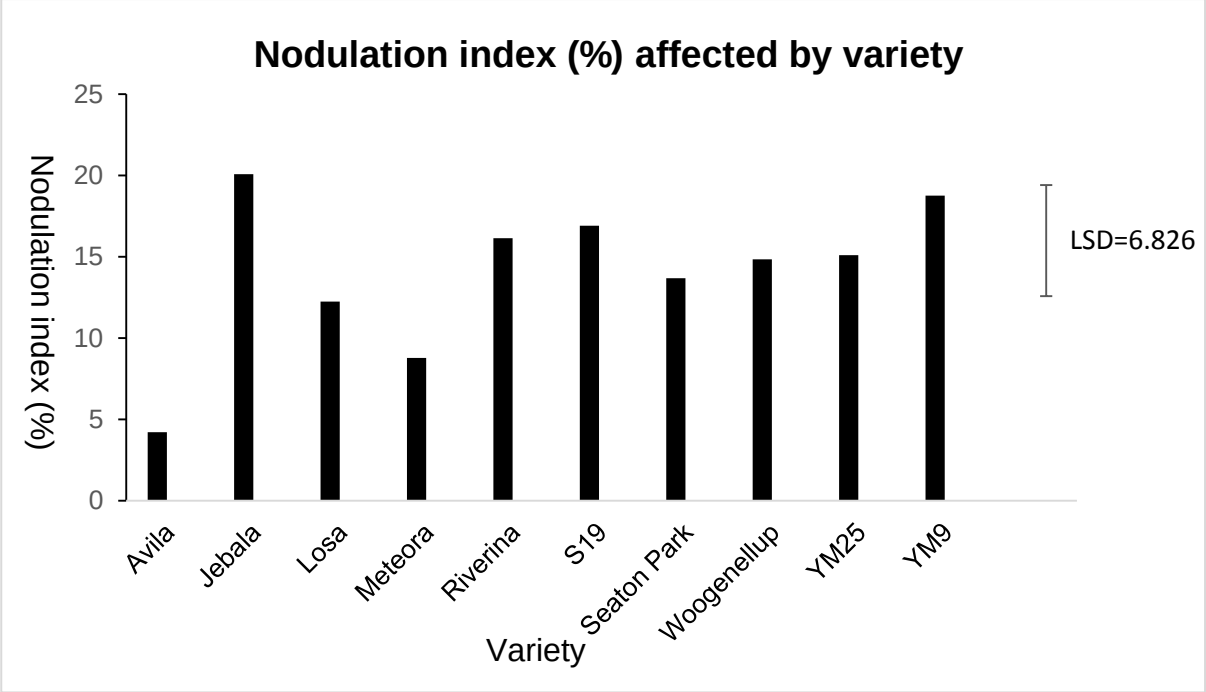
Second sampling results:

Statistical main effects fungicide spray (field and treatment), cultivation, fertilizer, variety and seed treatment on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%) and root and shoot dry weights (RDW and SDW) (mg/p).

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Field spray	NS	*	0.01	8.83	NS	*	NS	*	NS	*
Spray treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	NS	*	NS	*	NS	*	NS	*	NS	*
Fertilizer	NS	*	NS	*	NS	*	NS	*	NS	*
Variety	NS	*	0.004	8.266	< 0.001	6.826	NS	*	NS	*
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*


Lateral root disease index (%) of field subterranean clover affected by fungicide spray application
Lateral root disease index (%)
95
90
85
80
75
70
65
LSD=8.83
Metalaxyl
Phos-Inject
Nil
Fungicide


Lateral root disease index (%) affected by varieties
Lateral root disease index (%)
100
90
80
70
60
50
40
30
20
10
0
LSD=8.266
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety

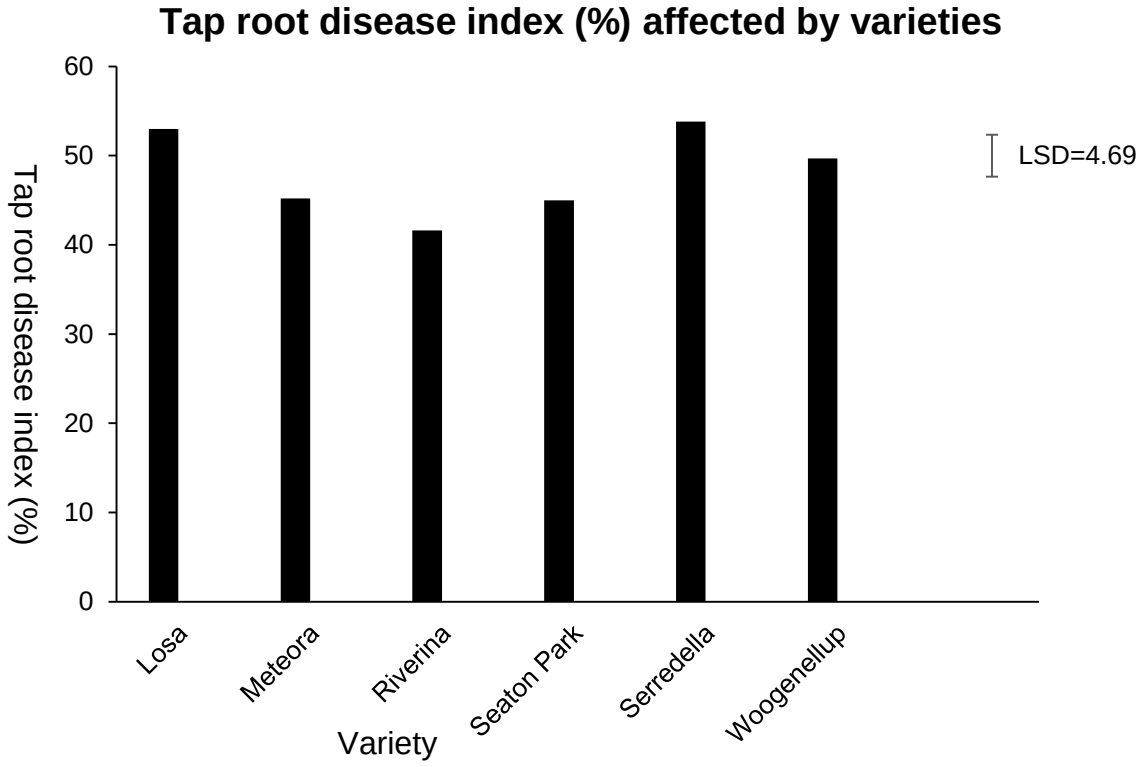

Nodulation index (%) affected by variety
Nodulation index (%)
25
20
15
10
5
0
Avila
Jebala
Losa
Meteora
Riverina
S19
Seaton Park
Woogenellup
YM25
YM9
Variety
LSD=6.826

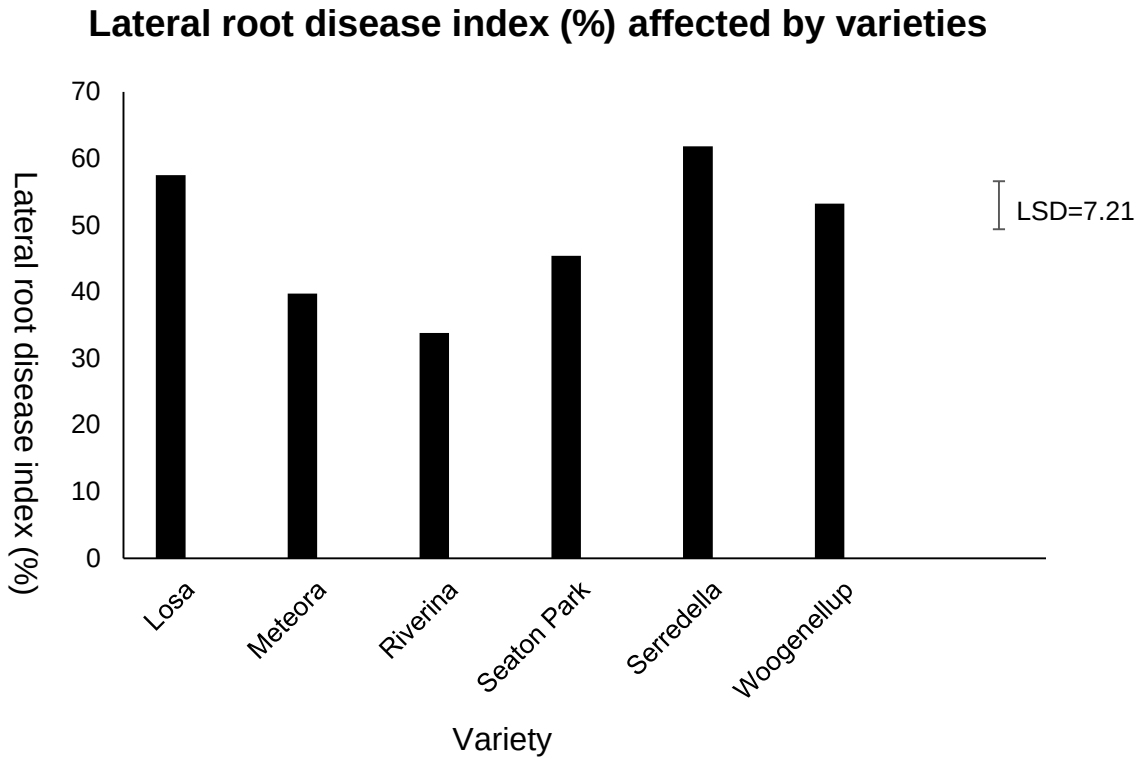
APPENDIX 7 – 2015 Denmark WA: First and second sampling results

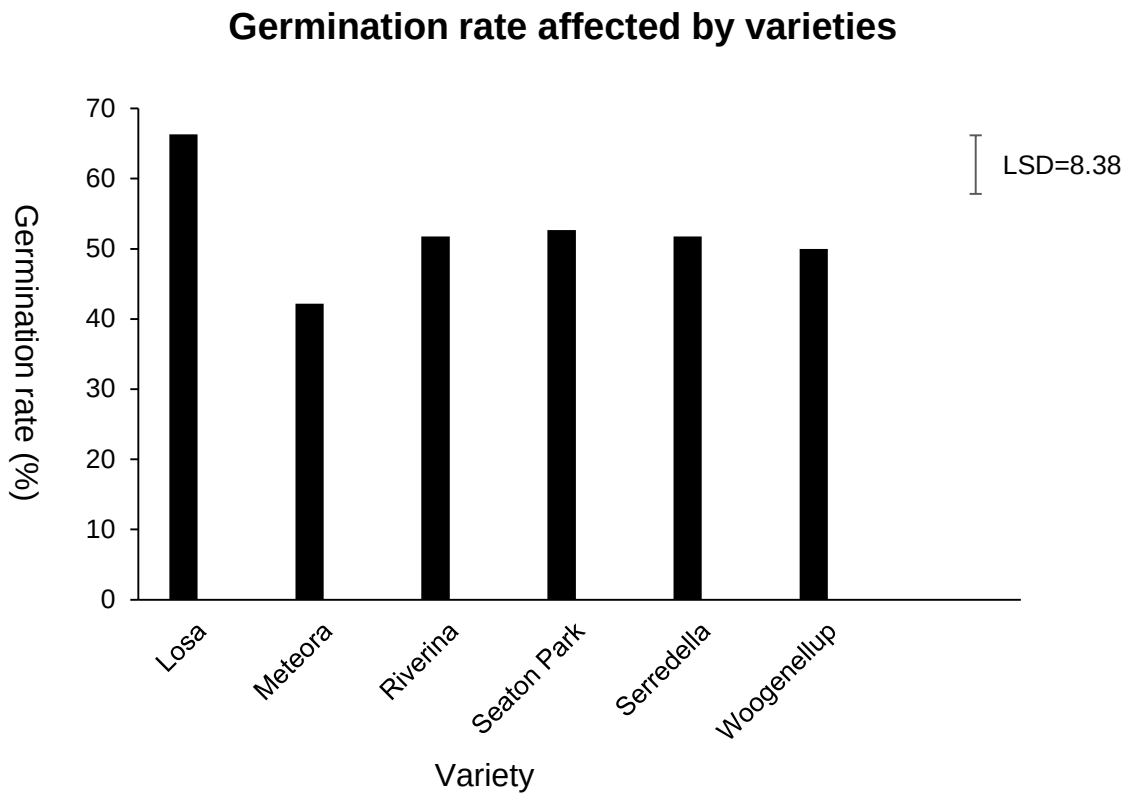
First sampling results:

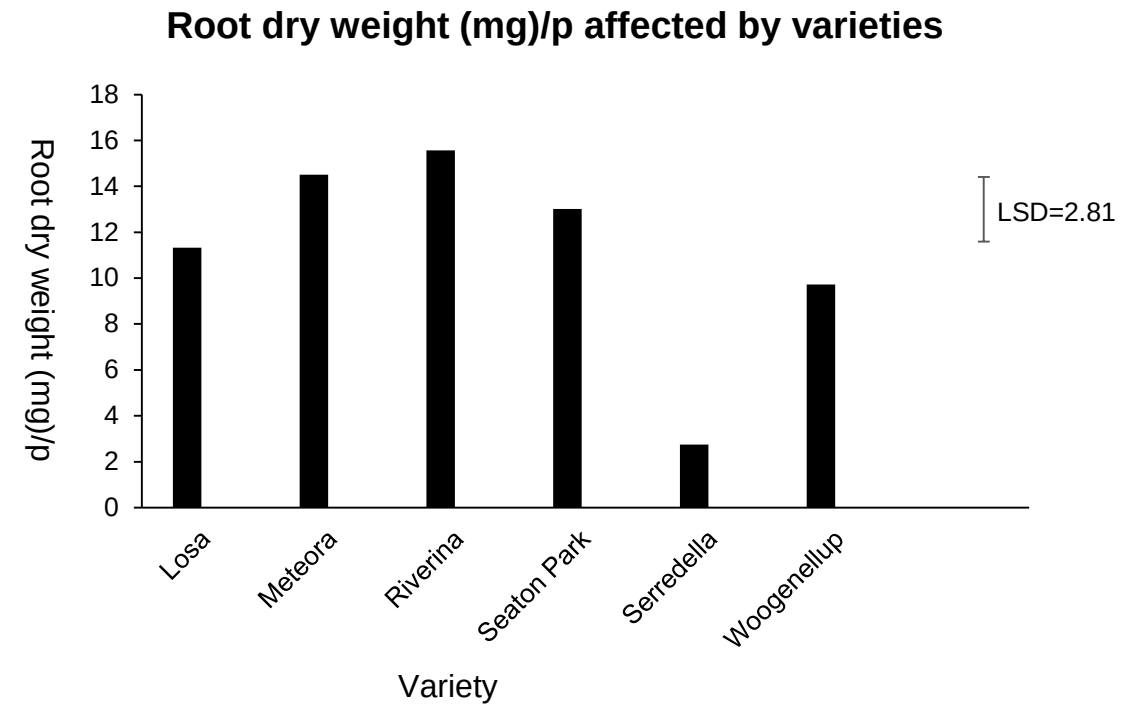
Statistical main effects of variety, seed treatment and fertilizer on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%), root and shoot dry weights (RDW and SDW) (mg/p) and germination rate (GR%)

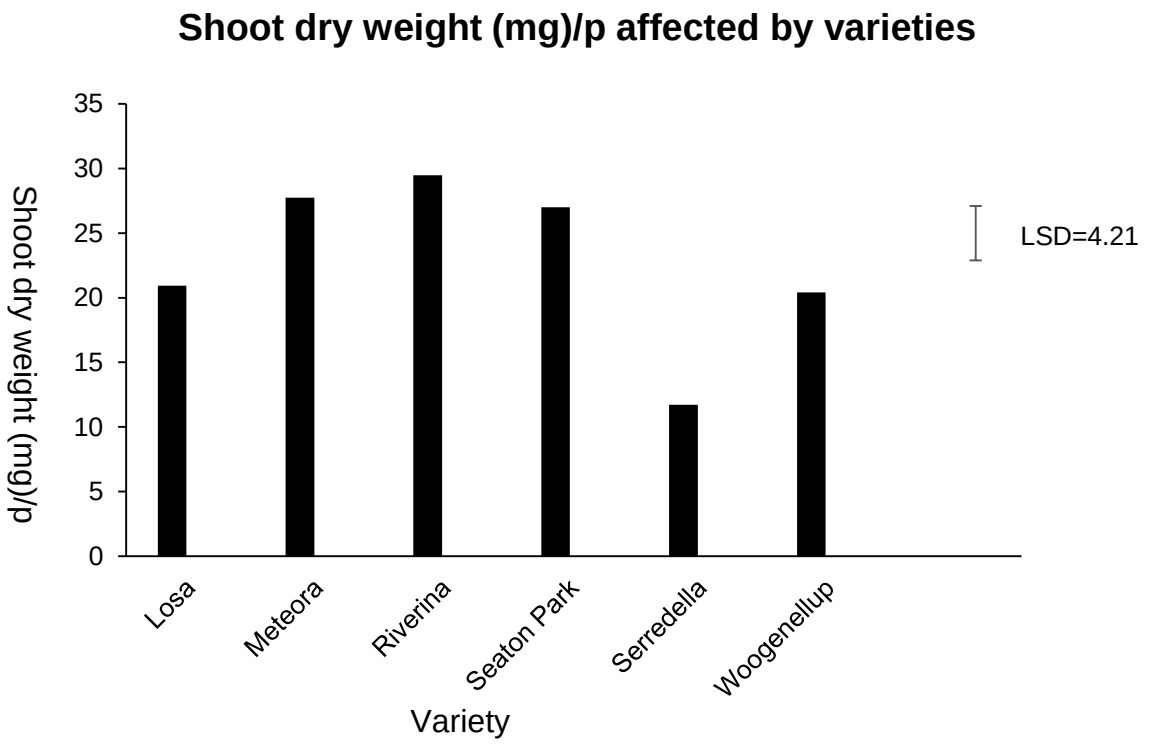
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		GR%	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	4.69	<0.001	7.21	NS	*	<0.001	2.814	<0.001	4.208	< 0.001	8.382
Seed treatment	0.009	6.98	NS	*	NS	*	0.014	3.654	<.001	6.128	NS	*
Fertilizer	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*

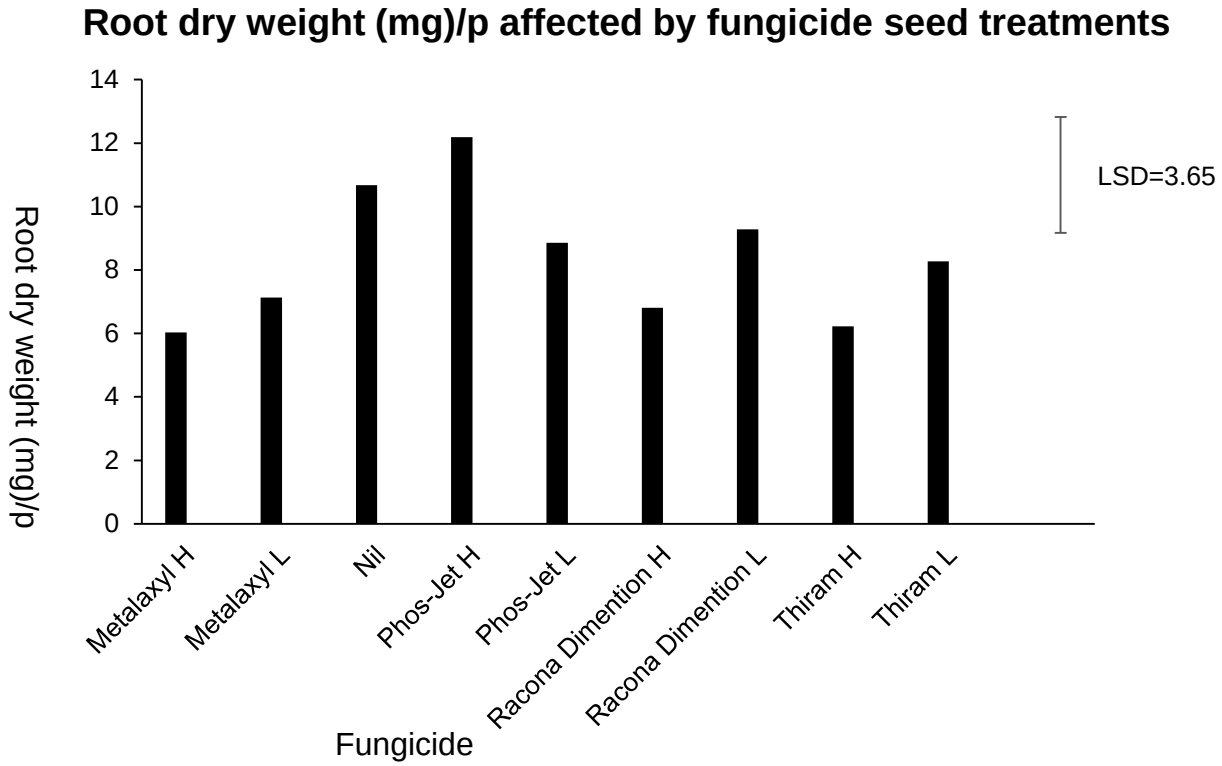

Tap root disease index (%) affected by varieties
Tap root disease index (%)
60
50
40
30
20
10
0
LSD=4.69
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety


Lateral root disease index (%) affected by varieties
Lateral root disease index (%)
70
60
50
40
30
20
10
0
LSD=7.21
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety


Germination rate affected by varieties
Germination rate (%)
70
60
50
40
30
20
10
0
LSD=8.38
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety


Root dry weight (mg)/p affected by varieties
Root dry weight (mg)/p
18
16
14
12
10
8
6
4
2
0
LSD=2.81
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety

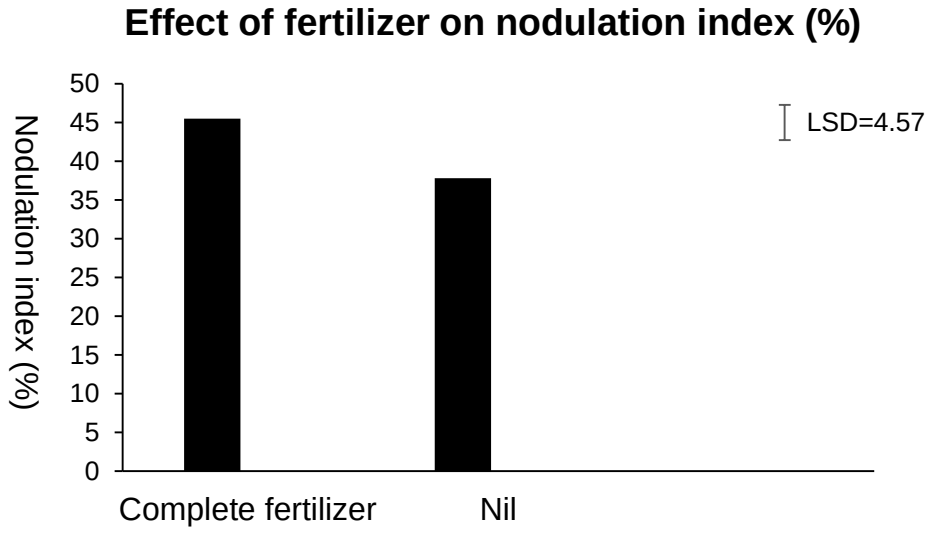

Shoot dry weight (mg)/p affected by varieties
Shoot dry weight (mg)/p
35
30
25
20
15
10
5
0
LSD=4.21
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety

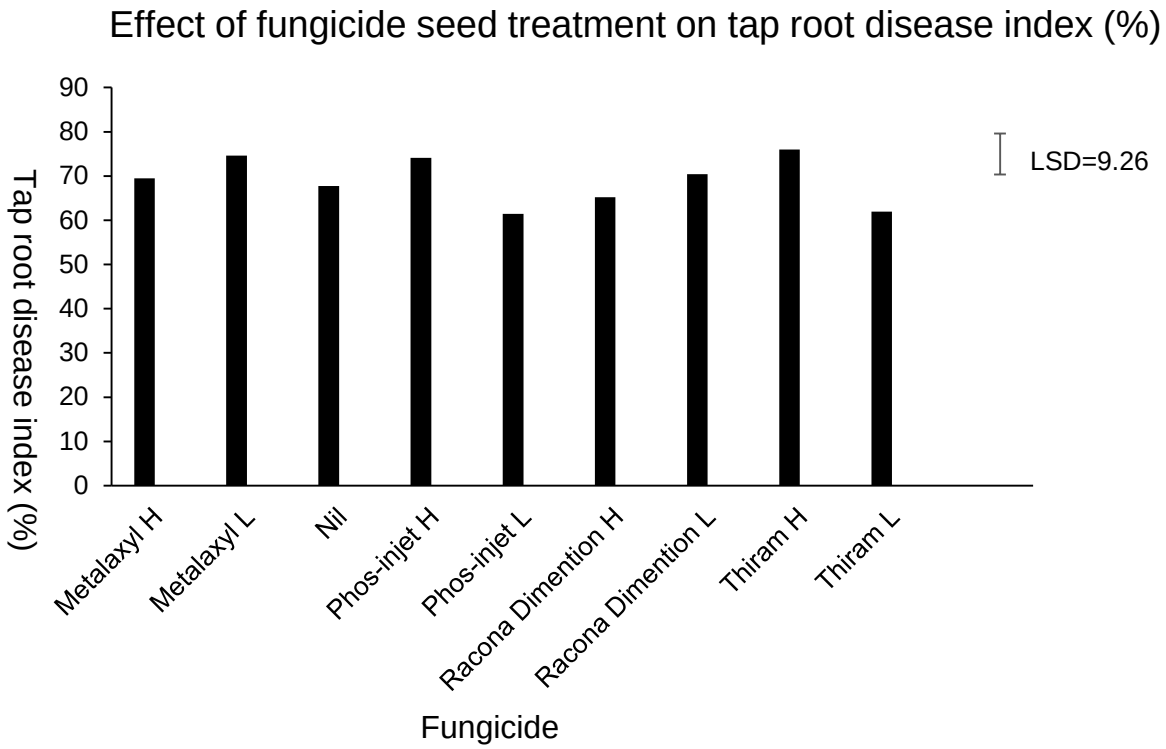

Root dry weight (mg)/p affected by fungicide seed treatments
Root dry weight (mg)/p
14
12
10
8
6
4
2
0
LSD=3.65
Metalaxyl H
Metalaxyl L
Nil
Phos-Jet H
Phos-Jet L
Racona Dimention H
Racona Dimention L
Thiram H
Thiram L
Fungicide

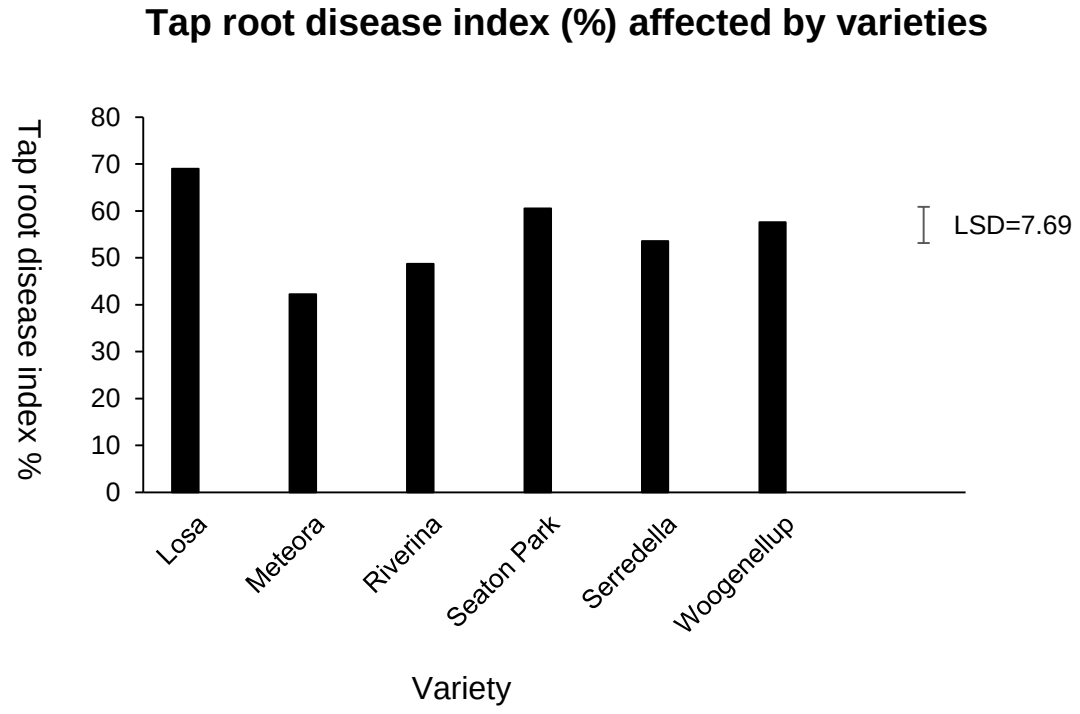
Denmark second sampling results:

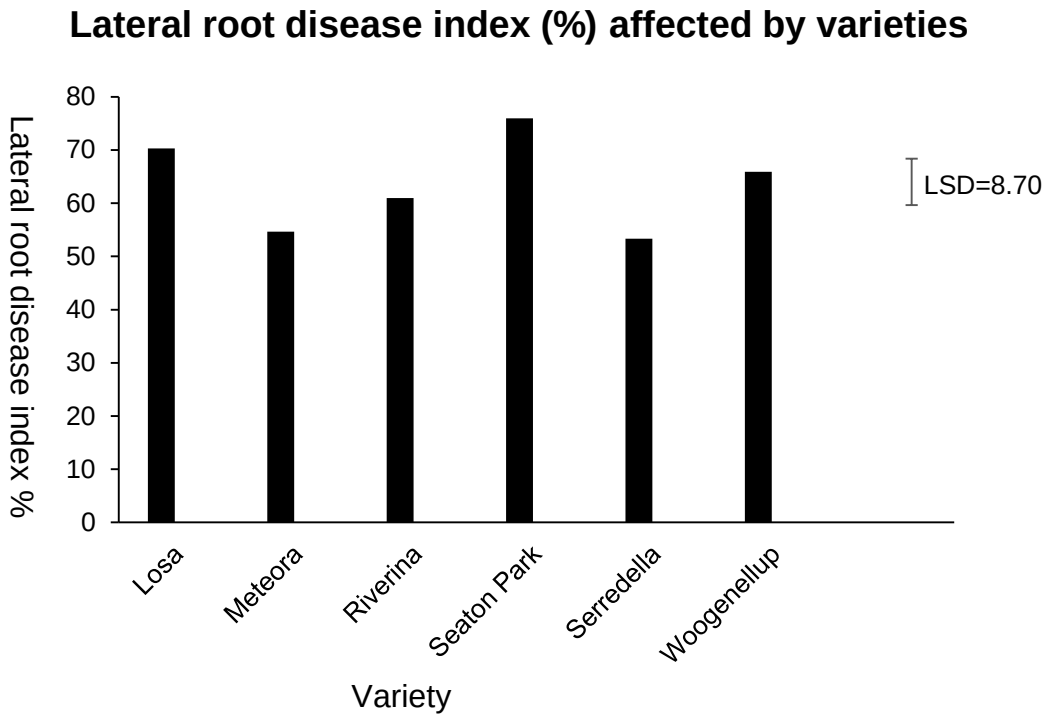
Statistical main effects fungicide spray, fertilizer, seed treatment and variety on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%) and root and shoot dry weights (RDW and SDW) (mg/p)

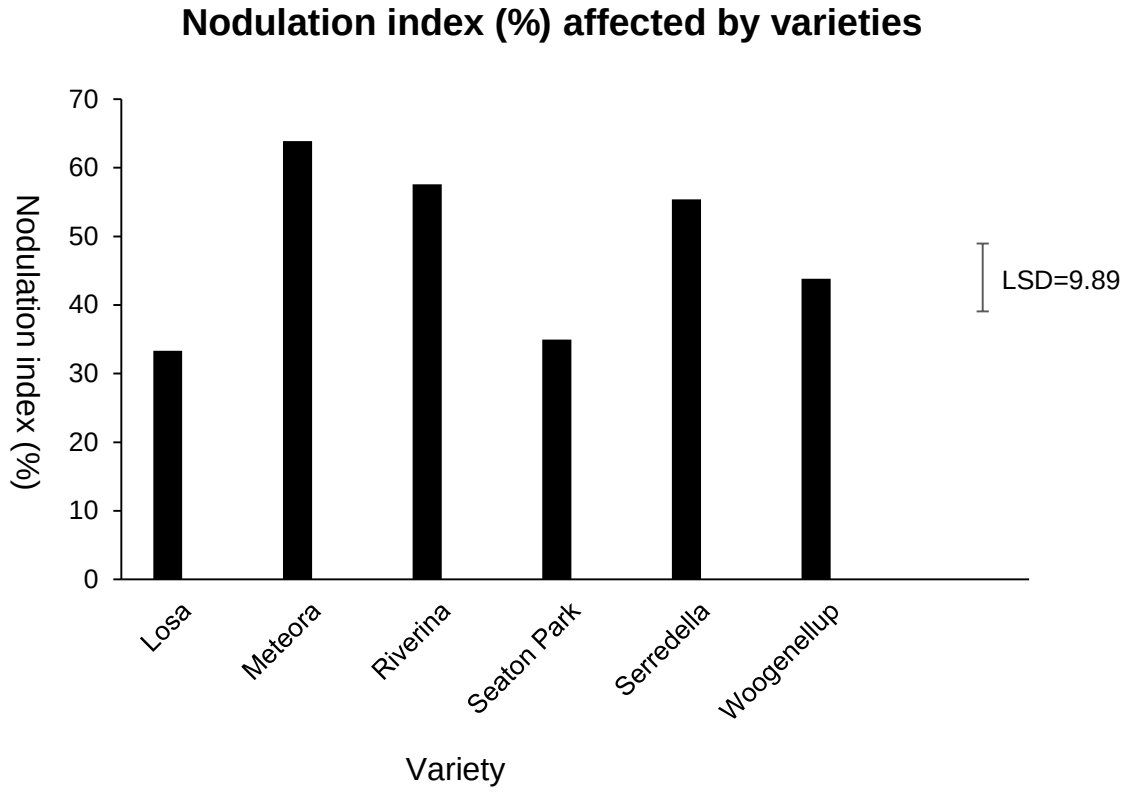
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
fungicide spray	NS	*	NS	*	NS	*	NS	*	NS	*
Fertilizer	NS	*	NS	*	0.005	4.571	NS	*	NS	*
Seed treatment	0.019	9.258	NS	*	NS	*	NS	*	NS	*
Variety	< 0.001	7.69	< 0.001	8.704	< 0.001	9.028	< 0.001	19.14	< 0.001	131.9

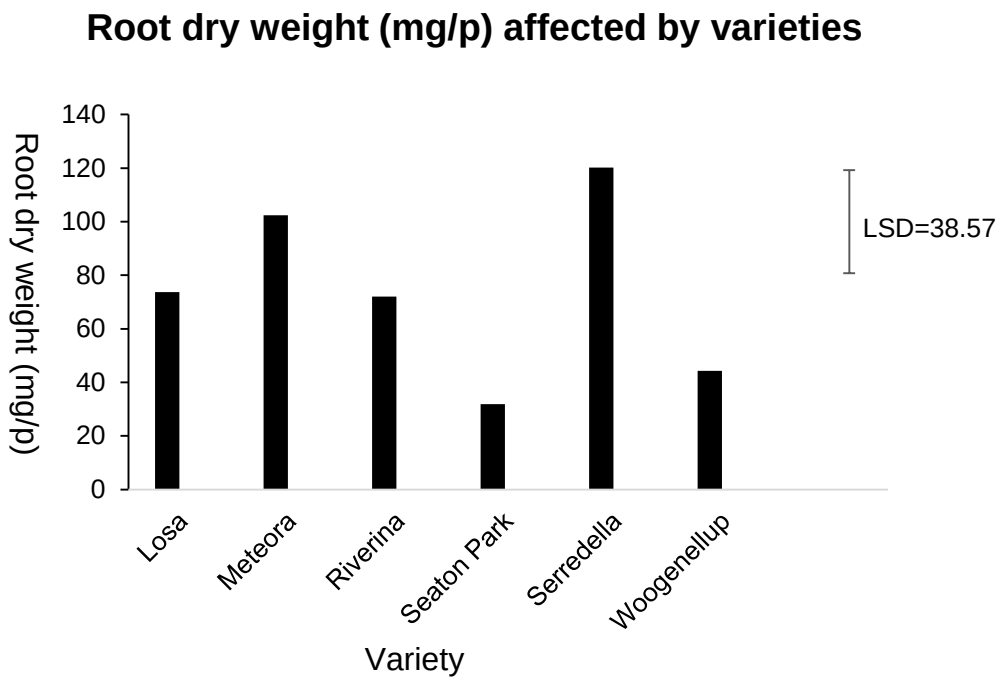


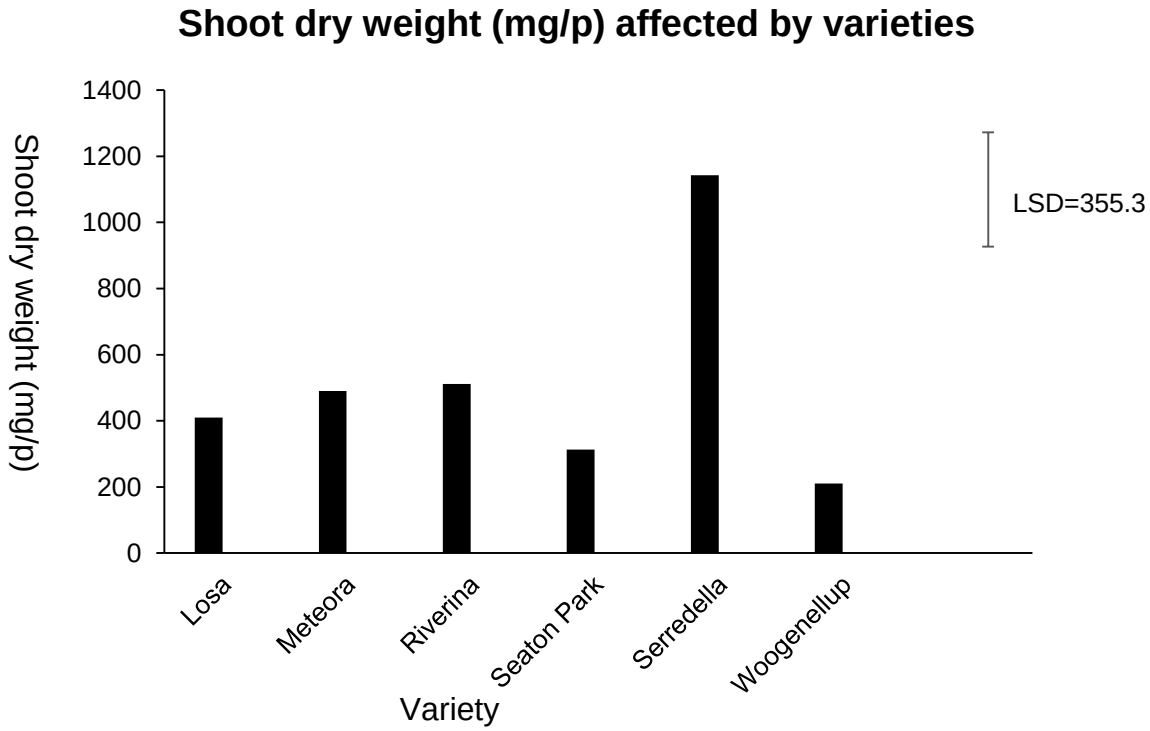

Effect of fungicide seed treatment on tap root disease index (%)
Tap root disease index (%)
90
80
70
60
50
40
30
20
10
0
LSD=9.26
Metalaxyl H
Metalaxyl L
Nil
Phos-injet H
Phos-injet L
Racona Dimention H
Racona Dimention L
Thiram H
Thiram L
Fungicide


Tap root disease index (%) affected by varieties
Tap root disease index %
80
70
60
50
40
30
20
10
0
LSD=7.69
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety


Lateral root disease index (%) affected by varieties
Lateral root disease index %
80
70
60
50
40
30
20
10
0
LSD=8.70
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety


Nodulation index (%) affected by varieties
Nodulation index (%)
70
60
50
40
30
20
10
0
LSD=9.89
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety


Root dry weight (mg/p) affected by varieties
Root dry weight (mg/p)
140
120
100
80
60
40
20
0
LSD=38.57
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety

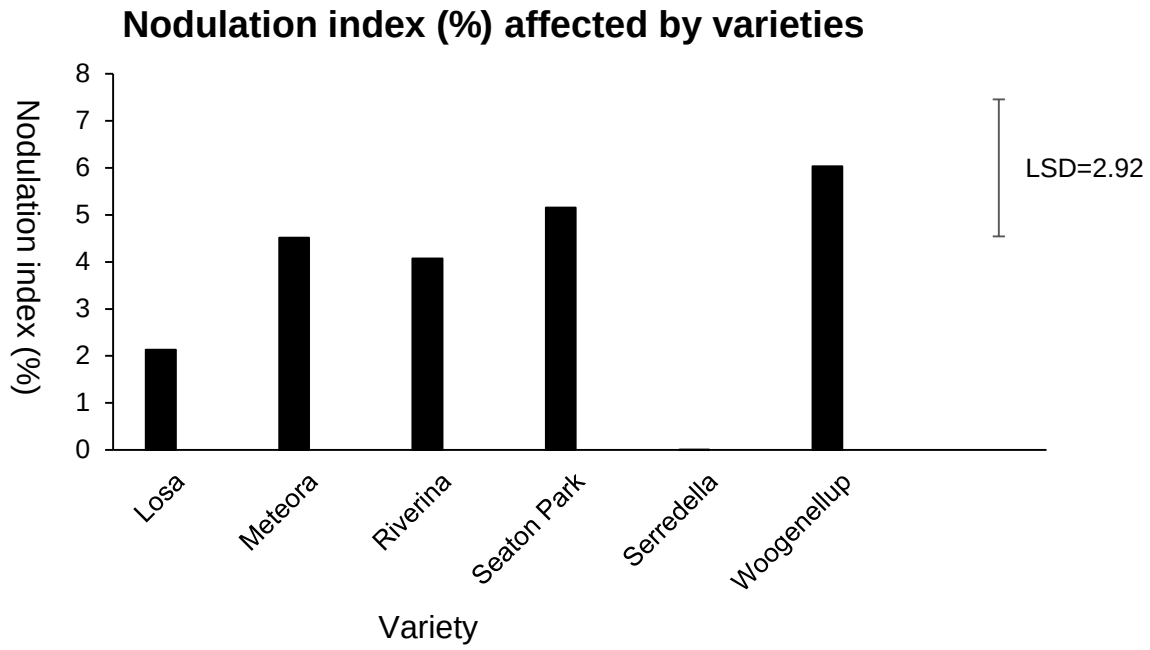

Shoot dry weight (mg/p) affected by varieties
Shoot dry weight (mg/p)
1400
1200
1000
800
600
400
200
0
LSD=355.3
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety

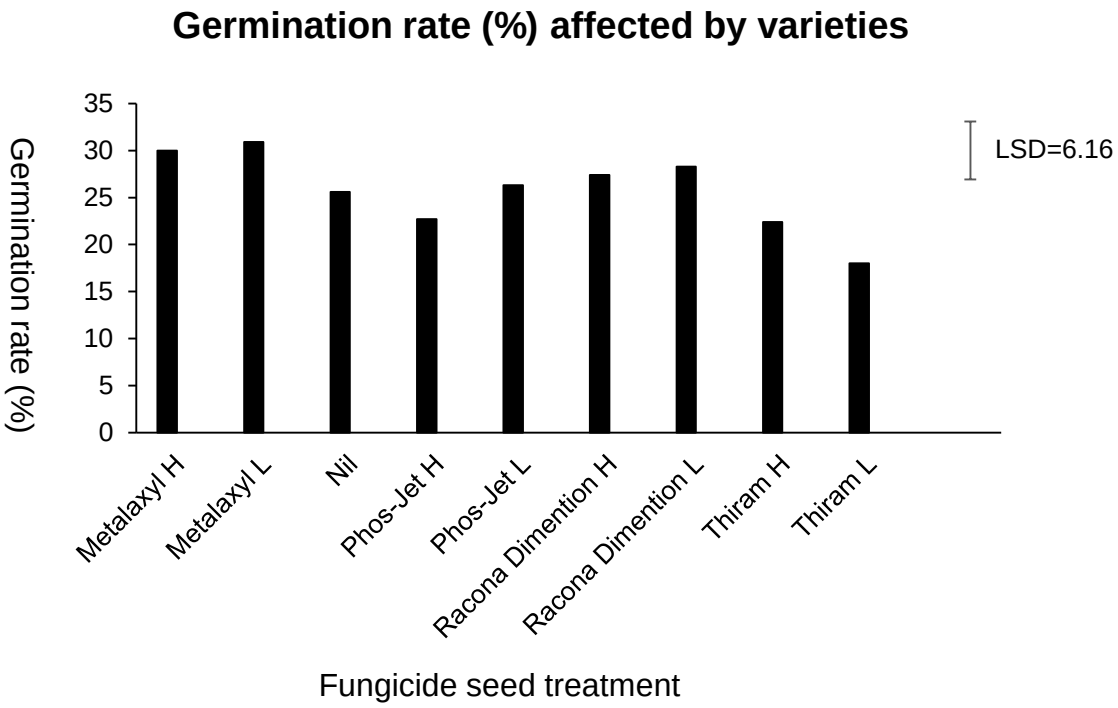
APPENDIX 8 – 2015 NSW: First and second sampling results

Wagin first sampling results:

Statistical main effects of variety, seed treatment and fertilizer on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%), root and shoot dry weights (RDW and SDW) (mg/p) and germination rate (Gr%)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		GR (%)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	NS	*	NS	*	0.002	2.92	NS	*	0.007	4.581	NS	*
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	0.002	6.16
Fertilizer	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*

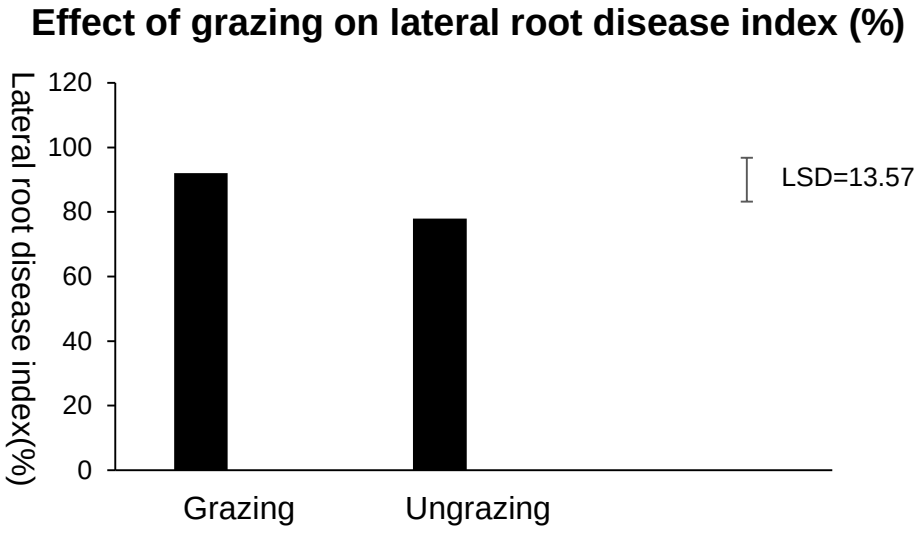

Nodulation index (%) affected by varieties
Nodulation index (%)
0
1
2
3
4
5
6
7
8
LSD=2.92
Losa
Meteora
Riverina
Seaton Park
Serredella
Woogenellup
Variety

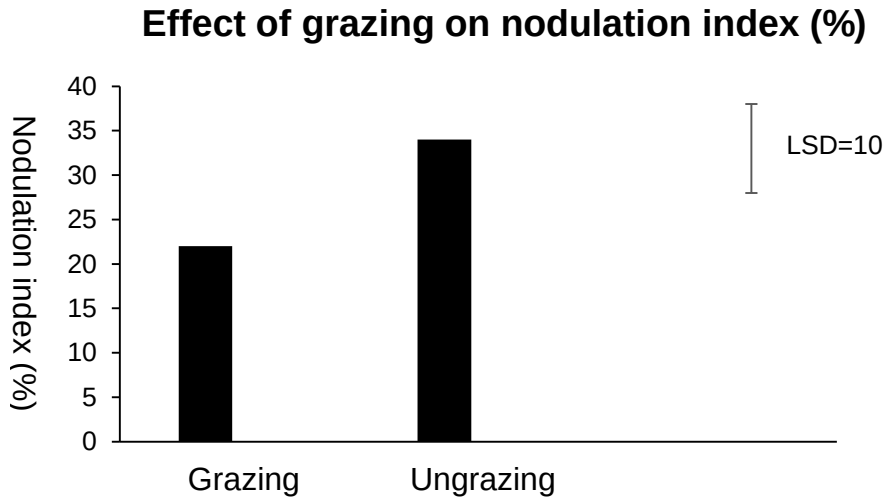

Germination rate (%) affected by varieties
Germination rate (%)
0
5
10
15
20
25
30
35
LSD=6.16
Metalaxyl H
Metalaxyl L
Nil
Phos-Jet H
Phos-Jet L
Racona Dimention H
Racona Dimention L
Thiram H
Thiram L
Fungicide seed treatment

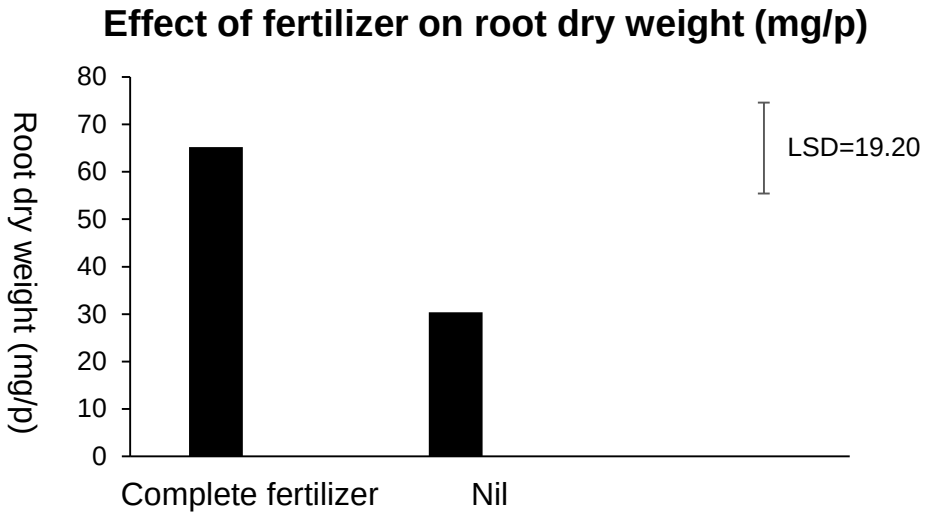
Wagin second sampling results:

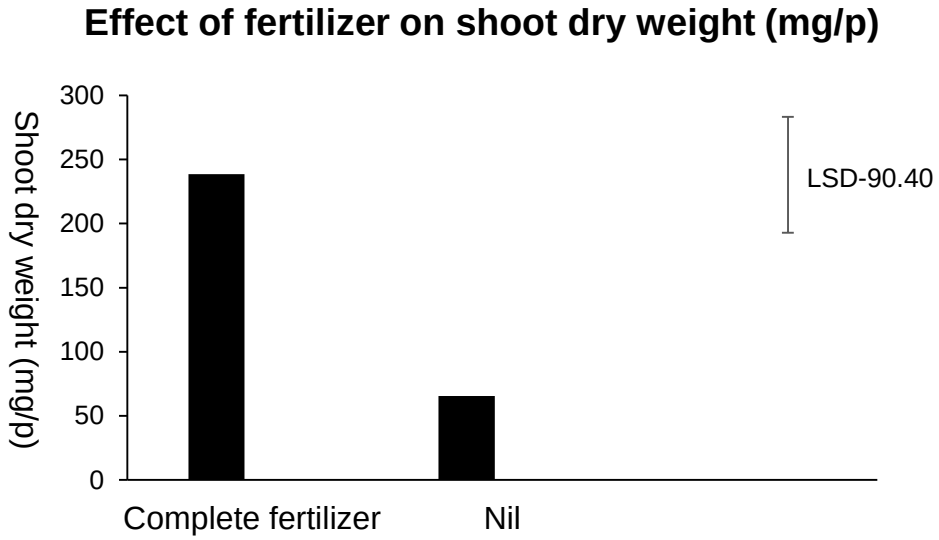
Table. Statistical main effects of grazing, fungicide spray, fertilizer, seed treatment and variety on subterranean clover tap and lateral roots and nodulation indices (TI%, LI% and NI%) and root and shoot dry weights (RDW and SDW) (mg/p)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Grazing	NS	*	0.045	13.57	0.024	10	NS	*	NS	*
fungicide spray	NS	*	NS	*	NS	*	NS	*	NS	*
Fertilizer	NS	*	NS	*	NS	*	0.003	19.2	0.002	90.4
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Variety	NS	*	NS	*	NS	*	NS	*	NS	*


Effect of grazing on lateral root disease index (%)
Lateral root disease index(%)
120
100
80
60
40
20
0
LSD=13.57
Grazing
Ungrazing


Effect of grazing on nodulation index (%)
Nodulation index (%)
40
35
30
25
20
15
10
5
0
LSD=10
Grazing
Ungrazing


Effect of fertilizer on root dry weight (mg/p)
Root dry weight (mg/p)
80
70
60
50
40
30
20
10
0
LSD=19.20
Complete fertilizer
Nil


Effect of fertilizer on shoot dry weight (mg/p)
Shoot dry weight (mg/p)
300
250
200
150
100
50
0
LSD-90.40
Complete fertilizer
Nil

APPENDIX 9 – 2016 NSW/ACT: First and second sampling results

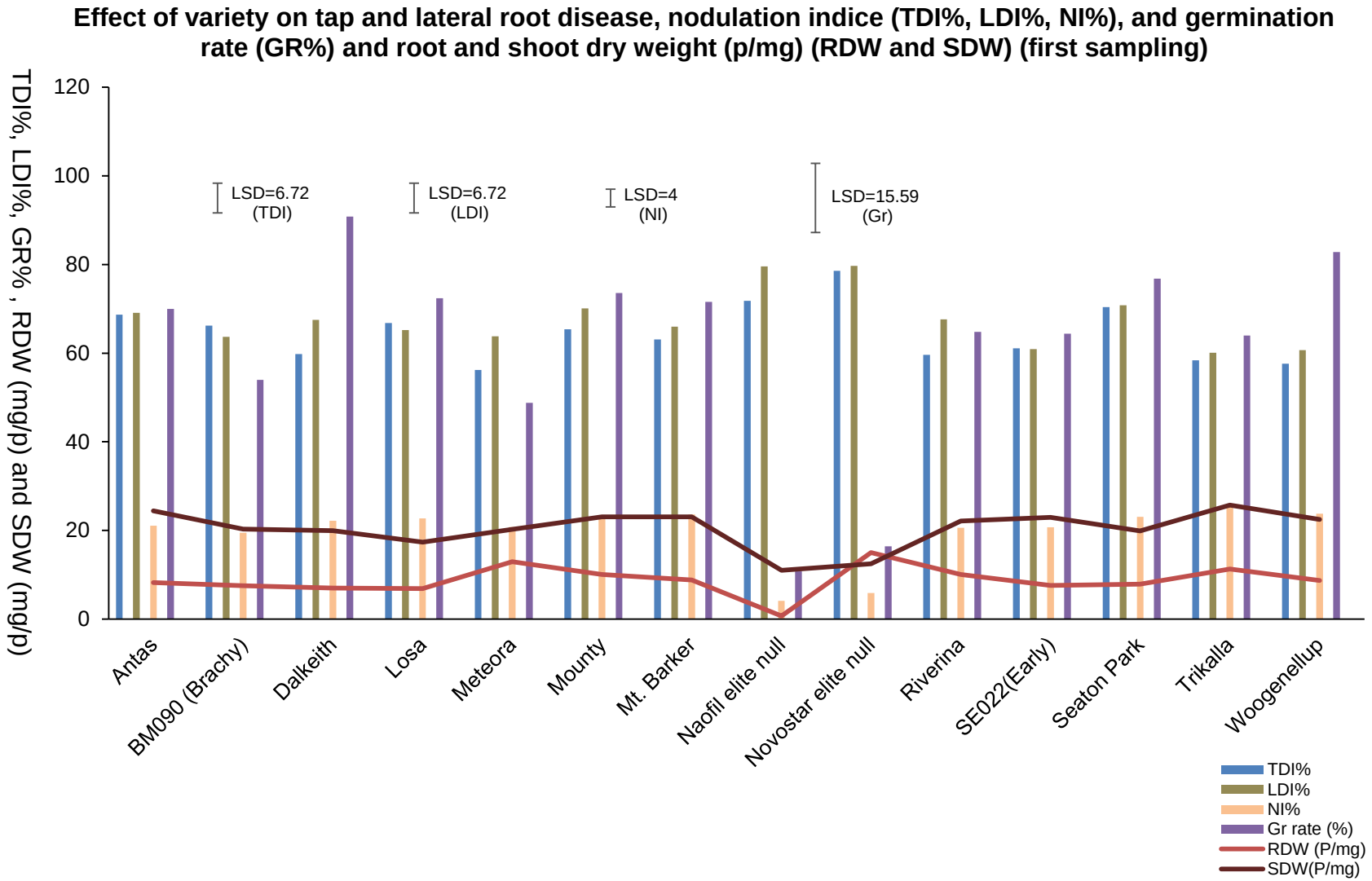
First sampling (young seedlings)

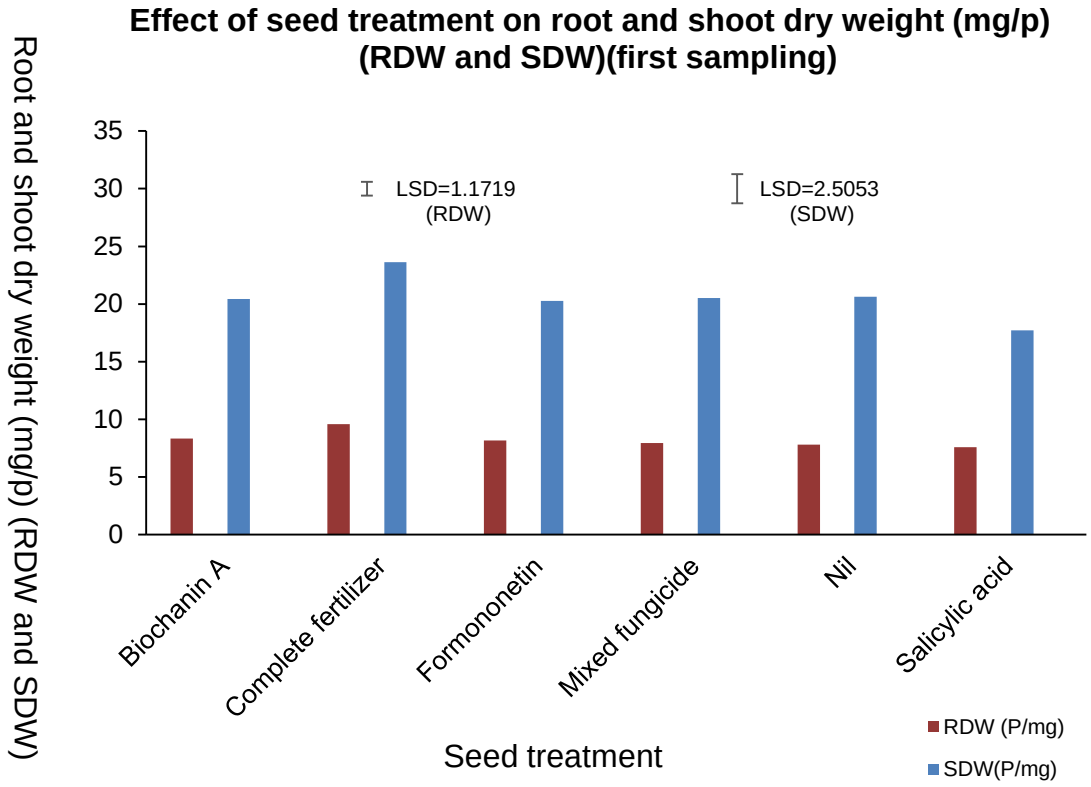
Table 1. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TDI%; LDI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	TDI %		LDI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr rate (%)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	6.72	<0.001	6.72	<0.001	4	NS	*	NS	*	<0.001	15.59
Seed treatment	NS	*	NS	*	NS	*	0.029	1.1719	0.004	2.5053	NS	*
Cultivation	NS	*	NS	*	NS	*	0.034	1.3053	NS	*	<0.001	13.22

Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	Variety	TDI%	LDI%	NI%	RDW (P/mg)	SDW(P/mg)	Gr rate (%)
Variety	Antas	68.7	69.1	21.1	8.261	24.45	70
	BM090 (Brachy)	66.2	63.7	19.5	7.524	20.317	54
	Dalkeith	59.8	67.5	22.2	7.036	19.945	90.8
	Losa	66.8	65.2	22.7	6.888	17.352	72.4
	Meteora	56.2	63.8	20.8	12.973	20.27	48.8
	Mounty	65.4	70.1	23.5	10.049	23.107	73.6
	Mt. Barker	63.1	66	23.6	8.863	23.083	71.6
	Naofil elite null	71.8	79.6	4.1	0.74	11.02	10.8
	Novostar elite null	78.6	79.7	5.9	14.994	12.471	16.4
	Riverina	59.6	67.6	20.6	10.073	22.133	64.8
	SE022(Early)	61.1	60.9	20.7	7.619	22.954	64.4
	Seaton Park	70.4	70.8	23.1	7.881	19.91	76.8
	Trikalla	58.4	60.1	25.3	11.289	25.704	64
	Woogenellup	57.6	60.7	23.8	8.745	22.468	82.8
Seed treatment	Biochanin A	58.5	53.2	27.3	8.337	20.424	75.2
	Complete fertilizer	57.2	63.1	28.5	9.573	23.629	72.4
	Formononetin	52.5	56.8	21.1	8.174	20.262	84
	Mixed fungicide	56	60.8	25.1	7.934	20.521	68.8
	Nil	56.1	57.8	21.8	7.807	20.634	80
	Salicylic acid	55.9	60.1	24.6	7.585	17.72	75.2
Cultivation	Cultivated	62	63.3	24	9.436	21.329	52
	Non-cultivated	66.5	68.5	20.4	7.991	18.583	84


Effect of variety on tap and lateral root disease, nodulation indice (TDI%, LDI%, NI%), and germination rate (GR%) and root and shoot dry weight (p/mg) (RDW and SDW) (first sampling)
TDI%, LDI%, GR% , RDW (mg/p) and SDW (mg/p)
120
100
80
60
40
20
0
LSD=6.72 (TDI)
LSD=6.72 (LDI)
LSD=4 (NI)
LSD=15.59 (Gr)
Antas
BM090 (Brachy)
Dalkeith
Losa
Meteora
Mounty
Mt. Barker
Naofil elite null
Novostar elite null
Riverina
SE022(Early)
Seaton Park
Trikalla
Woogenellup
TDI%
LDI%
NI%
Gr rate (%)
RDW (P/mg)
SDW(P/mg)


Effect of seed treatment on root and shoot dry weight (mg/p) (RDW and SDW)(first sampling)
Root and shoot dry weight (mg/p) (RDW and SDW)
35
30
25
20
15
10
5
0
LSD=1.1719 (RDW)
LSD=2.5053 (SDW)
Biochanin A
Complete fertilizer
Formononetin
Mixed fungicide
Nil
Salicylic acid
Seed treatment
RDW (P/mg)
SDW(P/mg)

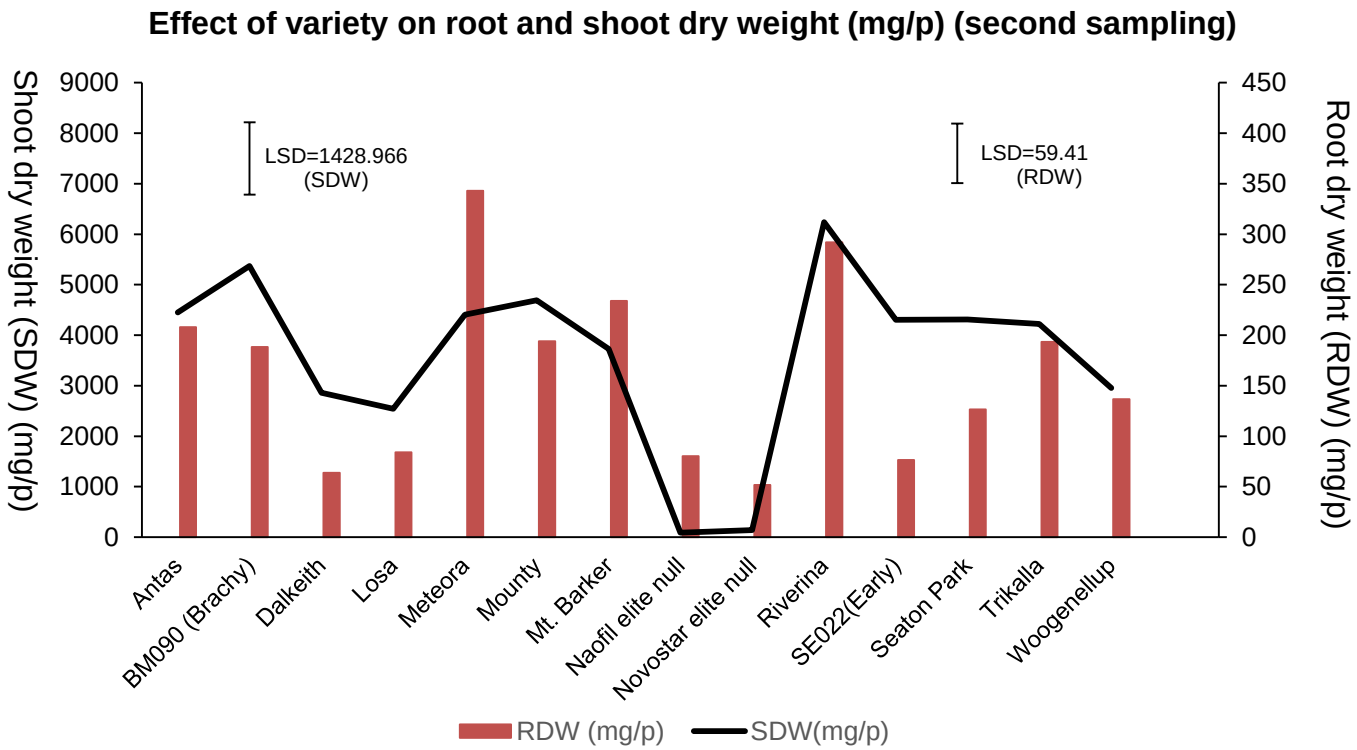
Second sampling (adult plants)

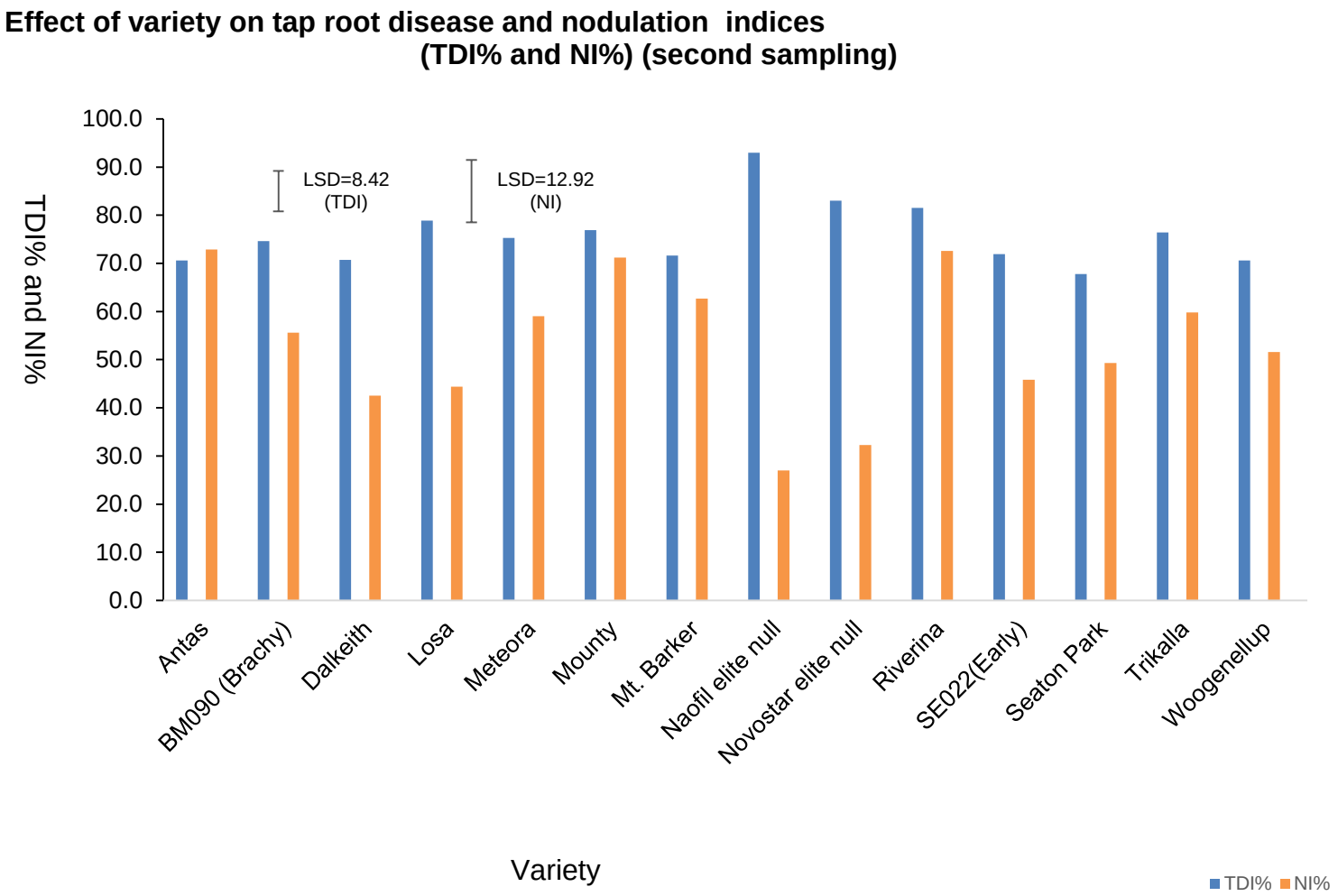
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW).

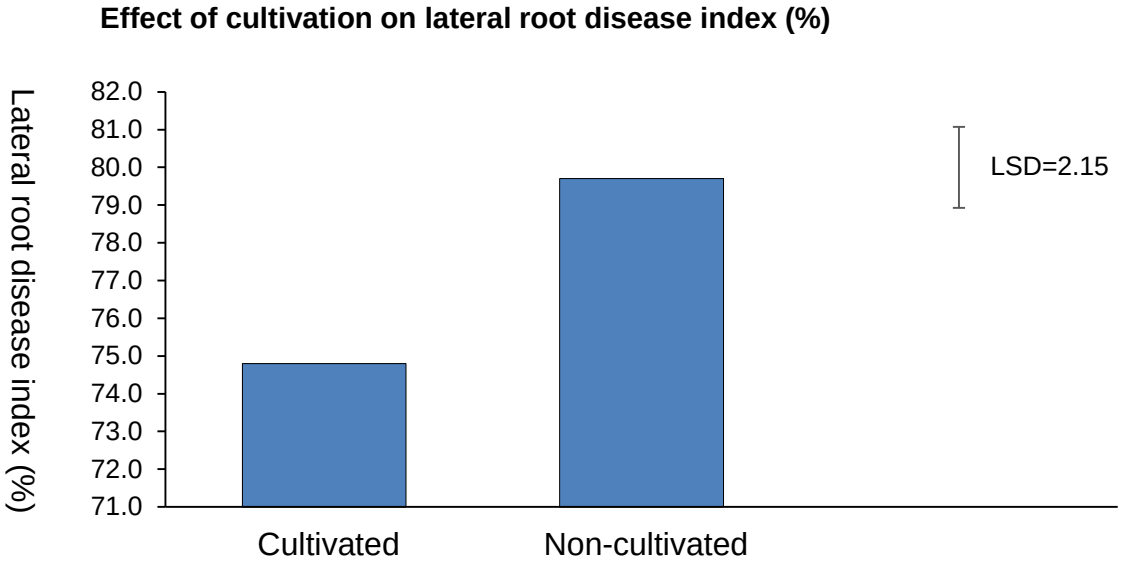
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr rate (%)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	8.42	NS	*	<0.001	12.92	<0.001	59.4088	<0.001	1428.966		
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*		
Cultivation	NS	*	<0.001	2.15	NS	*	NS	*	NS	*		
Spray	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*

Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and dry weights (RDW and SDW).

Factor		TDI%	LDI%	NI%	RDW (P/mg)	SDW(P/mg)
Variety	Antas	70.6	78.1	72.9	208.1	4453.4
	BM090 (Brachy)	74.6	73.8	55.6	188.2	5371.7
	Dalkeith	70.7	80.7	42.5	63.7	2861.9
	Losa	78.9	78.1	44.4	84.1	2542.9
	Meteora	75.3	79.8	59.0	342.9	4404.6
	Mounty	76.9	79.9	71.2	194.1	4693.9
	Mt. Barker	71.6	77.8	62.7	234.1	3727.0
	Naofil elite null	93.0	86.0	27.0	80.2	91.8
	Novostar elite null	83.0	80.0	32.3	51.7	138.7
	Riverina	81.5	76.6	72.6	292.1	6239.1
	SE022(Early)	71.9	77.9	45.8	76.4	4307.1
	Seaton Park	67.8	79.0	49.3	126.5	4312.5
	Trikkala	76.4	79.5	59.8	193.5	4220.4
	Woogenellup	70.6	77.8	51.6	136.7	2953.2
Cultivation	Cultivation	NS	74.8	NS	NS	NS
	Non-cultivation	NS	79.7	NS	NS	NS


Effect of variety on root and shoot dry weight (mg/p) (second sampling)
Shoot dry weight (SDW) (mg/p)
Root dry weight (RDW) (mg/p)
LSD=1428.966 (SDW)
LSD=59.41 (RDW)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
450
400
350
300
250
200
150
100
50
0
Antas
BM090 (Brachy)
Dalkeith
Losa
Meteora
Mounty
Mt. Barker
Naofil elite null
Novostar elite null
Riverina
SE022(Early)
Seaton Park
Trikalla
Woogenellup
RDW (mg/p)
SDW(mg/p)


Effect of variety on tap root disease and nodulation indices (TDI% and NI%) (second sampling)
TDI% and NI%
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
LSD=8.42 (TDI)
LSD=12.92 (NI)
Antas
BM090 (Brachy)
Dalkeith
Losa
Meteora
Mounty
Mt. Barker
Naofil elite null
Novostar elite null
Riverina
SE022(Early)
Seaton Park
Trikalla
Woogenellup
Variety
TDI%
NI%


Effect of cultivation on lateral root disease index (%)
Lateral root disease index (%)
82.0
81.0
80.0
79.0
78.0
77.0
76.0
75.0
74.0
73.0
72.0
71.0
LSD=2.15
Cultivated
Non-cultivated

APPENDIX 10 – 2016 Barossa SA: First and second sampling results

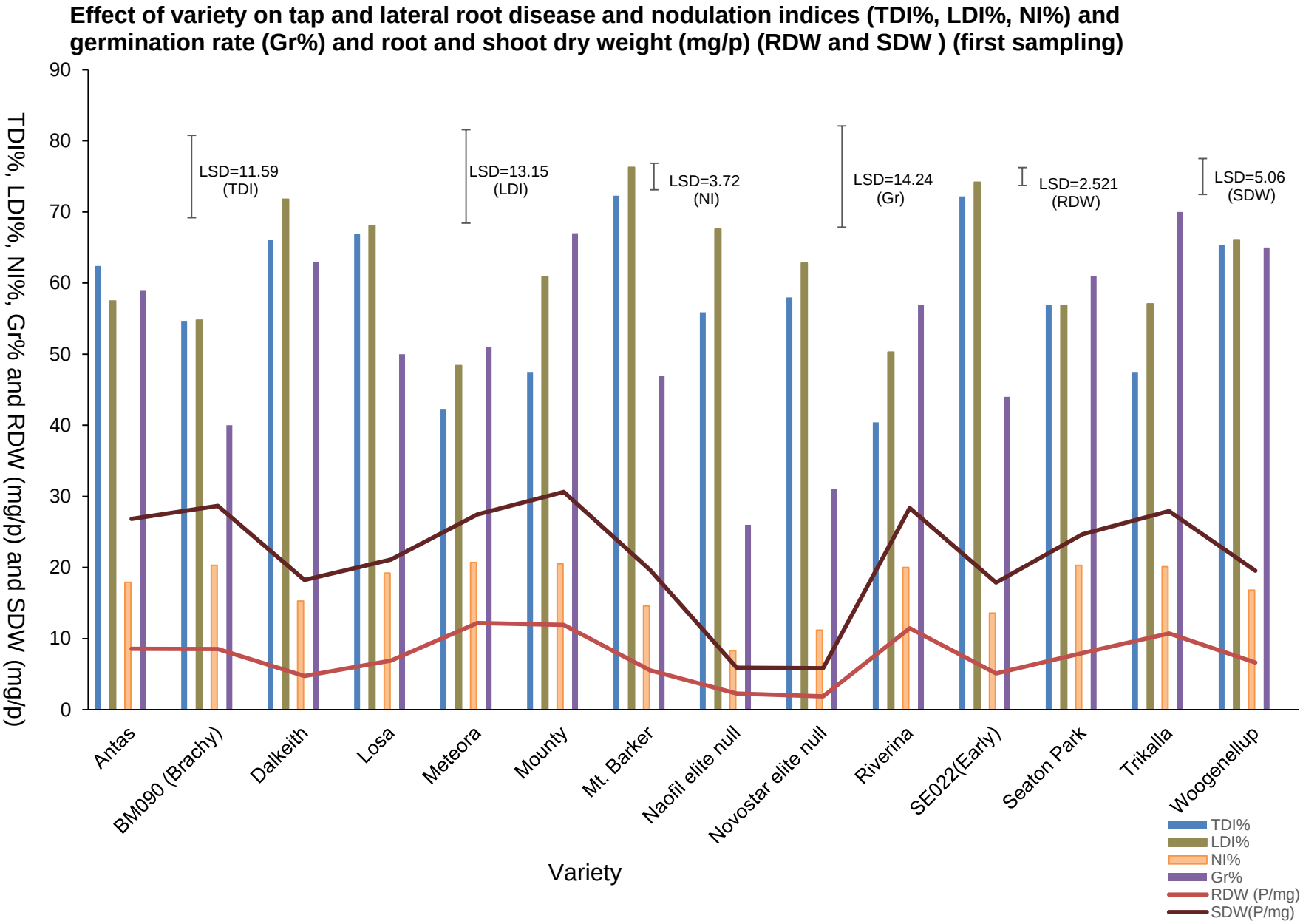
First sampling (young seedlings)

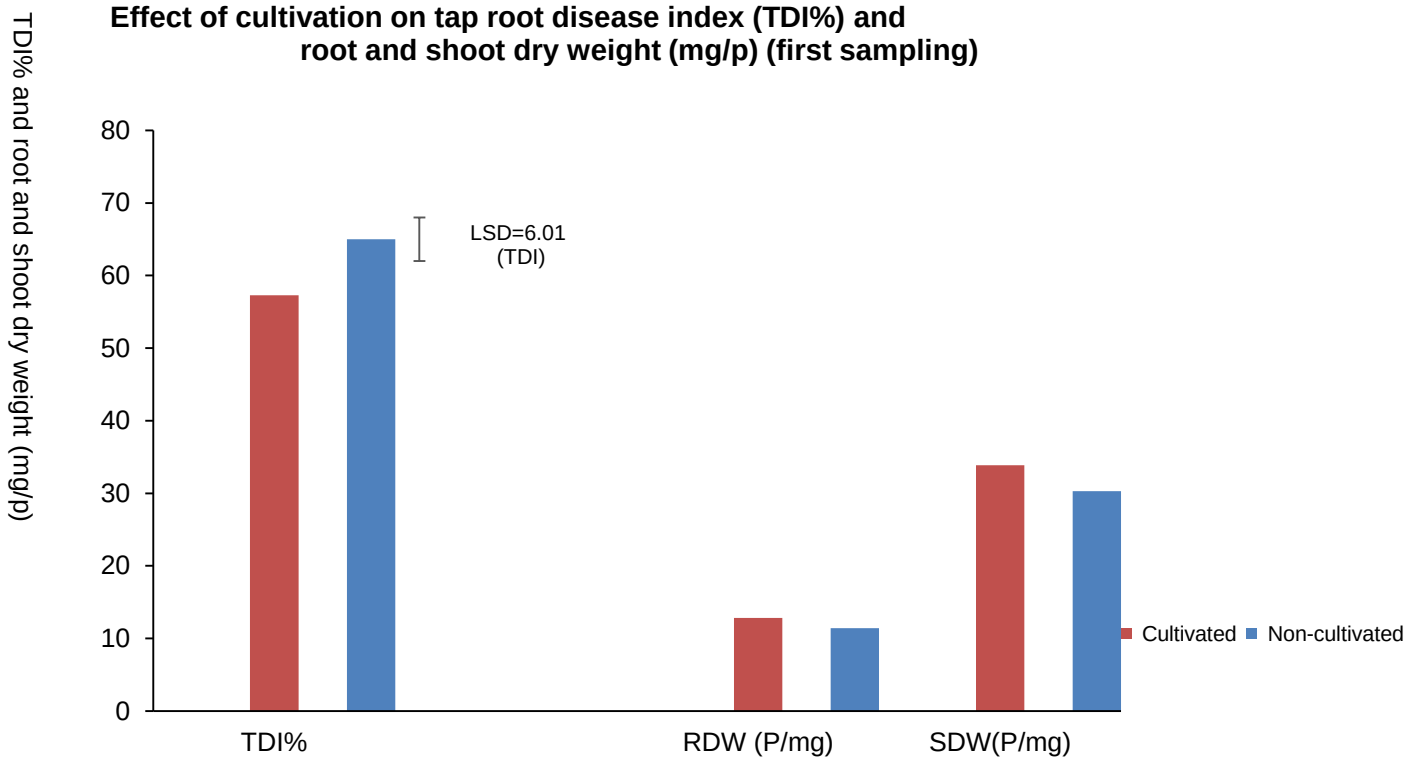
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr rate (%)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	11.59	<0.001	13.15	<0.001	3.72	<0.001	2.521	<0.001	5.0623	<0.001	14.24
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	0.018	6.01	NS	*	0.002	6	NS	*	NS	*	0.039	8.97

Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	Treatment	TDI%	LDI%	NI%	RDW (P/mg)	SDW(P/mg)	Gr rate (%)
Variety	Antas	62.4	57.5	17.9	8.551	26.84	59
	BM090 (Brachy)	54.7	54.8	20.3	8.523	28.67	40
	Dalkeith	66.1	71.8	15.3	4.734	18.25	63
	Losa	66.9	68.1	19.2	6.884	21.12	50
	Meteora	42.3	48.4	20.7	12.184	27.47	51
	Mounty	47.5	60.9	20.5	11.939	30.62	67
	Mt. Barker	72.3	76.3	14.6	5.527	19.64	47
	Naofil elite null	55.9	67.6	8.3	2.288	5.901	26
	Novostar elite null	58	62.8	11.2	1.869	5.821	31
	Riverina	40.4	50.3	20	11.463	28.37	57
	SE022(Early)	72.2	74.2	13.6	5.111	17.9	44
	Seaton Park	56.9	56.9	20.3	7.979	24.67	61
	Trikkala	47.5	57.1	20.1	10.713	27.93	70
	Woogenellup	65.4	66.1	16.8	6.643	19.54	65
Cultivation	Cultivated	57.3	55.1	28.7	12.833	33.88	29.6
	Non-cultivated	65	57.2	40.3	11.4	30.3	39.2


Effect of variety on tap and lateral root disease and nodulation indices (TDI%, LDI%, NI%) and germination rate (Gr%) and root and shoot dry weight (mg/p) (RDW and SDW) (first sampling)
TDI%, LDI%, NI%, Gr% and RDW (mg/p) and SDW (mg/p)
0
10
20
30
40
50
60
70
80
90
LSD=11.59 (TDI)
LSD=13.15 (LDI)
LSD=3.72 (NI)
LSD=14.24 (Gr)
LSD=2.521 (RDW)
LSD=5.06 (SDW)
Antas
BM090 (Brachy)
Dalkeith
Losa
Meteora
Mounty
Mt. Barker
Naofil elite null
Novostar elite null
Riverina
SE022(Early)
Seaton Park
Trikalla
Woogenellup
Variety
TDI%
LDI%
NI%
Gr%
RDW (P/mg)
SDW(P/mg)


Effect of cultivation on tap root disease index (TDI%) and root and shoot dry weight (mg/p) (first sampling)
TDI% and root and shoot dry weight (mg/p)
80
70
60
50
40
30
20
10
0
LSD=6.01
(TDI)
TDI%
RDW (P/mg)
SDW(P/mg)
Cultivated
Non-cultivated

Second sampling (adult plants)

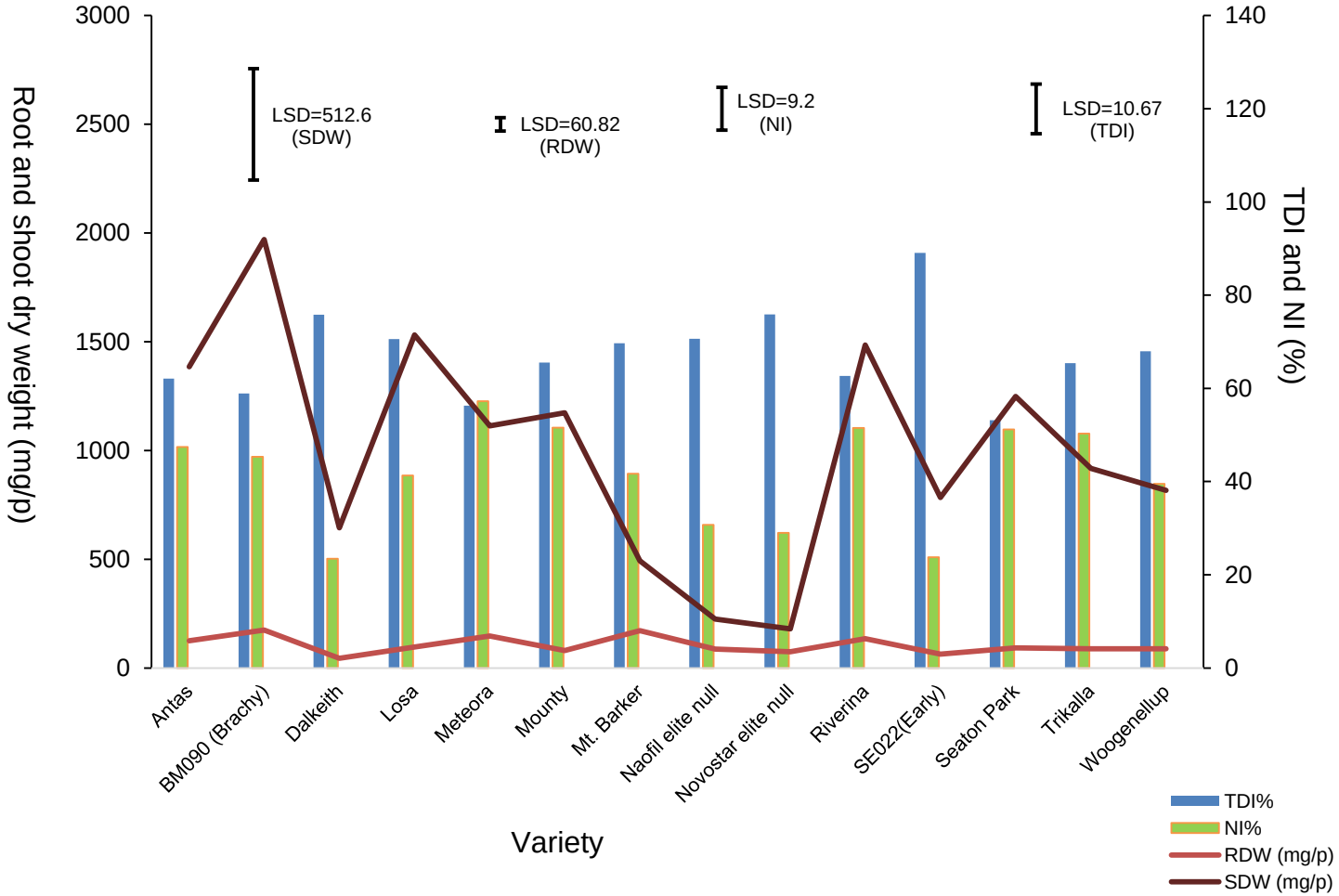
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW).

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	< 0.001	10.67	NS	*	< 0.001	9.201	< 0.001	60.82	< 0.001	512.6
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	NS	*	0.02	5.12						
Fungicide spray	NS	*	NS	*	NS	*	NS	*	NS	*

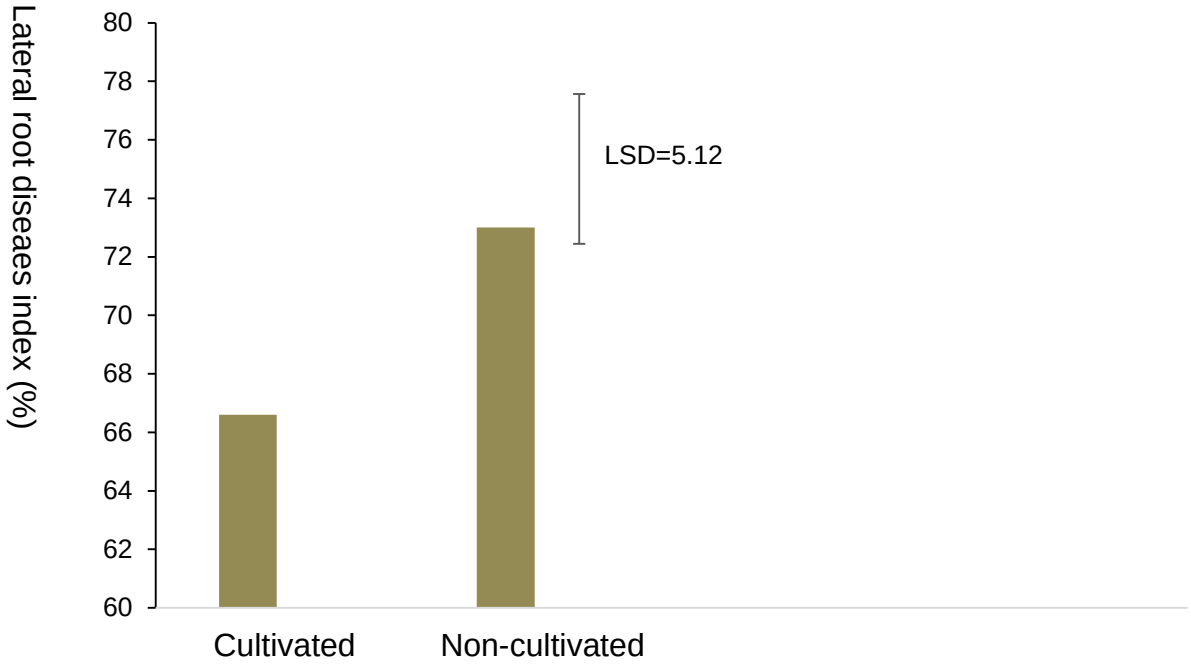
Table. Effect of variety and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW)

Factor		TDI%	LDI%	NI%	RDW (mg/p)	SDW (mg/p)
Variety	Antas	62.1	79.53	47.45	125.5	1384.7
	BM090 (Brachy)	58.94	74.43	45.3	174.6	1970.7
	Dalkeith	75.83	81.23	23.48	44.4	645.2
	Losa	70.58	76.48	41.3	97.2	1531
	Meteora	56.3	75.02	57.25	147.5	1112.8
	Mounty	65.53	78.13	51.55	80.8	1174.1
	Mt. Barker	69.7	79.43	41.7	171.9	493
	Naofil elite null	70.64	77	30.72	87.7	224.4
	Novostar elite null	75.87	77.2	28.97	74.2	180.6
	Riverina	62.69	76.55	51.49	135.4	1485.4
	SE022(Early)	89.07	82	23.76	63.5	784.2
	Seaton Park	53.2	78.4	51.17	92.3	1248.5
	Trikkala	65.41	78.57	50.27	88.9	917.4
	Woogenellup	67.97	78.99	39.51	88.2	816.5
Cultivation	Cultivated	*	66.6	*	*	*
	Non-cultivated	*	73	*	*	*

Effect of variety on tap root disease index (TDI%), nodulation index (NI%) and root and shoot dry weight (mg/p) (RDW abd SDW



Effect of cultivation on lateral root disease index (%) (second sampling)



APPENDIX 11 – 2016 Denmark WA: First and second sampling results

First sampling (young seedlings)

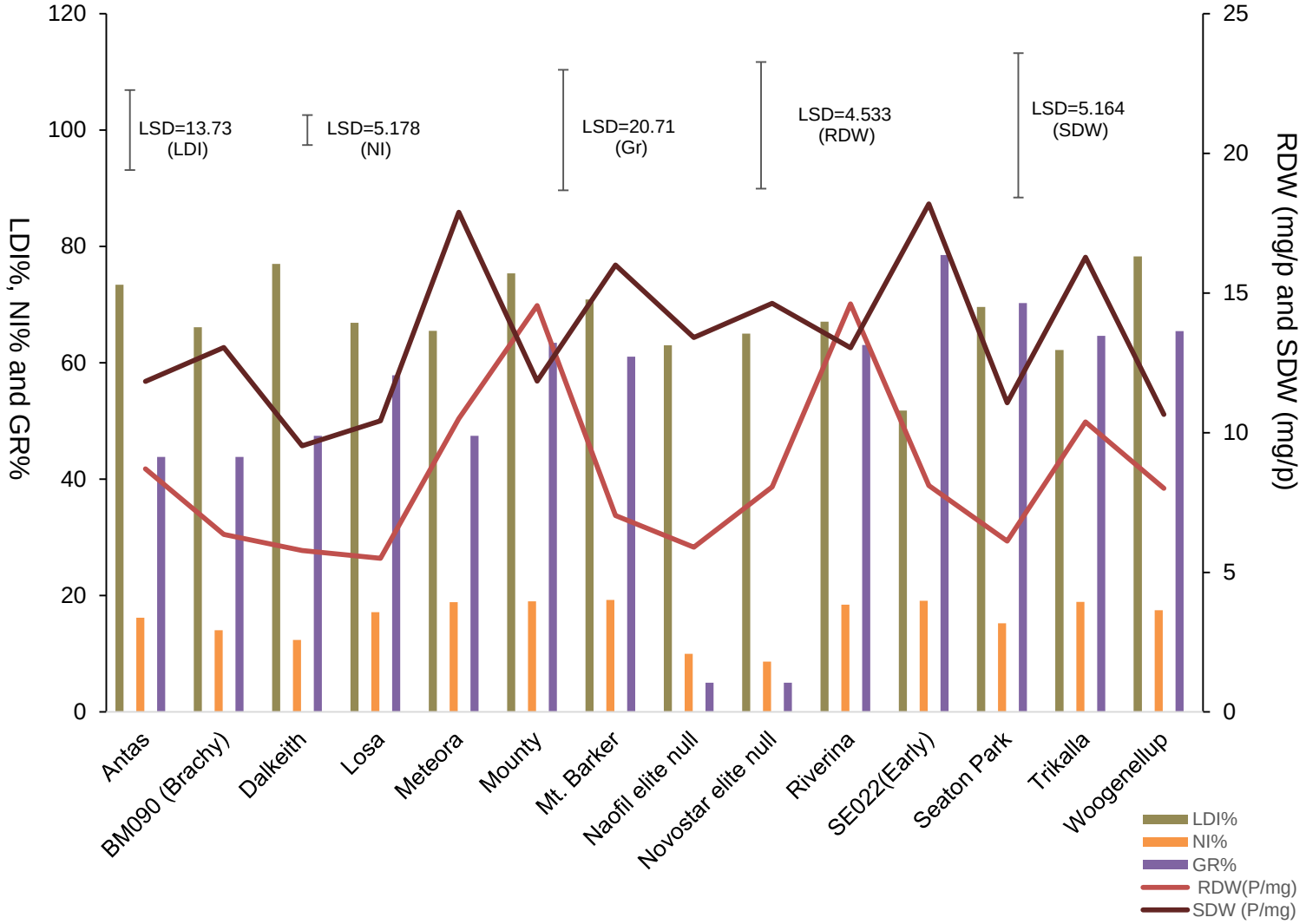
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

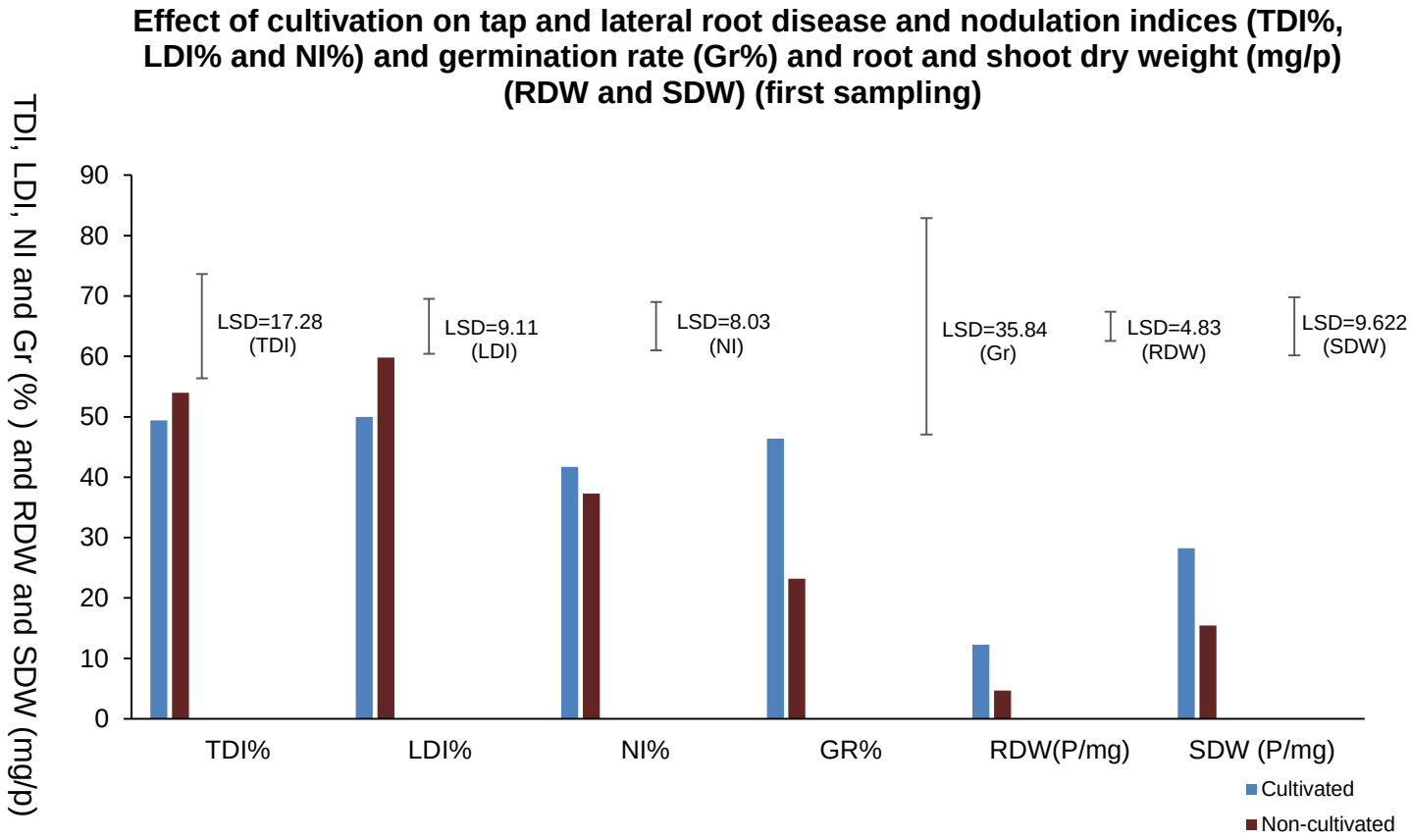
	TDI%		LDI%		NI%		RDW (P/mg)		SDW (P/mg)		GR%	
Factor	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$
Variety	NS	*	0.049	13.73	< 0.001	5.178	< 0.001	4.533	0.015	5.164	< 0.001	20.71
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	NS	*	0.041	9.11	NS	*	0.012	4.8301	0.021	9.6219	NS	*

Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor		TDI%	LDI%	NI%	RDW(P/mg)	SDW (P/mg)	GR%
Variety	Antas	75.44	73.39	16.18	8.699	11.83	43.85
	BM090 (Brachy)	70.34	66.09	14.07	6.354	13.05	43.85
	Dalkeith	71.59	76.97	12.39	5.776	9.53	47.45
	Losa	69.29	66.86	17.16	5.5	10.42	57.85
	Meteora	62.19	65.49	18.88	10.506	17.89	47.45
	Mounty	71.53	75.37	19.01	14.549	11.84	63.45
	Mt. Barker	68.94	70.88	19.24	7.035	16	61.05
	Naofil elite null	69.72	63.02	9.98	5.896	13.41	5.05
	Novostar elite null	65.39	65.02	8.64	8.062	14.63	5.05
	Riverina	67.14	67.09	18.45	14.606	13.04	63.05
	SE022(Early)	62.75	51.79	19.12	8.108	18.19	78.5
	Seaton Park	69.92	69.61	15.26	6.12	11.07	70.25
	Trikkala	62.12	62.22	18.91	10.384	16.28	64.65
	Woogenellup	74.33	78.27	17.47	8.01	10.65	65.45
Seed treatment	Biochanin A	70.9	78.8	18.1	6.75	12.388	54.4
	Complete fertilizer	67	73.5	17.5	7.426	15.174	52
	Formononetin	58.9	71.9	19.9	10.732	23.668	36
	Mixed fungicide	62.3	75.1	17.8	8.409	16.214	44.8
	Nil	66.5	77.2	19.1	8.452	15.275	43.2
	Salicylic acid	71.4	76.2	18.7	8.321	14.557	51.6
Cultivation	Cultivated	49.4	50	41.7	12.278	28.229	46.4
	Non-cultivated	54	59.8	37.3	4.682	15.46	23.2

Effect of variety on lateral root disease and nodulation index (LDI% and NI%) and germination rate (and root and shoot dry weight (mg/p) (first sampling)




Effect of cultivation on tap and lateral root disease and nodulation indices (TDI%, LDI% and NI%) and germination rate (Gr%) and root and shoot dry weight (mg/p) (RDW and SDW) (first sampling)
TDI, LDI, NI and Gr (%) and RDW and SDW (mg/p)
90
80
70
60
50
40
30
20
10
0
LSD=17.28 (TDI)
LSD=9.11 (LDI)
LSD=8.03 (NI)
LSD=35.84 (Gr)
LSD=4.83 (RDW)
LSD=9.622 (SDW)
TDI%
LDI%
NI%
GR%
RDW(P/mg)
SDW (P/mg)
Cultivated
Non-cultivated

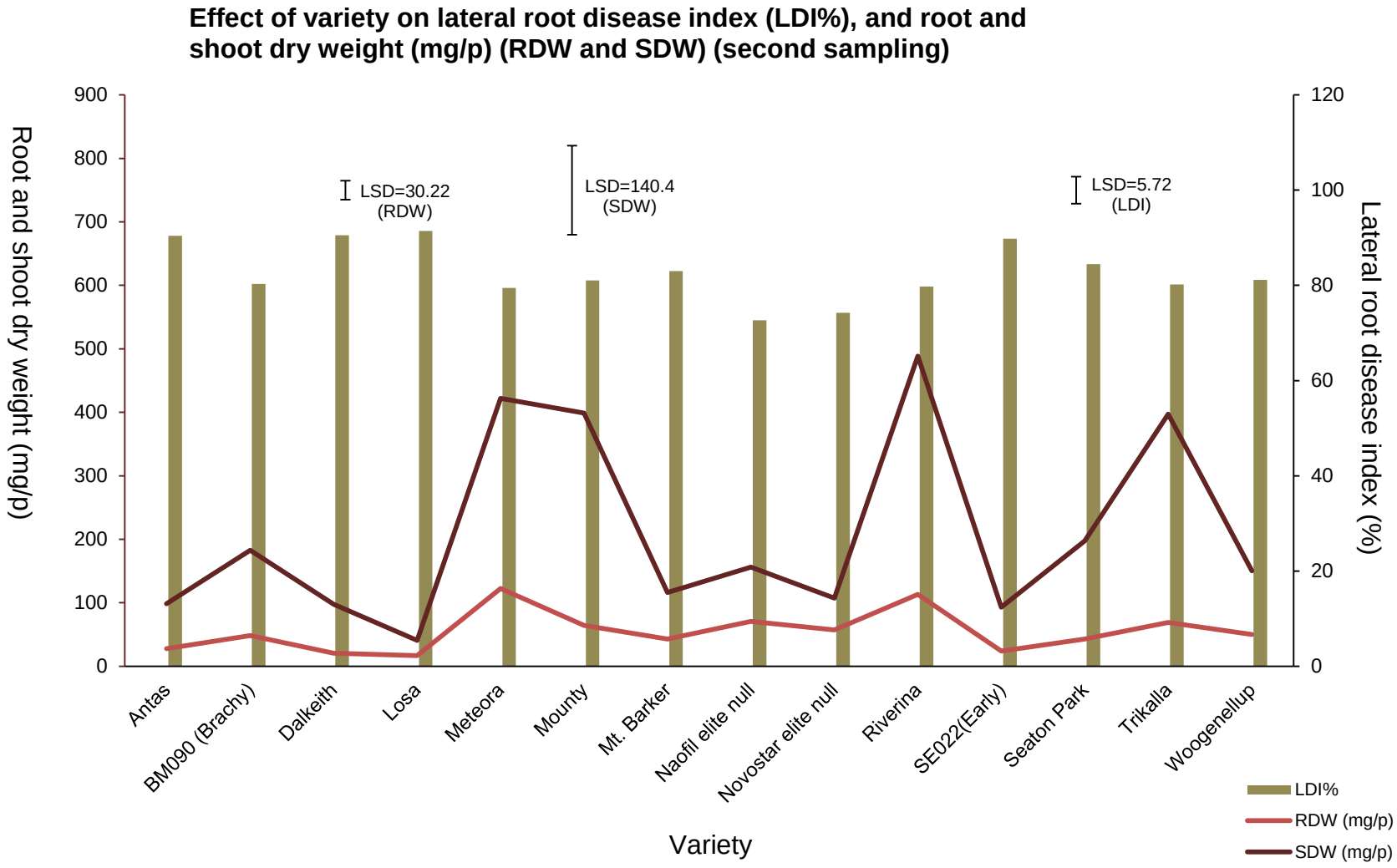
Second sampling (adult plants)

Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW).

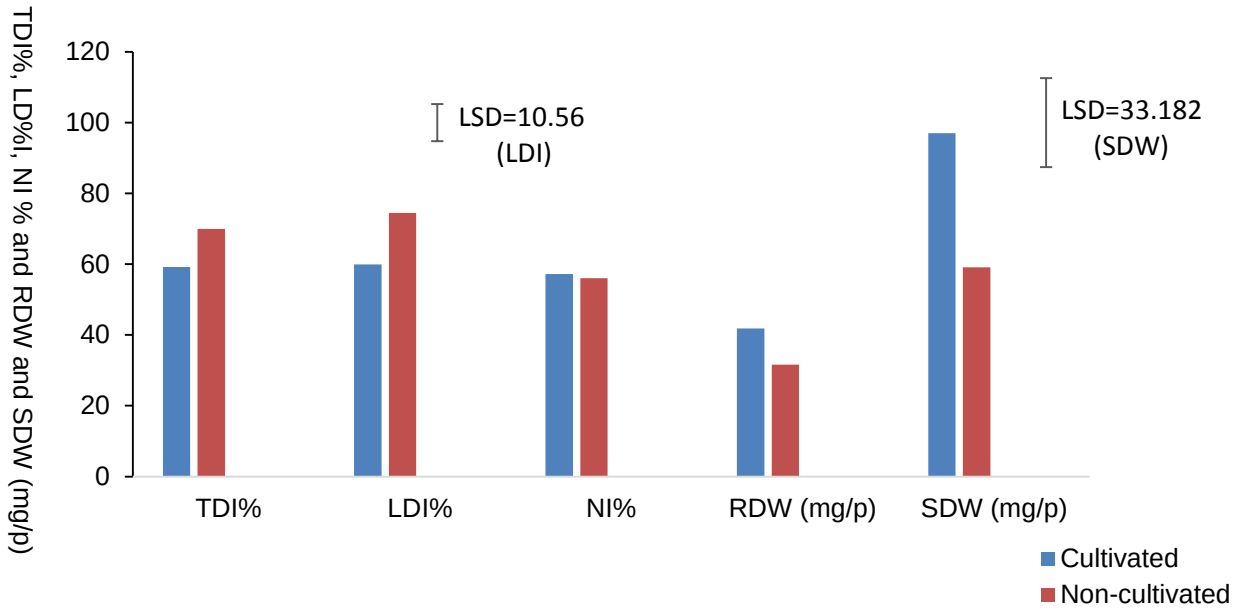
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	NS	*	< 0.001	5.715	NS	*	< 0.001	30.22	< 0.001	140.4
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	NS	*	0.012	10.56	NS	*	NS	*	0.03	33.182
Spray	NS	*	NS	*	NS	*	NS	*	0.041	52.8645

Table. Effect of variety, cultivation and spray on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW)

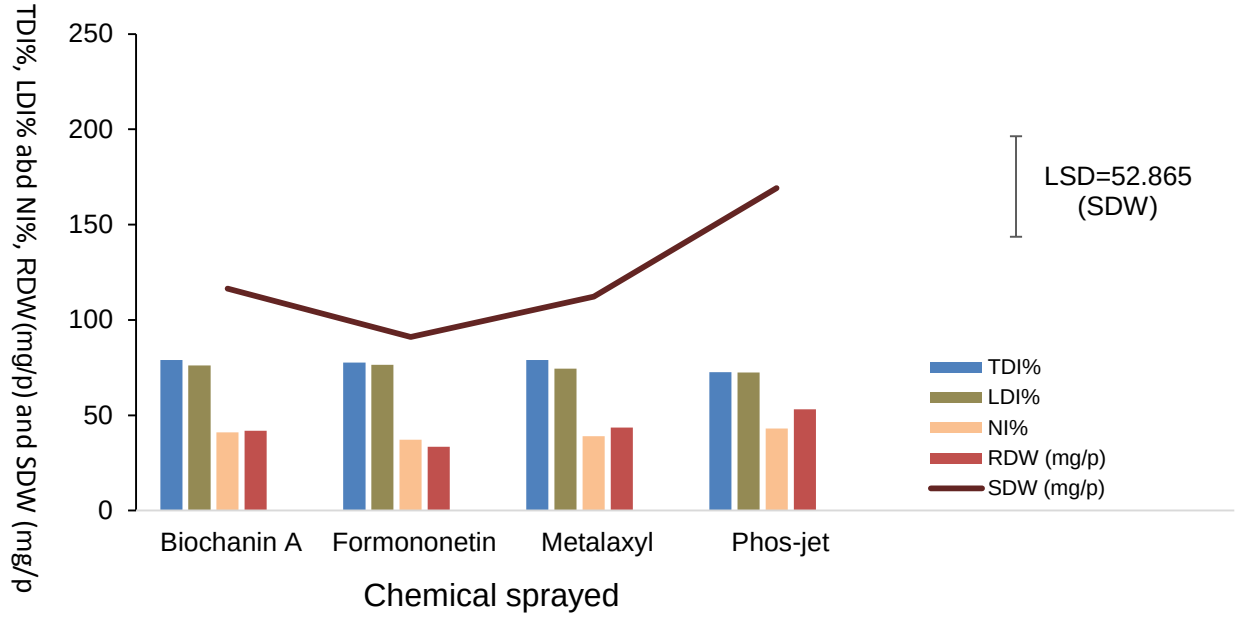
Factor		TDI%	LDI%	NI%	RDW (mg/p)	SDW (mg/p)
Variety	Antas	80.03	90.42	*	27.5	98.5
	BM090 (Brachy)	80.03	80.31	*	48.11	182.7
	Dalkeith	78.2	90.52	*	20.33	97.8
	Losa	76.27	91.42	*	16.74	40.7
	Meteora	79.6	79.46	*	122.43	422.1
	Mounty	80.05	81.05	*	64.33	399
	Mt. Barker	79.65	82.98	*	42.85	116.2
	Naofil elite null	86.71	72.65	*	70.62	156.2
	Novostar elite null	80.03	74.26	*	56.96	107.1
	Riverina	80.21	79.73	*	113.33	488.6
	SE022(Early)	79.16	89.82	*	23.96	92.8
	Seaton Park	72.03	84.45	*	42.63	197.6
	Trikkala	80.05	80.21	*	69.15	397
	Woogenellup	79.48	81.16	*	50.19	150.3
Cultivation	Cultivated	59.2	59.9	57.2	41.87	96.984
	Non-cultivated	70	74.5	56	31.65	59.15
Spray	Biochanin A	78.9	76.2	41.1	41.871	116.45
	Formononetin	77.6	76.5	37.1	33.462	91.059
	Metalaxyl	78.9	74.5	39	43.563	112.212
	Phos-jet	72.6	72.4	43	53.127	169.209


Effect of variety on lateral root disease index (LDI%), and root and shoot dry weight (mg/p) (RDW and SDW) (second sampling)
Root and shoot dry weight (mg/p)
Lateral root disease index (%)
LSD=30.22 (RDW)
LSD=140.4 (SDW)
LSD=5.72 (LDI)
900
800
700
600
500
400
300
200
100
0
120
100
80
60
40
20
0
Antas
BM090 (Brachy)
Dalkeith
Losa
Meteora
Mounty
Mt. Barker
Naofil elite null
Novostar elite null
Riverina
SE022(Early)
Seaton Park
Trikalla
Woogenellup
Variety
LDI%
RDW (mg/p)
SDW (mg/p)

Effect of cultivation on tap, lateral and nodulation indices (TDI%, LDI% and NI%) and root and shoot dry weight (mg/p)(RDW and SDW)



Effect of chemical spray on tap, lateral and nodulation indices (TDI%, LDI% and NI% and root and shoot dry weight (mg/p) (RDW and SDW)



APPENDIX 12 – 2016 South Eastern SA: First and second sampling results

First sampling (young seedlings)

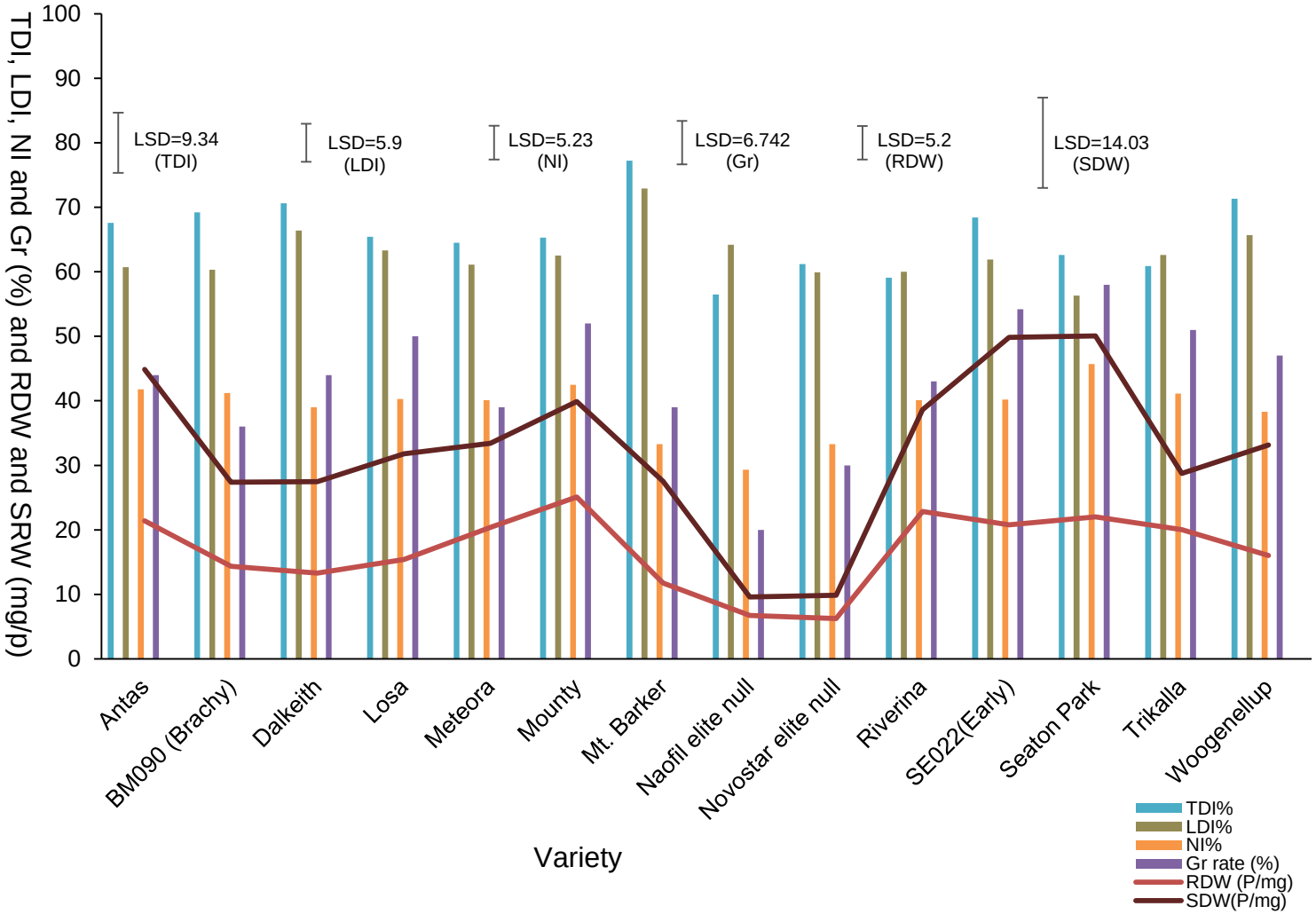
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr rate (%)	
	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$	$P_{0.05}$	$LSD_{0.05}$
Variety	0.004	9.34	<.001	5.9	<.001	5.23	<.001	5.1962	<.001	14.0317	<.001	6.742
Seed treatment	0.044	8.525	NS	*	0.007	7.951	NS	*	NS	*	0.001	10.21
Cultivation	NS	*	NS	*	NS	*	0.001	3.1227	0.007	7.8074	NS	*

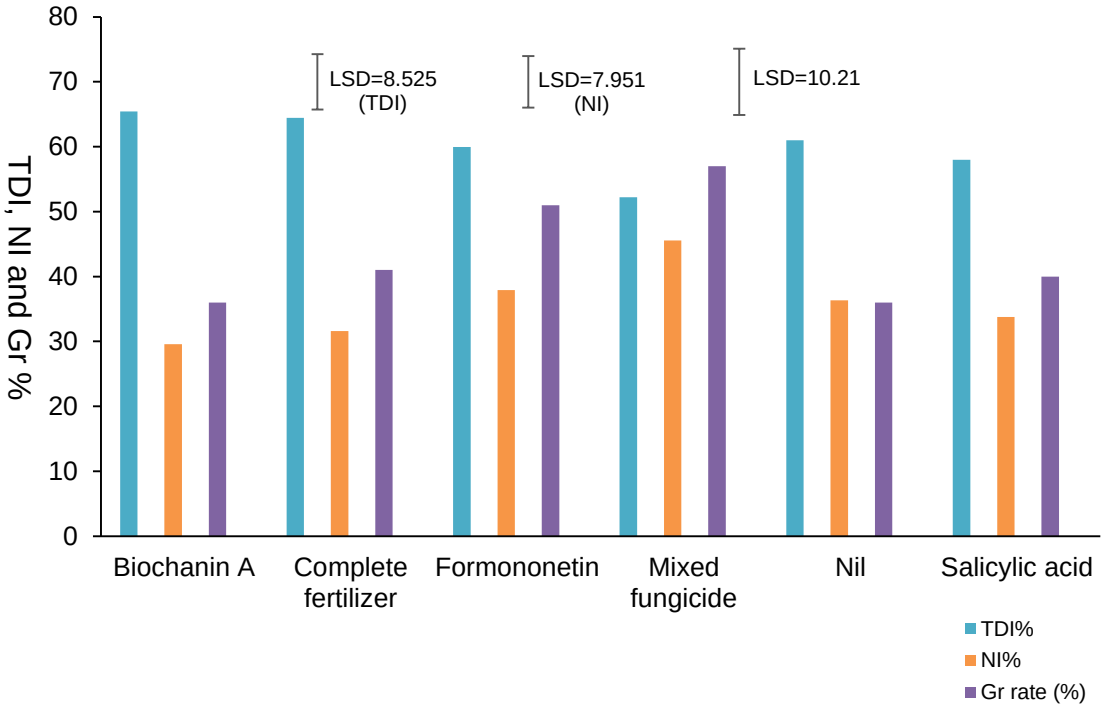
Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	Treatment	TDI%	LDI%	NI%	RDW (P/mg)	SDW(P/mg)	Gr rate (%)
Variety	Antas	67.6	60.7	41.8	21.383	44.869	44
	BM090 (Brachy)	69.2	60.3	41.2	14.369	27.361	36
	Dalkeith	70.6	66.4	39	13.289	27.453	44
	Losa	65.4	63.3	40.3	15.386	31.793	50
	Meteora	64.5	61.1	40.1	20.361	33.423	39
	Mounty	65.3	62.5	42.5	25.109	39.868	52
	Mt. Barker	77.2	72.9	33.3	11.745	27.525	39
	Naofil elite null	56.5	64.2	29.3	6.725	9.594	20
	Novostar elite null	61.2	59.9	33.3	6.242	9.873	30
	Riverina	59.1	60	40.1	22.837	38.597	43
	SE022(Early)	68.4	61.9	40.2	20.766	49.849	54.2
	Seaton Park	62.6	56.3	45.7	22.018	50.051	58
	Trikalla	60.9	62.6	41.1	20.03	28.743	51
	Woogenellup	71.3	65.7	38.3	16.04	33.155	47
Seed treatment	Biochanin A	65.41	73.16	29.58	14.05	29.25	36
	Complete fertilizer	64.46	70.6	31.6	15.32	37.01	41
	Formononetin	59.95	64.62	37.89	17.91	37.32	51
	Mixed fungicide	52.23	65.4	45.58	17.98	40.58	57
	Nil	61	69.32	36.33	13.96	29.47	36
	Salicylic acid	57.99	68.92	33.78	14.61	30.38	40
Cultivation	Cultivation	59.7	74.1	35.7	17.908	33.83	34.8
	Non-cultivation	61.2	75.6	36.4	11.68	21.969	38.8

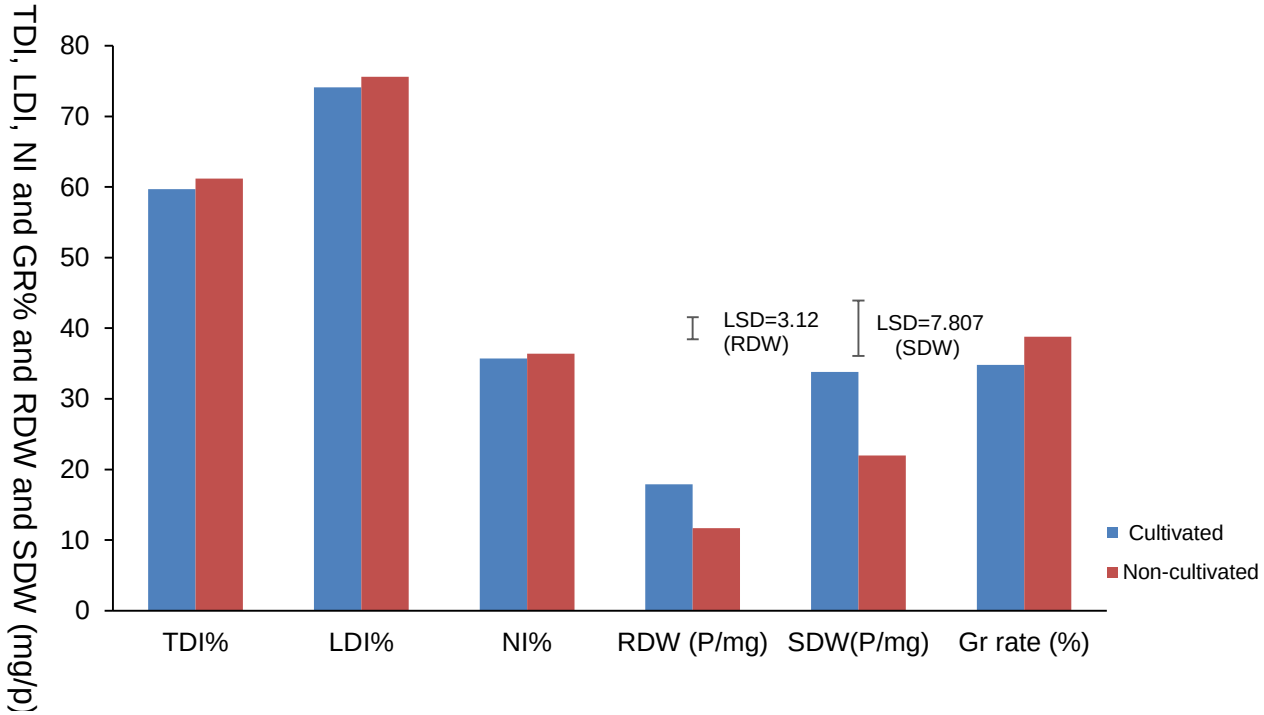
Effect of variety on tap and lateral root disease and nodulation indices (TDI, LDI and NI %) and germination rate (Gr%) and root and shoot dry weight (mg/p) (RDW and SDW) (first sampling)



Effect of seed treatment on tap root disease and nodulation indices (TDI% and NI%) and germination rate (Gr%) (first sampling)



Effect of cultivation on tap and lateral root and nodulation indices (TDI, LDI, NI%), and root and shoot dry weight (RDW and SDW (p/mg) and Gr rate% (first sampling)



Second sampling (adult plants)

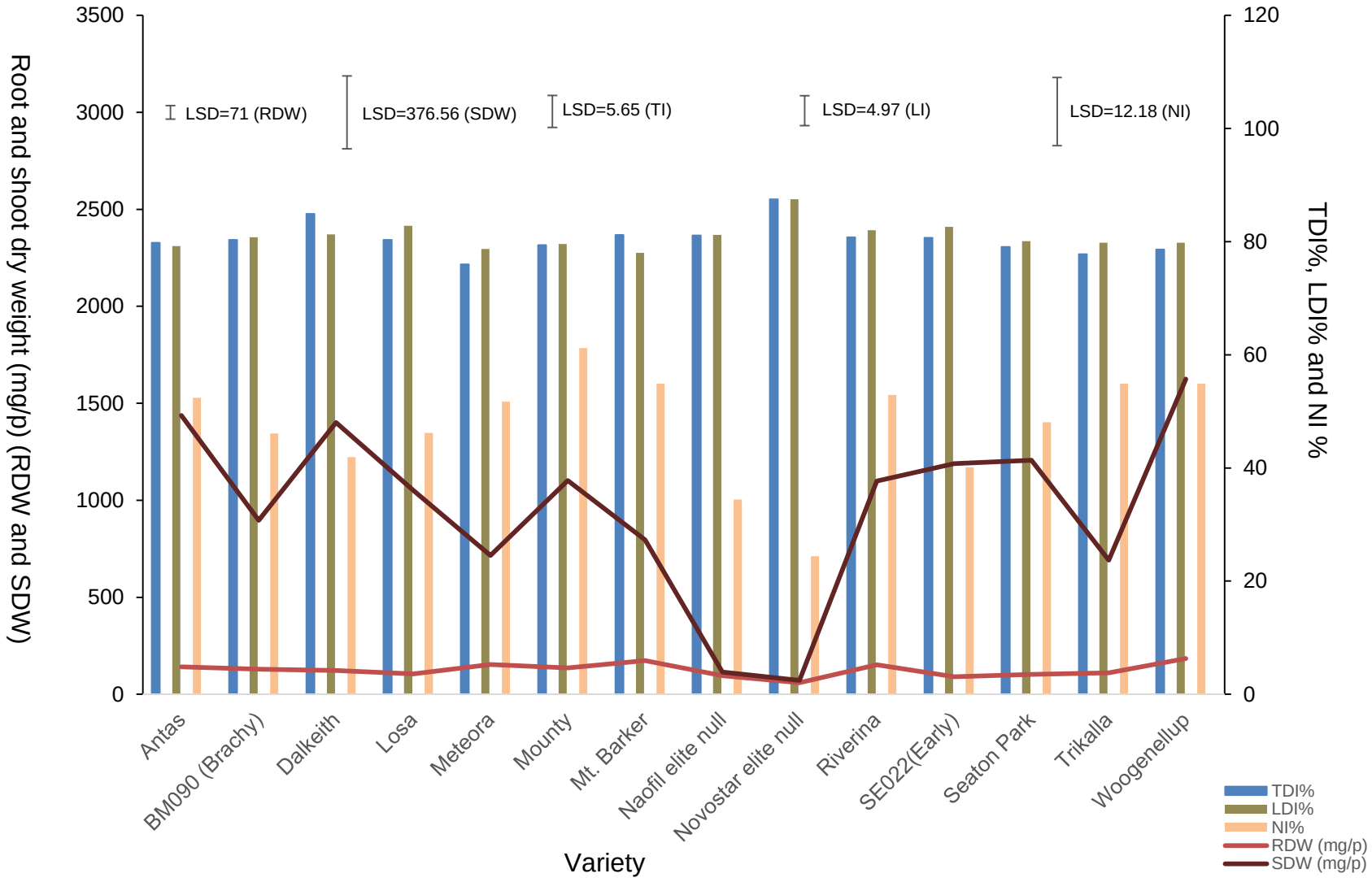
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW).

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	0.038	5.65	0.065	4.97	<0.001	12.18	0.056	70.996	0.003	376.56
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	NS	*	NS	*	NS	*	NS	*	NS	*
Spray	NS	*	NS	*	NS	*	NS	*	NS	*

Table. Effect of variety, on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW)

Factor		TDI%	LDI%	NI%	RDW (mg/p)	SDW (mg/p)
Variety	Antas	79.8	79.2	52.4	141.571	1437.067
	BM090 (Brachy)	80.3	80.8	46.1	129.736	896.895
	Dalkeith	84.9	81.3	41.9	123.133	1400.568
	Losa	80.3	82.8	46.2	104.534	1051.265
	Meteora	76	78.7	51.7	153.608	714.973
	Mounty	79.4	79.6	61.2	135.461	1101.765
	Mt. Barker	81.2	78	54.9	172.832	795.859
	Naofil elite null	81.1	81.2	34.4	95.033	114.381
	Novostar elite null	87.5	87.5	24.4	59.234	71.575
	Riverina	80.8	82	52.9	151.521	1099.594
	SE022(Early)	80.7	82.6	40.1	90.362	1189.316
	Seaton Park	79.1	80.1	48.1	102.504	1206.044
	Trikkala	77.8	79.8	54.9	110.446	690.983
	Woogenellup	78.6	79.8	54.9	183.57	1625.388

Effect of variety on root and shoot dry weight (mg/p) (RDW and SDW) and tap and lateral root disease index (TDI% and LDI%) and nodulatioon index (NI%) (second sampling)



APPENDIX 13 – 2016 NSW: First and second sampling results

First sampling (young seedlings)

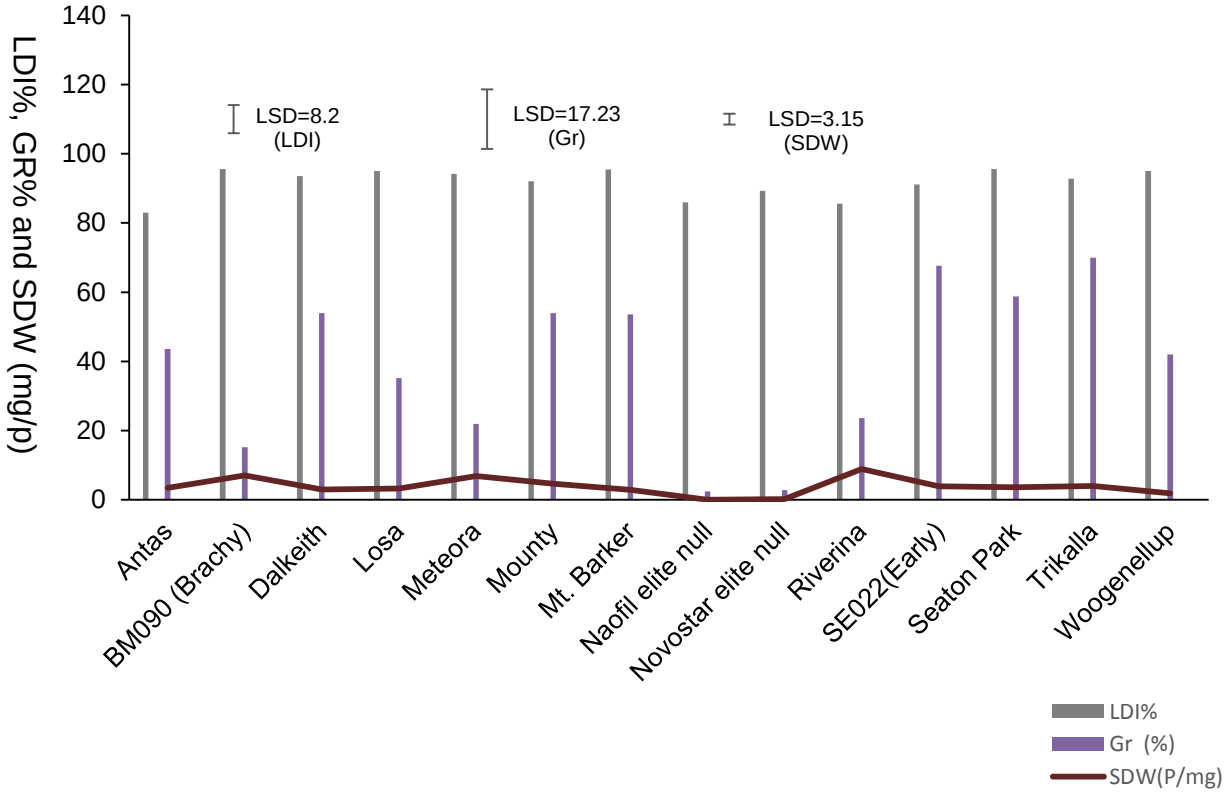
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr rate (%)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	NS	*	0.025	8.1	NS	*	NS	*	<0.001	3.1525	<0.001	17.23
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*

Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	Treatment	TDI%	LDI%	NI%	RDW (P/mg)	SDW(P/mg)	Gr rate (%)
Variety	Antas	78.2	83	0	2.653	3.497	43.6
	BM090 (Brachy)	86.4	95.6	0	3.573	7.027	15.2
	Dalkeith	80.5	93.5	0	1.982	2.952	54
	Losa	86.1	95	0	2.815	3.266	35.2
	Meteora	79.7	94.2	0.2	5.662	6.852	22
	Mounty	74.1	92.1	0	3.281	4.671	54
	Mt. Barker	80.8	95.5	0	2.457	2.874	53.6
	Naofil elite null	87	86	0	0	0	2.4
	Novostar elite null	85.3	89.3	0	0.1	0.2	2.8
	Riverina	75.4	85.6	0.2	4.597	8.873	23.6
	SE022(Early)	78.2	91.1	0	1.619	3.935	67.6
	Seaton Park	85.7	95.6	0.3	2.283	3.67	58.8
	Trikkala	80.6	92.8	0	2.788	4	70
	Woogenellup	81.7	95	0	0.808	1.906	42
Seed treatment	Biochanin A	86.8	98.4	0.4	1.384	1.821	53.2
	Complete fertilizer	90.5	98.8	1.8	1.419	2.378	56.4
	Formononetin	83.4	96.7	1.1	1.467	3.499	47.6
	Mixed fungicide	84.9	95	0.7	2.592	2.83	65.6
	Nil	87.6	99.1	1.1	1.555	2.158	72.4
	Salicylic acid	87.2	96.6	2.4	1.823	2.286	65.6
Cultivation	Cultivated	71.9	75.6	7.8	2.256	5.309	27.2
	Non-cultivated	74.5	77.9	6.5	1.251	4.321	27.8

Effect of variety on lateral root disease index (LDI%), germination rate (Gr%) and shoot dry weight (SDW (mg/p)) (first sampling)



Second sampling (adult plants)

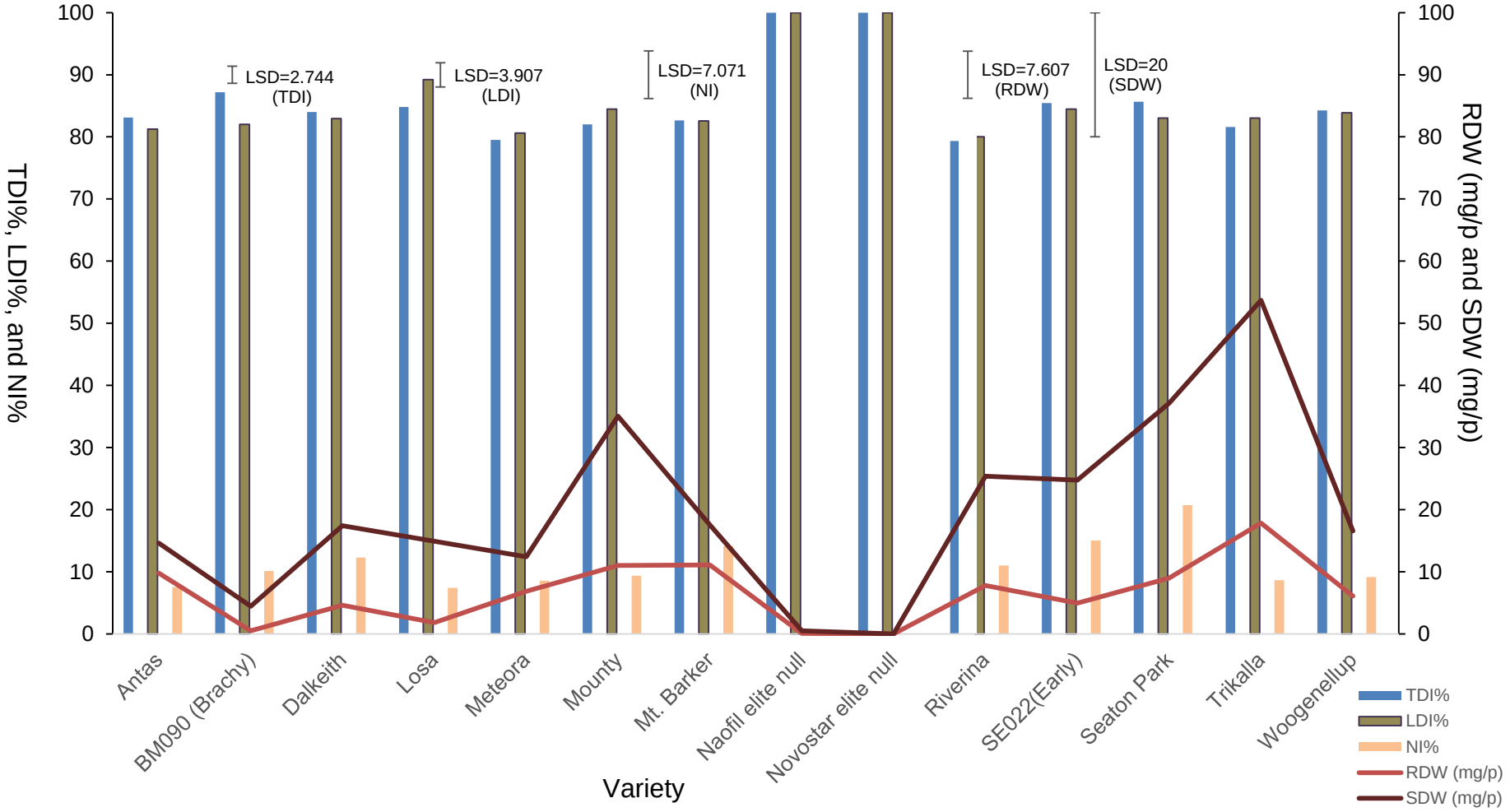
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW).

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	< 0.001	2.744	< 0.001	3.907	< 0.001	7.071	< 0.001	7.607	< 0.001	20
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	NS	*	NS	*	NS	*	NS	*	NS	*
Spray	NS	*	NS	*	NS	*	NS	*	NS	*

Table. Effect of variety, on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW)

Factor		TDI%	LDI%	NI%	RDW (mg/p)	SDW (mg/p)
	Antas	83.1	81.25	7.56	9.818	14.61
	BM090 (Brachy)	87.19	82.01	10.141	0.5	4.47
	Dalkeith	83.99	82.94	12.318	4.619	17.44
	Losa	84.82	89.23	7.407	1.839	14.94
	Meteora	79.5	80.63	8.576	6.877	12.41
	Mounty	82.03	84.48	9.355	11.01	35.05
Variety	Mt. Barker	82.66	82.57	14.174	11.131	17.57
	Naofil elite null	99.99	100.	0.007	0.012	0
	Novostar elite null	100	100	0	0	0
	Riverina	79.33	80.01	11.007	7.803	25.38
	SE022(Early)	85.43	84.46	15.074	4.963	24.77
	Seaton Park	85.67	83.02	20.736	8.974	37.14
		81.58	83.04	8.672	17.867	53.71
	Woogenellup	84.25	83.88	9.167	6.11	16.59

Effect of variety on tap root disease, lateral root disease and nodulation indices (TDI%, LDI% and NI%) and root and shoot dry weight (mg/p) (RDW and SDW)



APPENDIX 14 – 2016 Bendigo region Victoria: First and second sampling results

First sampling (young seedlings)

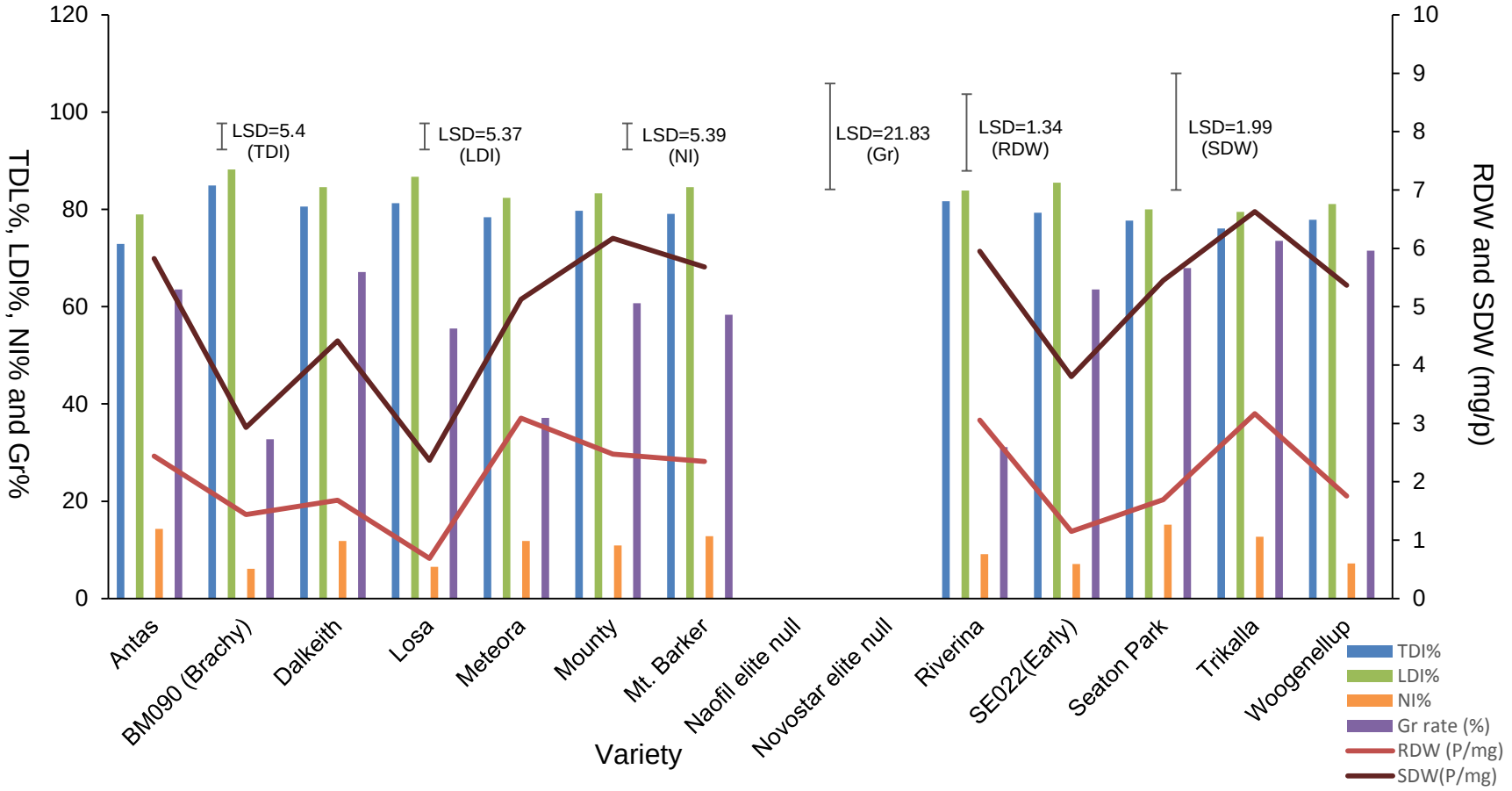
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr rate (%)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	0.015	5.4	0.02	5.37	0.008	5.39	0.005	1.3401	0.001	1.9909	< 0.001	21.83
Seed treatment	NS	*	NS	*	NS	*	NS	*	0.024	1.3117	NS	*
Cultivation	NS	*	0.042	4.68	NS	*	0.033	0.84	NS	*	NS	*

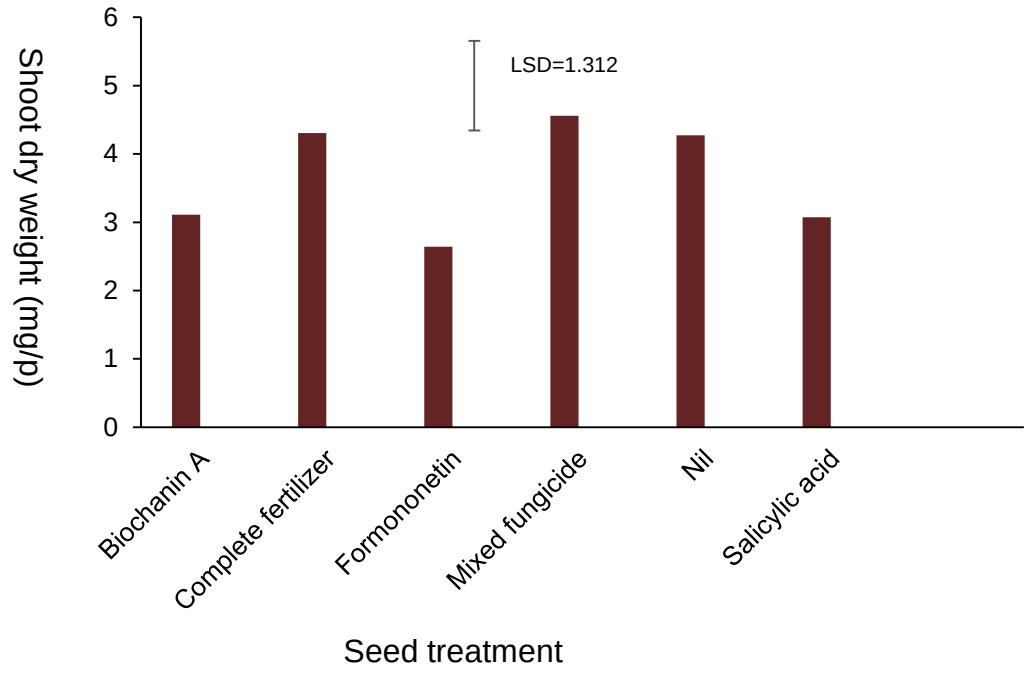
Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	Treatment	TDI%	LDI%	NI%	RDW (P/mg)	SDW(P/mg)	Gr rate (%)
Variety	Antas	72.9	79	14.3	2.438	5.825	63.54
	BM090 (Brachy)	84.9	88.2	6.1	1.44	2.93	32.74
	Dalkeith	80.6	84.6	11.8	1.683	4.412	67.14
	Losa	81.3	86.7	6.5	0.689	2.366	55.54
	Meteora	78.4	82.4	11.8	3.088	5.125	37.14
	Mounty	79.7	83.3	10.9	2.475	6.17	60.74
	Mt. Barker	79.1	84.6	12.8	2.35	5.681	58.34
	Naofil elite null	*	*	*	*	*	0
	Novostar elite null	*	*	*	*	*	0
	Riverina	81.7	83.9	9.1	3.055	5.949	31.14
	SE022(Early)	79.3	85.5	7.1	1.149	3.803	63.54
	Seaton Park	77.7	80	15.2	1.691	5.45	67.94
	Trikkala	76.1	79.5	12.7	3.168	6.631	73.54
	Woogenellup	77.9	81.1	7.2	1.754	5.369	71.54
Seed treatment	Biochanin A	81.1	86.8	14.4	1.546	3.109	60.4
	Complete fertilizer	80.4	86.7	12.2	2.117	4.307	60.4
	Formononetin	80.3	87.3	13.4	0.641	2.644	64
	Mixed fungicide	77.6	81.8	16	1.583	4.556	61.2
	Nil	78.7	85.9	14.6	1.293	4.273	77.6
	Salicylic acid	79.4	87	14.4	1.133	3.075	68.8
Cultivation	Cultivated	67.3	76.7	16.5	3.874	6.667	35.6
	Non-cultivated	67.8	81.6	15	2.94	5.763	40.4

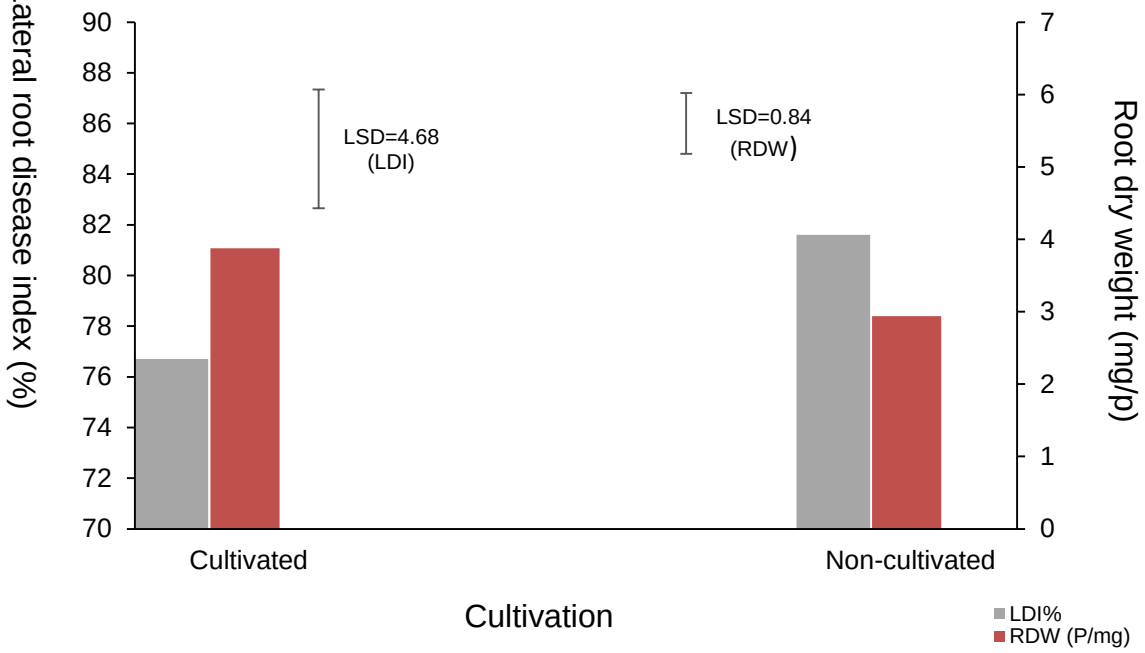
Effect of variety on tap and root disease and nodulation indices (TDI%, LDI% and NI%) and germination rate (Gr and root and shoot dry weight (mg/p) (RDW and SDW) (first sampling)



Effect of seed treatment on shoot dry weight (mg/p)



Effect of cultivation on lateral root disease index (LDI%) and root dry weight (RDW(mg/p))



Second sampling (adult plants)

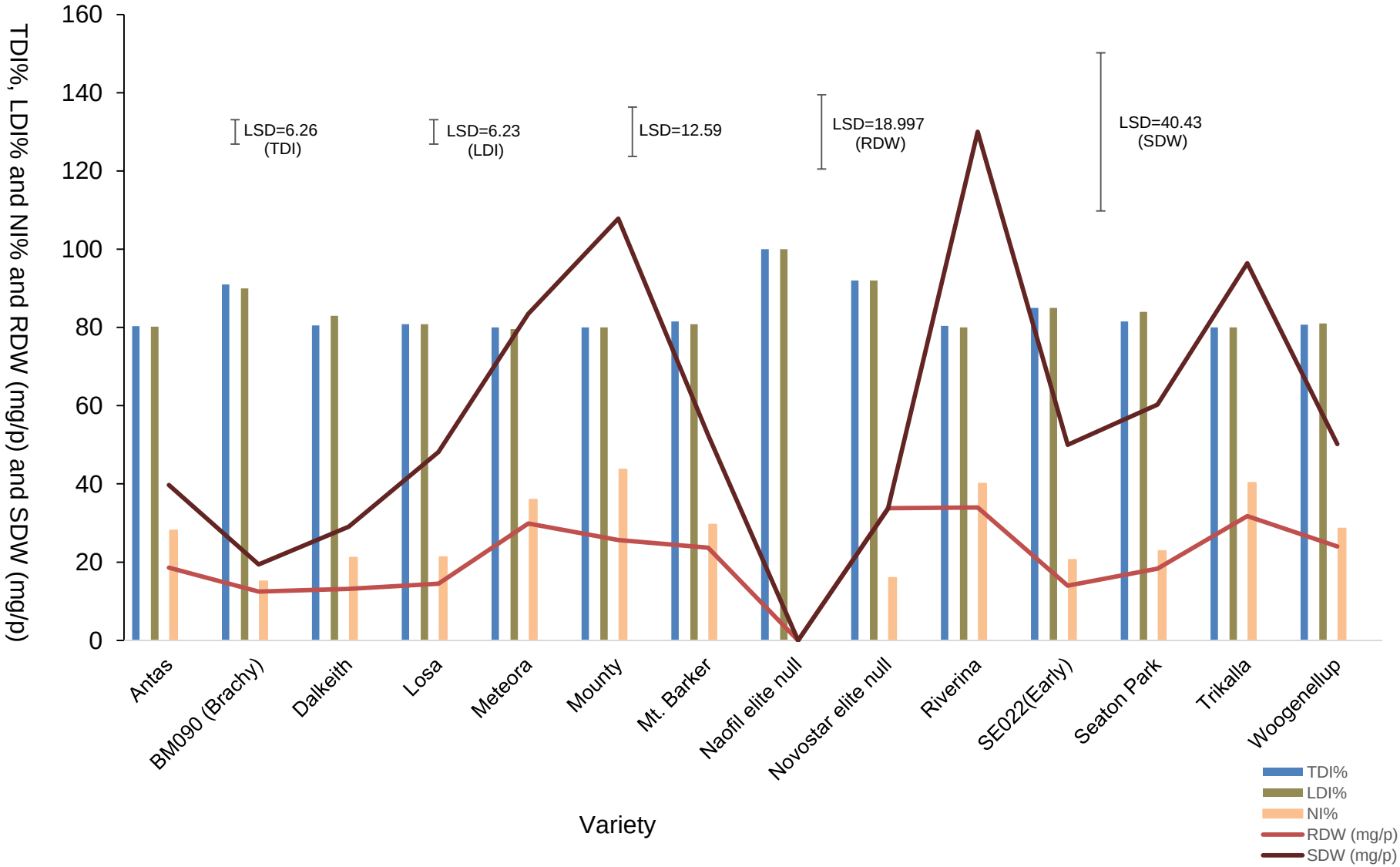
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW).

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	6.26	<0.001	6.23	<0.001	12.59	0.027	18.9974	<.001	40.4297
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	0.054	7.12	0.21	2.97	0.934	6.89	0.188	7.89	0.182	29.7629
Spray	NS	*	NS	*	NS	*	NS	*	NS	*

Table. Effect of variety and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW)

Factor		TDI%	LDI%	NI%	RDW (mg/p)	SDW (mg/p)
Variety	Antas	80.3	80.2	28.1	18.585	39.703
	BM090 (Brachy)	91	90	15.1	12.477	19.403
	Dalkeith	80.5	83	21.2	13.195	29.088
	Losa	80.8	80.8	21.3	14.465	48.181
	Meteora	80	79.6	36	29.899	83.459
	Mounty	80	80	43.7	25.641	107.842
	Mt. Barker	81.5	80.8	29.6	23.687	52.519
	Naofil elite null	100	100	0	0	0
	Novostar elite null	92	92	16	33.8	33.6
	Riverina	80.4	80	40.1	34.003	130.001
	SE022(Early)	85	85	20.6	13.996	49.967
	Seaton Park	81.5	84	22.9	18.36	60.259
	Trikkala	80	80	40.3	31.794	96.375
	Woogenellup	80.7	81	28.6	24.035	50.158
Cultivation	Cultivated	68.1	76.6	31	28.188	88.028
	Non-cultivated	75	78.4	31	23.211	69.008

Effect of variety on tap and lateral root disease indices and nodulation index (TDI%, LDI% and NI%) and root and shoot dry weight (mg/p) (RDW and SDW)



APPENDIX 15 – 2016 Western Victoria: First and second sampling results

First sampling (young seedlings)

Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr rate (%)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<.001	9.98	<.001	10.23	<.001	6.32	<.001	6.0826	<.001	19.239	<.001	11.02
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	NS	*	NS	*	NS	*	NS	*	NS	*	NS	*

Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	Treatment	TDI%	LDI%	NI%	RDW (P/mg)	SDW(P/mg)	Gr rate (%)
Variety	Antas	65.7	64.6	28.7	15.047	43.541	53
	BM090 (Brachy)	63.3	63.9	30.4	16.815	49.992	45
	Dalkeith	56	74.4	30.4	14.183	71.528	70
	Losa	63.2	64.7	35	16.5	59.348	61
	Meteora	54.9	60.5	29.1	21.099	46.764	53
	Mounty	55.4	70.5	36.1	20.593	55.481	63
	Mt. Barker	74.8	76.2	23.7	18.533	63.717	45
	Naofil elite null	59.6	58	18.1	0	8.808	19
	Novostar elite null	63.4	62.2	20.5	7.342	10.599	26
	Riverina	48.1	53	33.3	23.295	47.259	50
	SE022(Early)	71.6	54.9	29	19.843	65.678	62
	Seaton Park	57.1	66.5	30.8	17.06	52.243	64
	Trikkala	45.8	52	32	22.707	56.668	65
	Woogenellup	57.1	69.2	31.5	15.238	51.6	64
Seed treatment	Biochanin A	62.8	73	31	14.913	36.044	55
	Complete fertilizer	57.6	76.7	42.2	16.986	44.417	57
	Formononetin	58.4	67.6	37.7	16.501	40.035	49
	Mixed fungicide	60.9	68.5	37.2	16.839	42.544	45
	Nil	56.3	71.5	38.8	19.003	34.79	51
	Salicylic acid	57.1	71.1	35.6	14.279	35.057	49
Cultivation	Cultivated	57.1	71.8	36.4	12.979	49.092	32.8
	non-cultivated	59.8	73.9	41.1	8.554	35.433	40.4

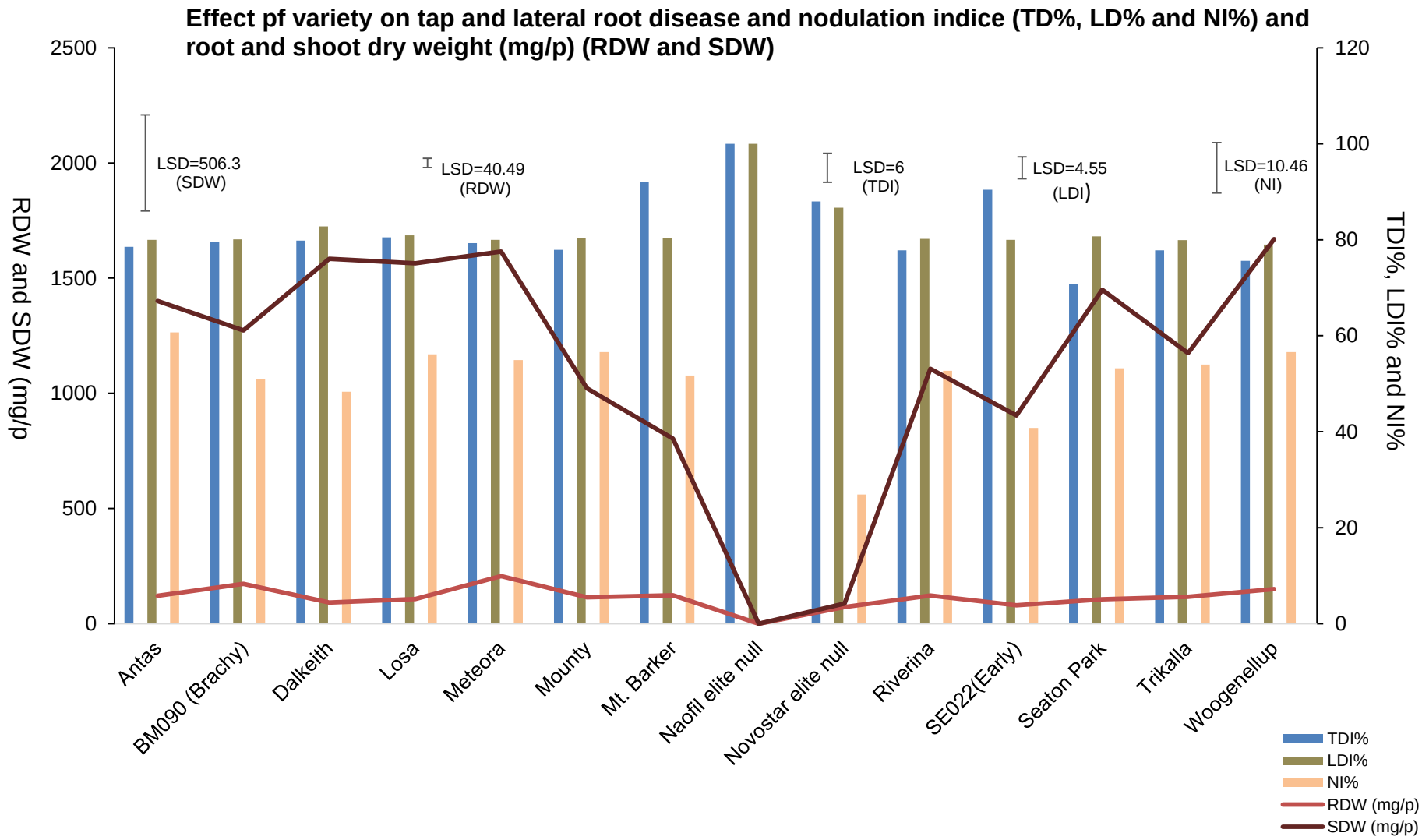
Second sampling (adult plants)

Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW).

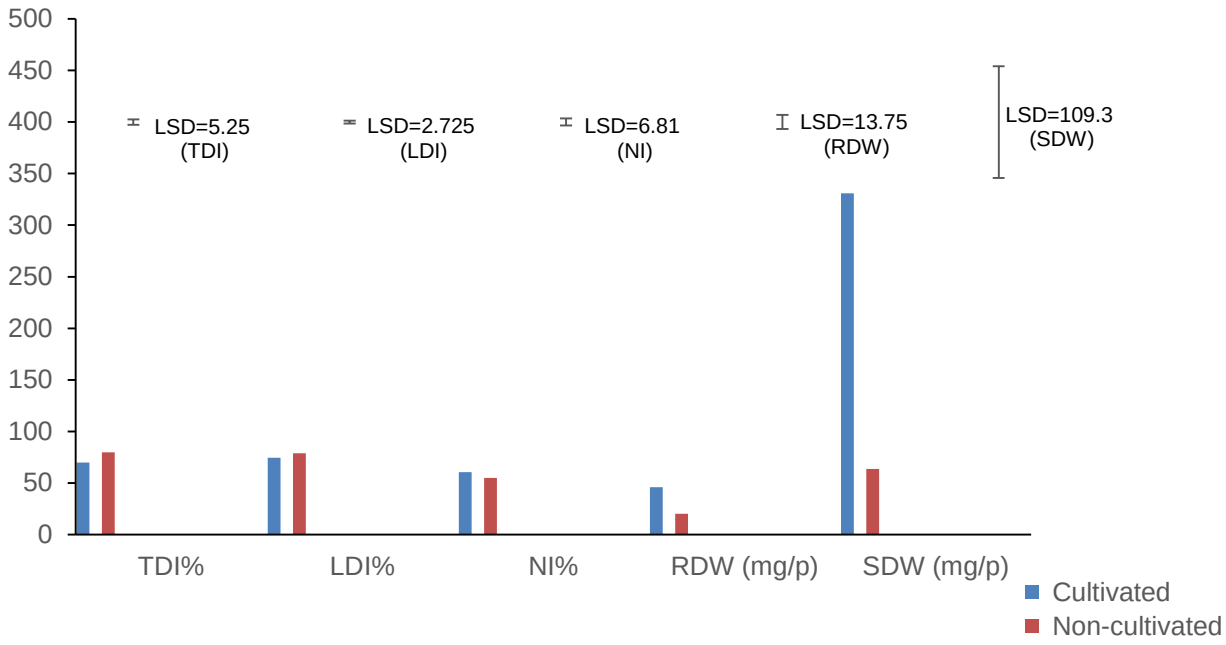
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	6	<0.001	4.55	<0.001	10.46	<0.001	40.4892	<0.001	506.2924
Seed treatment	NS	*	NS	*	NS	*	NS	*	NS	*
Cultivation	0.002	5.25	0.005	2.72	0.091	6.81	0.002	13.7474	<0.001	109.3045
Spray	NS	*	NS	*	NS	*	NS	*	NS	*

Table. Effect of variety and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW)

Factor		TDI%	LDI%	NI%	RDW (mg/p)	SDW (mg/p)
	Antas	78.5	80	60.7	120.438	1400.402
	BM090 (Brachy)	79.6	80.1	50.9	172.498	1272.915
	Dalkeith	79.8	82.8	48.3	91.823	1584.149
	Losa	80.5	80.9	56.1	107.031	1564.768
	Meteora	79.3	80	54.9	206.187	1615.24
	Mounty	77.9	80.4	56.6	114.085	1021.642
Variety	Mt. Barker	92.1	80.3	51.7	123.091	802.531
	Naofil elite null	100	100	0	0	0
	Novostar elite null	88	86.7	26.9	72.571	87.8
	Riverina	77.8	80.2	52.7	121.43	1106.352
	SE022(Early)	90.4	80	40.8	79.512	903.311
	Seaton Park	70.8	80.7	53.2	105.382	1450.072
		77.8	79.9	54	116.843	1174.625
	Woogenellup	75.6	79	56.6	150.385	1669.583
	Cultivated	69.8	74.4	60.6	46.008	330.929
	Non-cultivated	79.6	78.9	54.9	20.067	63.456


Effect pf variety on tap and lateral root disease and nodulation indice (TD%, LD% and NI%) and root and shoot dry weight (mg/p) (RDW and SDW)
RDW and SDW (mg/p
TDI%, LDI% and NI%
2500
2000
1500
1000
500
0
120
100
80
60
40
20
0
LSD=506.3 (SDW)
LSD=40.49 (RDW)
LSD=6 (TDI)
LSD=4.55 (LDI)
LSD=10.46 (NI)
Antas
BM090 (Brachy)
Dalkeith
Losa
Meteora
Mounty
Mt. Barker
Naofil elite null
Novostar elite null
Riverina
SE022(Early)
Seaton Park
Trikalla
Woogenellup
TDI%
LDI%
NI%
RDW (mg/p)
SDW (mg/p)

Effect of cultivation on tap and lateral root disease and nodulation indices (TDI%, LDI% and NI%) and root and shoot dry weight (mg/p) (RDW and SDW)



APPENDIX 16 – 2016 Wagin WA: First and second sampling results

First sampling (young seedlings)

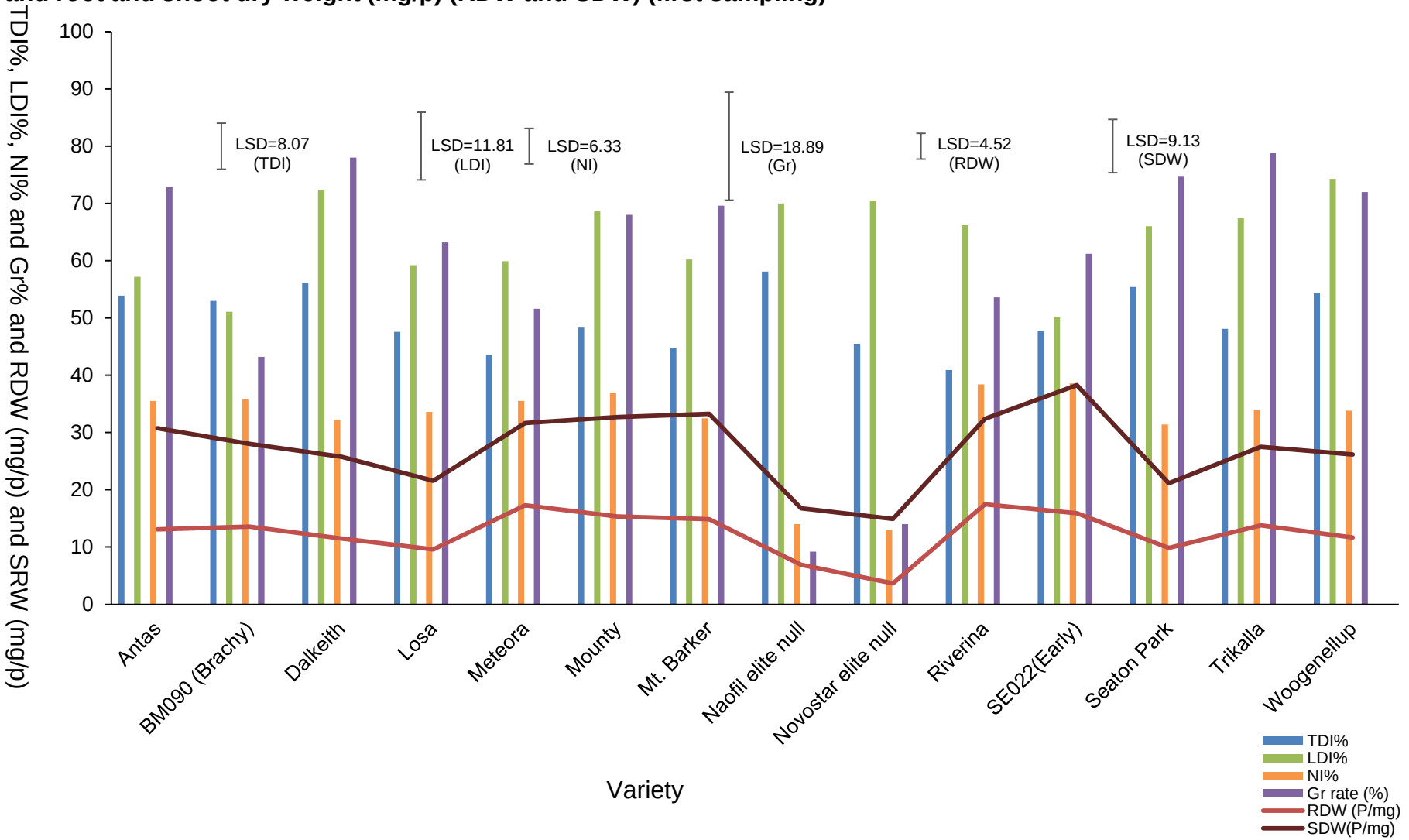
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)		Gr rate (%)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	8.07	<0.001	11.81	<0.001	6.33	<0.001	4.5234	<0.001	9.3145	<0.001	18.89
Seed treatment	0.631	6.55	0.262	7.1	0.004	2.43	<0.001	2.0574	<0.001	7.7865	0.241	21.52
Cultivation	0.028	3.25	0.304	9.43	0.084	10.01	0.013	4.1778	0.01	11.783	<0.001	11.41

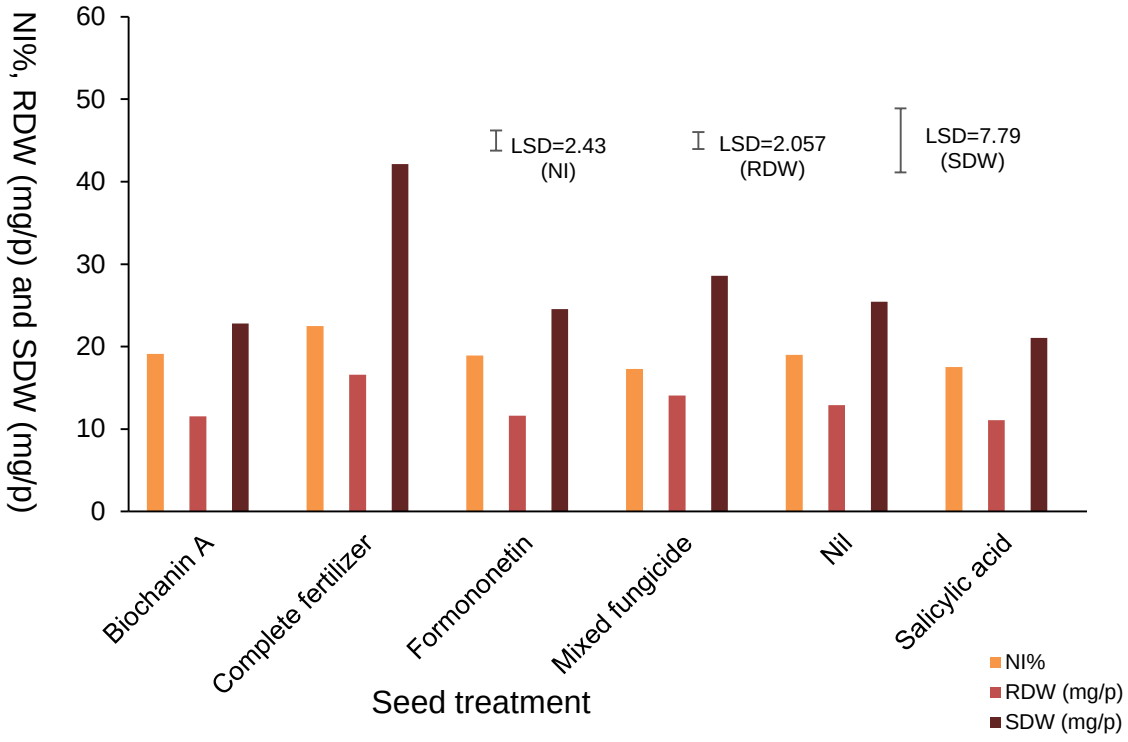
Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW) and germination rate (Gr rate %)

Factor	Treatment	TDI%	LDI%	NI%	RDW (P/mg)	SDW(P/mg)	Gr rate (%)
Cultivation	Cultivated	50	49.4	45	11.623	30.658	98.4
	Non-cultivated	46.1	53.4	36.8	5.216	11.152	50.4
Seed treatment	Biochanin A	63.7	68.1	19.1	11.546	22.785	73.2
	Complete fertilizer	60.3	62.6	22.5	16.57	42.14	69.2
	Formononetin	64.3	68	18.9	11.622	24.561	67.6
	Mixed fungicide	60.3	63.5	17.3	14.046	28.593	48.4
	Nil	61.7	63.6	19	12.883	25.426	70.4
	Salicylic acid	60.1	61.4	17.5	11.09	21.038	65.6
Variety	Antas	53.9	57.2	35.5	13.088	30.73	72.8
	BM090 (Brachy)	53	51.1	35.8	13.581	28.022	43.2
	Dalkeith	56.1	72.3	32.2	11.488	25.753	78
	Losa	47.6	59.2	33.6	9.567	21.582	63.2
	Meteora	43.5	59.9	35.5	17.3	31.639	51.6
	Mounty	48.3	68.7	36.9	15.328	32.692	68
	Mt. Barker	44.8	60.2	32.5	14.886	33.247	69.6
	Naofil elite null	58.1	70	14	6.907	16.763	9.2
	Novostar elite null	45.5	70.4	13	3.65	14.915	14
	Riverina	40.9	66.2	38.4	17.479	32.389	53.6
	SE022(Early)	47.7	50.1	38.6	15.902	38.29	61.2
	Seaton Park	55.4	66	31.4	9.849	21.13	74.8
	Trikkala	48.1	67.4	34	13.773	27.513	78.8
	Woogenellup	54.4	74.3	33.8	11.679	26.156	72

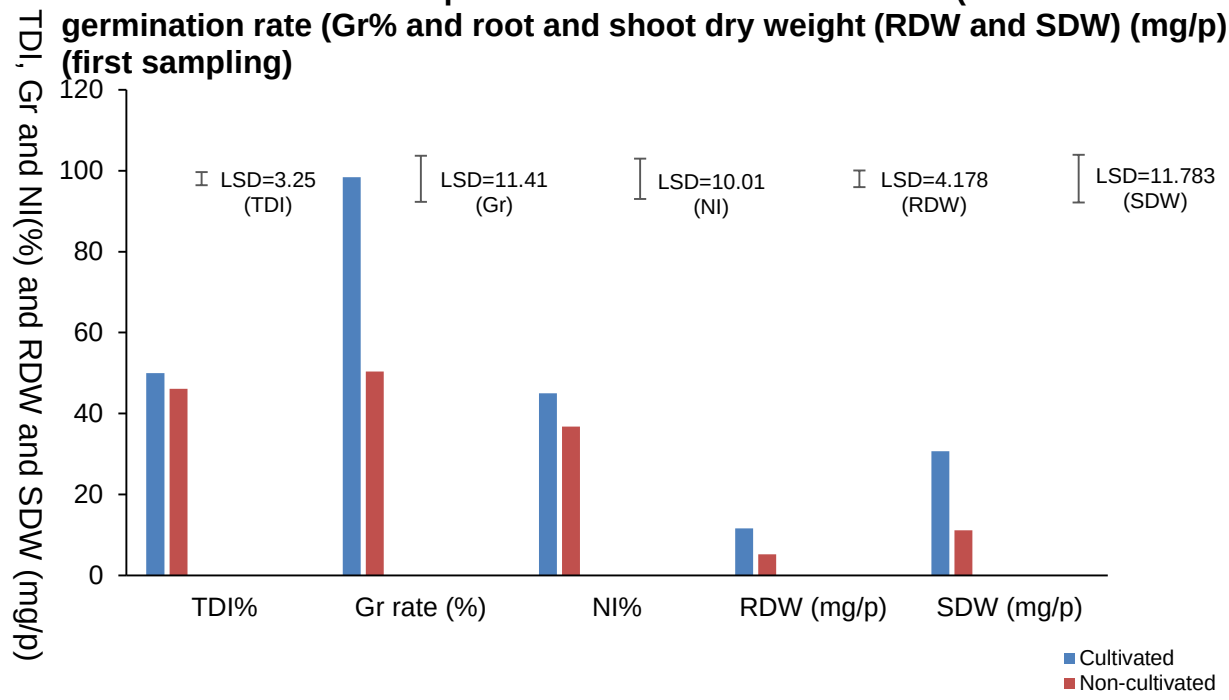
Effect of variety on tap and lateral root disease and nodulation indices (TDI%, LDI% and NI%) and germination rate (Gr% and root and shoot dry weight (mg/p) (RDW and SDW) (first sampling)



Effect of seed treatment on nodulation index (NI%) and root and sho dry weight (mg/p) (RDW and SDW) (first sampling)



Effect of cultivation on tap disease and nodulation indices (TDI% and NI% and germination rate (Gr% and root and shoot dry weight (RDW and SDW) (mg/p) (first sampling)



Second sampling (adult plants)

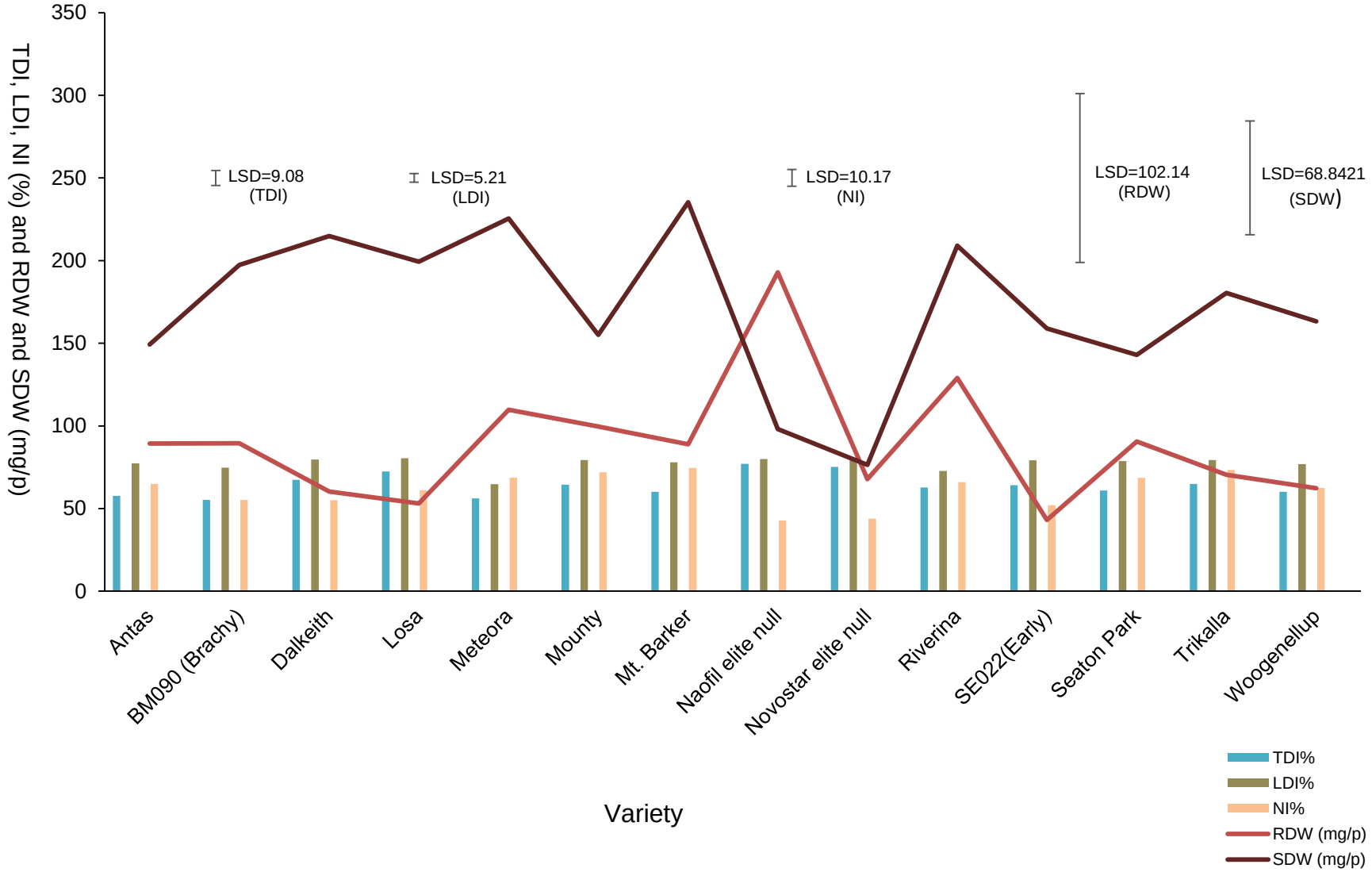
Table. Statistical effect of main factors on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW).

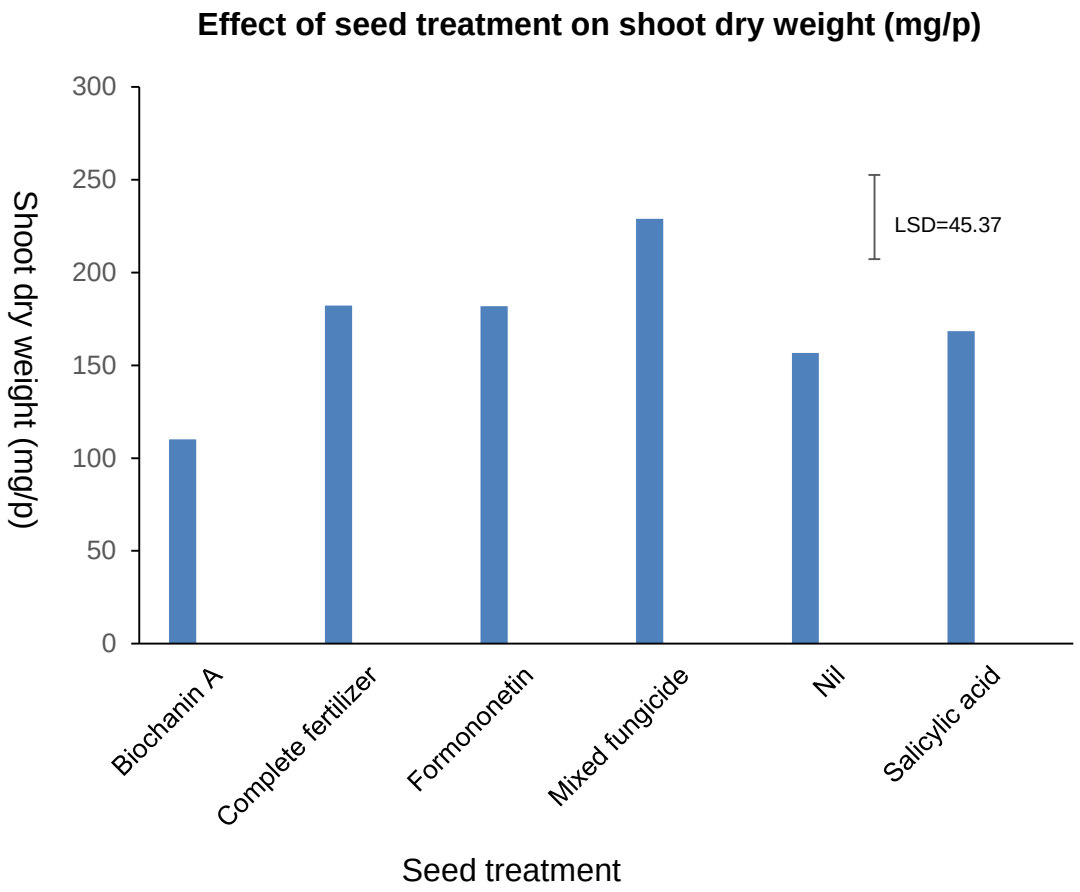
Factor	TI %		LI %		NI%		RDW (mg/p)		SDW (mg/p)	
	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$	$p_{0.05}$	$LSD_{0.05}$
Variety	<0.001	9.08	<0.001	5.21	<0.001	10.17	NS	*	<0.001	68.8421
Seed treatment	NS	*	NS	*	NS	*	NS	*	0.001	45.3697
Cultivation	NS	*	NS	*	NS	*	NS	*	NS	*
Spray	NS	*	NS	*	NS	*	NS	*	NS	*

Table. Effect of variety, seed treatment and cultivation on tap and lateral root disease and nodulation indices (TI%; LI% and NI%), and root and shoot dry weights (RDW and SDW)

Factor		TDI%	LDI%	NI%	RDW (mg/p)	SDW (mg/p)
Variety	Antas	57.7	77.3	64.9	89.313	149.299
	BM090 (Brachy)	55.2	74.8	55.2	89.458	197.292
	Dalkeith	67.3	79.7	55	60.248	214.868
	Losa	72.5	80.4	61	53.025	199.374
	Meteora	56.1	64.8	68.7	109.724	225.545
	Mounty	64.4	79.4	72	99.59	155.023
	Mt. Barker	60.2	77.9	74.6	88.928	235.243
	Naofil elite null	77.1	80	42.8	192.884	98.13
	Novostar elite null	75.2	79.3	43.8	67.833	76.367
	Riverina	62.8	72.7	66	128.956	209.032
	SE022(Early)	64.1	79.2	52	43.062	158.991
	Seaton Park	60.9	78.8	68.6	90.5	142.899
	Trikkala	64.9	79.4	73.4	70.439	180.402
	Woogenellup	60.2	76.9	62.5	62.359	163.249
Seed treatment	Biochanin A	*	*	*	*	110.097
	Complete fertilizer	*	*	*	*	182.259
	Formononetin	*	*	*	*	181.84
	Mixed fungicide	*	*	*	*	229.031
	Nil	*	*	*	*	156.722
	Salicylic acid	*	*	*	*	168.345
Cultivation	Cultivated	70.3	78.8	47.4	64.898	200.519
	Non-cultivated	69.2	80.4	42.7	61.441	185.938

Effect of variety on tap root and lateral root disease and nodulation indices(TDI%, LDI% and NI%a) and root and shoot dry weight (RDW and SDW) (mg/p)




Effect of seed treatment on shoot dry weight (mg/p)
Shoot dry weight (mg/p)
300
250
200
150
100
50
0
LSD=45.37
Biochanin A
Complete fertilizer
Formononetin
Mixed fungicide
Nil
Salicylic acid
Seed treatment