FUNGI CAUSING ROOT DISEASE OF SEEDLING LUCERNE IN THE MURRAY MALLEE REGION OF SOUTH AUSTRALIA

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INTRODUCTION

Lucerne (*Medicago sativa*) is a deep-rooted perennial fodder plant that is used to reduce groundwater recharge in dryland cropping systems. Financial subsidies are offered to encourage farmers to plant lucerne in the Murray Mallee Region of South Australia (SA). However, root diseases are believed to be affecting successful plant establishment (1). Root diseases have not previously been considered a significant problem in the drier regions, but have long been recognised as a problem for lucerne grown under irrigation. Pathogens implicated in root diseases of irrigated lucerne in Australia include *Acrocalymma medicaganis*, *Colletotrichum trifolii, Fusarium* spp., *Phytophthora medicaganis*, *Rhizoctonia* spp. and *Sclerotium* sp. (2, 3 and 4). The aim of the research outlined here was to identify the principal pathogens of seedling lucerne grown under dryland conditions in the Murray Mallee.

MATERIALS AND METHODS

Up to 100 plants were collected from each of twenty sites, newly planted to lucerne, in the Murray Mallee in 2005 and 2006. Roots were scored for disease severity (0=no disease; 5=dead roots) and examined microscopically for the presence of fungal structures and disease symptoms.

Roots, of up to 10 plants per site, were washed, excess water removed and 1-2 mm pieces of diseased roots were placed onto tap water agar and media selective for *Pythium*, *Fusarium* and *Rhizoctonia*. Pure cultures were established from hyphal tips, identified to at least genus level using morphological characteristics and grouped according to cultural characteristics.

The numbers of Pratylenchus neglectus were quantified in the dried root samples using real-time PCR by the Root Disease Testing Service at SARDI.

Pathogenicity testing. Isolates were first screened for pathogenicity to lucerne seedlings using an *in-vitro* Petri-dish test. Those isolates resulting in high disease scores were subsequently used in pathogenicity tests carried out in pots in controlled growth conditions. Inoculum for *in-planta* pot tests was grown on sterilised millet seed mixed with course sand. Soil, collected from the Murray Mallee, was steam sterilised and mixed with inoculum (0.5% w/w) from each isolate. Five lucerne seeds were sown in pots containing 400 g of inoculated soil and maintained at 20^{o}C (15^{o}C for *Rhizoctonia*) for 4 weeks, and assessed for plant survival and scored for root disease severity (0-5 scale). Shoot and root dry weight (DW) were also measured and data analysed using ANOVA.

RESULTS

A total of 591 fungal isolates was established from the lucerne roots. Of known pathogens to lucerne roots, 21 isolates of *Rhizoctonia* (including binucleate species), 14 *Pythium*, 17 *Phoma*, six *Sclerotinia* and over 200 *Fusarium* spp. were obtained. Other fungi included *Alternaria*, *Epicoccum*, *Bipolaris*, *Papulospora*, *Cylindrocarpon*, and 170 unidentified fungi. *P. neglectus* were detected in the dry roots from every sample site, and ranged from 10 to 24 800 nematodes/g dry root (median = 1223 nematodes/g root).

Between four and 87 isolates were obtained per site and up to nine genera isolated per plant. Isolates of *Fusarium*, particularly *F. oxysporum,* were ubiquitous, whereas *Rhizoctonia* was recovered from 13 sites, *Pythium* from seven and *Phoma* from four. Disease scores ranged from 1.5 to 4.5 and there was no obvious link between the relative numbers of *P. neglectus*, or frequency of isolation of *Rhizoctonia*, *Pythium* or *Fusarium* spp. at each site.

Pathogenicity tests indicate that isolates of *Rhizoctonia*, *Fusarium*, *F. oxysporum*, *Pythium* and several unidentified fungi are pathogenic. Virulence varied among isolates. Several, e.g. *F. oxysporum*, caused significant ($P< 0.05$) seedling death. Others, e.g. *R. solani* Ag8, caused extensive rotting and lesions on roots, decreased shoot and root DW but resulted in little seedling mortality.

DISCUSSION

Results indicate that the cause of root disease(s) of seedling lucerne in the Murray Mallee is likely to be a complex of *Rhizoctonia*, *Pythium* and *Fusarium* spp. High frequency of *P. neglectus* indicates it might be contributing to disease. However, the exact nature of the interaction and relative importance of each organism is yet to be elucidated.

The remaining isolates in the collection will undergo pathogenicity tests and isolates confirmed as pathogenic will be identified to species level. Further work is needed to quantify yield losses associated with lucerne root diseases and to understand how paddock management might be used to influence pathogen levels and disease severity.

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REFERENCES

1. Colmagro, S.F., Ballard, R.A., Kobelt, E., Humphries, A. and McKay, A., (2006). Root diseases affect newly established dryland lucerne in the Murray Mallee region of South Australia. 4th Australasian Soilborne Diseases Symposium, Queenstown, New Zealand, Sept 2006.
2. Irwin J.A.G. (1977) Factors contributing to poor lucerne persistence in southern Queensland. Australian Journal of Experimental Agriculture and Animal Husbandry **17**, 998-1003.
3. Marclay M.D. (1970) *Fusarium oxysporum* as a cause of lucerne decline in Western Australia. Plant Disease Reporter **54**, 1061-1063.
4. Irwin J.A.G., Mackie J.M., Marney T.S., Musial J.M., Roberts S. (2004) Incidence of *Stagonospora meliloti* and *Acrocalymma medicaginis* in lucerne crowns and roots in eastern Australia, their comparative aggressiveness to lucerne and inheritance reaction to *S. meliloti* in lucerne. Australasian Plant Pathology **33,** 61-67.