ROOT DISEASES AFFECT NEWLY ESTABLISHED DRYLAND LUCERNE IN THE MURRAY MALLEE REGION OF SOUTH AUSTRALIA.

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INTRODUCTION

Lucerne (*Medicago sativa*) is an important deep-rooted perennial plant used to control water recharge in dryland cropping systems. Its use in the Murray Mallee Region of South Australia is being encouraged with financial subsidies, because of its ability to reduce water recharge of the saline groundwater and ultimately the amount of salt seeping into the lower Murray River.

Observations of poor growth, diseased root systems and responses to fungicide application at seeding have led to speculation that soilborne diseases may be adversely impacting on lucerne establishment in the Murray Mallee.

Root diseases of lucerne have previously been reported in southern Queensland (1), Western Australia (2) and South Australia (3). The pathogens implicated have included *Rhizoctonia* spp., *Fusarium* spp., *Sclerotium* sp., *Phytopthora medicaganis*, *Stagonospora meliloti*, *Colletotrichum trifolii* (1, 4), *Acrocalymma medicaganis* (4) and *Phomopsis* sp. (3). These reports have primarily been of lucerne grown under irrigation or in areas with substantially higher rainfall than the Murray Mallee of South Australia. To date, root disease has not been considered a significant problem in the drier regions.

The aims of the survey reported here were to:
1. determine if root disease(s) were impacting on the establishment of lucerne in the Murray Mallee, and
2. detect and quantify several putative pathogens using DNA-based assays of roots collected from the field.

MATERIALS AND METHODS

Twenty-two farms, with paddocks newly planted (*ca* one month old) to lucerne, in the Murray Mallee Region of South Australia were surveyed in August 2005. Soil samples were collected and nutrient analysis, pH and conductivity measured by CSBP soil analysis service in Western Australia. Paddock management data was provided by the farmer for each site. Twenty plants, were collected from each site, roots were scored for disease severity (0=no disease; 5=dark brown and no lateral roots), the % of root lesioning and root tipping. Plants varied between spade leaf and 12 leaf growth stages between sites. The number of N2-fixing nodules per plant was counted and root and shoot dry weight measured. Amounts of *Rhizoctonia solani* AG 8, AG 2.1 and AG 2.2, *Pythium* clade F (5), *Pratylenchus neglectus* and *P. thornei* DNA were quantified in the dried root samples from each site by the Root Disease Testing Service at SARDI.

RESULTS

There was evidence of disease symptoms on the roots of some of the plants sampled from all sites, although mean disease score was negligible (≤1) for 12 of the sites (Table 1). Plant roots from six of the 22 sites had significant levels of root disease with a mean disease score >2 and were characterised by an increase in the mean incidence of lesions (11 to 15% of the root system covered) and damaged root tips (10 to 28%). Within each sample there was also large variation in the extent of root damage to individual plants. This was noticeable in the samples with higher root disease scores where individual plants had lesions on 50% of the root system.

The lesions and root-tipping symptoms on the roots from many sites were typical of infection by the root lesion nematode *P. neglectus* and *Rhizoctonia* spp. respectively, and the presence of these organisms was later confirmed by the DNA assays (Table 1). *P. neglectus* was detected in all the root samples (mean of 3721 nematodes per g root) with one of the samples in the highest disease score category having 11-fold the mean number. *R. solani* AG 8 DNA was detected in all but two of the samples (mean of 31000 pg of DNA per g root). DNA of *R. solani* AG 2.1 was detected in 13 samples (mean of 970 pg of DNA per g root). DNA of *Pythium* spp. (clade F) was detected in 12 samples (mean of 1600 pg of DNA per g root). However, regression analysis (data not shown) indicated that overall there was no significant correlation between the quantity of DNA for any single organism and root disease score. The aggregated data presented in Table 1 similarly show the inconsistency of relationship between the amount of DNA of the individual organisms tested across the classes of disease score.

Table 1. Number of lucerne root samples in four disease score classes and their corresponding incidence of lesioning, root tipping, number of N_2-fixing nodules and the amount of DNA of four potential pathogens. For each DNA value, the number of samples in which the organism was detected is shown in parentheses.

Variable	Disease score class			
	0 to 1	>1 to 2	>2 to 3	>3 to 4
Number of samples in each class	12	4	4	2
Lesions (% of root affected)	2	5	11	15
Damaged root tips (% of tips affected)	5	7	10	28
R. solani AG 8 (pg DNA/g root)	31377 (12)	23435 (3)	35753 (3)	44397 (2)
R. solani AG 2.1 (pg DNA/g root)	1663 (7)	93 (4)	248 (1)	34 (1)
Pythium spp. Clade F (pg DNA/g root)	1905 (8)	704 (3)	2384 (1)	0 (0)
P. neglectus (no./g root)	2441 (12)	1551 (4)	1033 (4)	21121 (2)
Number of N_2-fixing nodules	2	3	4	1

No DNA of either *R. solani* AG 2.2 or *P. thornei* was detected in the root samples from any site.

There was no significant correlation between any of the measured soil characteristics and disease score (data not shown). Further, there was no obvious relationship between disease severity and paddock management.

There was considerable variation in the mean number of N_2-fixing nodules per plant at the different sites (0.2 to 7.8) but there was no apparent relationship between this parameter and disease score.

DISCUSSION

Moderate levels of disease symptoms were observed on the roots of young lucerne plants collected from six of the 22 sample sites in the Murray Mallee of South Australia. In many of the remaining samples high amounts of *Rhizoctonia, Pythium* and *P. neglectus* DNA were detected which may suggest that plants from some of the sites were collected before the development of visual damage to the roots.

One site measured a disease score of 2.5 yet the DNA assays detected a negligible level of the four organisms tested. This finding suggests that other pathogens, apart from those tested, may also be contributing to the disease symptoms and/or a pathogen has caused disease earlier and is no longer in the root system, therefore not detected by the DNA assays. Plants from each sample site were not at the same growth stage, which may affect the pathogen population and relative susceptibility of lucerne roots to infection. Several assays over time may be needed to detect temporal changes in pathogen populations and numbers.

DNA-based assays are useful tools to assist with the detection and quantification of pathogens in roots collected from the field, especially for those pathogens which are difficult to isolate on artificial media. Work is continuing to understand how the amount of pathogen DNA in roots relates to the observed disease symptoms and the effect on plants in the field.

The failure to establish simple relationships between any individual organism and disease score is not surprising given that similar studies of annual *Medicago* spp. (6) have found that root-rots are often the result of complexes of pathogens, for example, species of *Pythium*, *Fusarium* and *Phoma*. Results of this study support the suggestion that a complex of pathogens may be contributing to root disease in young lucerne as the four organisms, namely, *Pythium* spp., *P. neglectus* and *R. solani* AG 8 and 2.2, were all detected in the roots at many of the sites.

Plant nodulation was sub-optimal for many samples, with individual plants frequently having no N_2-fixing nodules. Whilst not immediately related to any issue of root disease, it is probably an area demanding of further attention to ensure it is not restricting plant establishment.

The results of this survey have shown that young lucerne plants in the Murray Mallee Region appear to be affected by root diseases. Overall, the visual symptoms, frequent occurrence and sometimes high levels of *P. neglectus* and *R. solani* AG 8 DNA in the dried root samples suggests that these organisms are likely to be contributing to the root damage.

Another root disease survey is planned for 2006 and extensive isolation of fungi and nematodes from the roots of young lucerne plants will be carried out. Parallel development of DNA-based tests to detect and quantify multiple pathogens in root samples collected from the field is viewed as a critical aspect of the work that will provide a better understanding of the pathogen complexes involved and allow comprehensive and timely surveys in the future.

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