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**Rural Industries Research and
Development Corporation**

Bacterial Wilt of Lucerne

— *Management strategies* —

RIRDC Publication No. 10/009





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Development Corporation**

Bacterial Wilt of Lucerne: Management strategies

by Alan McKay, James De Barro and Kathy Ophel Keller

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Foreword

Lucerne seed exports are worth \$30 million pa to Australia (RIRDC Pub. No. 08/103). The major export markets for lucerne are United States of America, Argentina, Saudi Arabia, South Africa, Asia and Europe (RIRDC Pub. No. 08/103). Overseas markets require phytosanitary declarations for bacterial wilt in lucerne. In addition, Western Australia has a legislative requirement for freedom from bacterial wilt of lucerne seed.

Bacterial wilt of lucerne is caused by the bacterium, *Clavibacter michiganensis* subsp. *insidiosus* (*Cmi*). The bacterium has been found in lucerne stands in the main lucerne seed production areas in South-Eastern South Australia. Although the bacterium causes little apparent yield loss, its presence in seed production areas means that annual phytosanitary inspections and testing are undertaken to ensure that seed crops are free of *Cmi* (SAR 48-A).

Little is known about the survival of *Cmi* in soil and plant debris, so this research was undertaken to assist seed producers develop management strategies to control the *Cmi*.

A series of strategies can be recommended to reduce the risk of *Cmi* infecting new lucerne stands. These include implementing a 2 year break between lucerne crops, longer if one or more of the break years is a drought, management of volunteer lucerne plants, cultivation to encourage degradation of the old lucerne crowns, avoidance of *Cmi* susceptible cultivars, and testing of basic seed for *Cmi*.

This project was funded from industry revenue which is matched by funds provided by the Australian Government. This report, an addition to RIRDC's diverse range of over 1900 research publications, forms part of our Pasture Seeds R&D program, which aims to improve seed production technologies to maximise yield, quality and processing efficiency.

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Peter O'Brien
Managing Director
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Abbreviations

Cmi- *Clavibacter michigenense* subsp. *insidiosus*, the causal agent of bacterial wilt of lucerne

PCR- Polymerase Chain Reaction, DNA- based diagnostic technology

SARDI- South Australian Research and Development Institute

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Executive Summary

What the report is about

This report is about the development of management strategies to control the bacterium *Clavibacter michiganensis* subsp. *insidiosus* (*Cmi*).

Who is the report targeted at?

This report is targeted at lucerne seed producers and their consultants.

Background

Lucerne seed exports are worth \$30 million pa to Australia (RIRDC Pub. No. 08/103). The major export markets for lucerne are United States of America, Argentina, Saudi Arabia, South Africa, Asia and Europe (RIRDC Pub. No. 08/103). Overseas markets require phytosanitary declarations for bacterial wilt in lucerne. In addition, Western Australia has a legislative requirement for freedom from bacterial wilt of lucerne seed.

Bacterial wilt of lucerne is caused by the bacterium, *Clavibacter michiganensis* subsp. *insidiosus* (*Cmi*). The bacterium has been found in lucerne stands in the main lucerne seed production area of South Australia where over 90% of Australia's export lucerne seed is produced. Although the bacterium causes little apparent yield loss, its presence in seed production areas means that annual phytosanitary inspections and testing are undertaken to ensure that seed crops are free of *Cmi*. Little is known about the survival of *Cmi* in soil and plant debris, and there was a need to develop management strategies to reduce inoculum.

Aims/objectives

The aim of this work was to better understand the survival of *Cmi* so that management strategies can be developed to reduce the inoculum of *Cmi* to a very low level, to reduce risk of seed infection in subsequent seed crops.

Methods used

This research used two methodologies-

1. A seed producer survey to correlate bacterial wilt occurrence with crop management.
2. Field trials to evaluate management strategies for bacterial wilt, including factors influencing inoculum decline in infected paddocks

A survey was carried out by accredited phytosanitary inspectors. The survey focused on seed producers who had both infected and uninfected lucerne stands. The survey examined whether there was a correlation between occurrence of bacterial wilt and factors such as rotation history, cultivar, age of stand, soil type and irrigation method. Trials were established in infected paddocks at Keith and Mundulla to monitor inoculum decline in infected paddocks. Treatments were cultivation, rotation crops and chemical fallow. Crowns, plants and soil were sampled over 3 years to monitor *Cmi* inoculum levels.

Results/key findings

This project found that *Cmi* levels in dead lucerne crowns declined dramatically in the 3 years after the plants had been killed, however low levels could still be detected most likely in the old root fragments. The old crowns persisted longer in free draining sandy soil compared to clay loam and in chemical fallowed plots compared to cultivated plots. These old crowns are very fragile during winter when the soil is damp and cultivation during winter helped to break them up and increase degradation.

Cmi was also detected in root systems below the cultivation layer so the bacterium may persist longer in this layer, however it is unknown if this poses a significant risk to new lucerne stands.

A survey of seed producers with paired infected and uninfected paddocks indicated more uninfected paddocks used a combination of cultivation plus spraying to control the lucerne when establishing break than infected crops. However, most infected paddocks had no previous history of lucerne suggesting that seed or spread from infected paddocks by mechanical or other means plays an important role.

Implications for relevant stakeholders:

To reduce the risk of *Cmi* infecting new lucerne stands, seed producers should plan to:

- implement at least a 2 year break between lucerne crops, longer if one or more of the break years is a drought
- ensure all live lucerne plants are killed at the start of the break period
- cultivate when soil is damp to encourage degradation of the old lucerne crowns
- avoid sowing *Cmi* susceptible cultivars such as Hunter River
- ensure water does not pond in lucerne paddocks as these conditions are known to favour *Cmi*
- basic seed be tested for *Cmi*
 - Industry should test basic seed for *Cmi*
 - Industry should consider imposing a stand life on Hunter River. Management strategies for removal of volunteer lucerne plants and cultivation to encourage degradation of lucerne crowns should be promoted by industry.
 - Industry should consider discouraging maintenance of uncertified seed crops.

Recommendations

- Management recommendations are targeted at lucerne seed producers and their consultants

Introduction

The lucerne seed industry generates significant export income for Australia. The main seed production area is in the Upper South East of South Australia, producing over 90% of the Australian export seed crop. A number of the key markets for export seed (European Union, Tunisia and Uruguay) require field inspections to ensure freedom from a number of pathogens, including *Clavibacter michiganensis* subsp. *insidiosus* (*Cmi*).

Cmi was found in the seed production area in 2002, during a survey to authenticate area freedom status. This detection has resulted in a system of phytosanitary inspections being put in place. Lucerne stands are inspected in spring, and suspect material tested using a specific PCR test for *Cmi* developed in SAR 48-A (Marefat *et al.*, 2007). A small number of new infested properties are identified each year and on two occasions *Cmi* infection has been detected in seed.

The industry now needs to focus on reduction of *Cmi* inoculum in the seed production area, to reduce the number of new detections. It would be useful for the industry to know what factors are important in reducing *Cmi* levels between crops, to avoid infection of new stands.

There has been some work on survival of the bacterium in seed and lucerne debris demonstrating that the bacterium can survive for up to 10 years on dried plant material (McCormack, 1961)

However, there is no published literature about the survival of *Cmi* in plants in the field or in soil. There has been no work looking at bacterial survival under Australian conditions. This project was designed to determine *Cmi* survival in the field. The research was undertaken on two soil types, in naturally infested paddocks in the seed production area, and using crop management techniques relevant to grower practice in the region.

There are published reports overseas on cultivar susceptibility (Cho *et al.*, 1973) and increased incidence of infection in older stands. In Australia, older stands and certain varieties seem to be a greater risk. Subject to grower approval, the results of the phytosanitary surveys need to be examined to identify possible links to occurrence of bacterial wilt to management history of a stand. Factors such as stand age, cultivar, mowing, break between lucerne stands, crop history, soil type, irrigation practice can impact on occurrence of the pathogen.

Objectives

The objectives of this project were to:

- Assess impact of crop management on presence of bacterial wilt of lucerne.
- Determine factors affecting decline of bacterial wilt inoculum in an infected lucerne stand (s).

Chapter 1: Methodology

1.2 Survey Design

Eleven seed producers with infected paddocks were surveyed in 2006 via their crop inspectors to identify management strategies used by seed producers that may reduce the incidence of *Cmi* (Appendix 1). Seed producers were asked to also compare management of an uninfected paddock on their farms that had been tested by the phytosanitary scheme (Grower details have been kept confidential).

The survey covered factors such as rotation, cultivar, stand age, soil type, irrigation method, hay program and grazing.

Results were analysed using SPSS (Statistical Package for Social Sciences) Version 17, using a series of non-parametric tests including Chi Squared tests, Likelihood Ratio, Fisher's Exact test, to test whether the observed values differ significantly from the expected values if there were no treatment effect.

Evaluation of management practices

1.2.1 Field sites details

In 2006, two field sites were established in infected Hunter River lucerne paddocks. The core site was near Mundulla, the soil type was clay loam, and annual average rainfall was 500 mm. This paddock had a history of being very wet during winter, though 2006 and 2008 were drought years.

The second site was established closer to Keith on free draining deep sand, annual rainfall 450 mm.

1.2.2 Trial design

The core trial design was randomised block design with 3 replicates and 4 treatments; continuous cropping, mechanical fallow, chemical fallow and untreated. Treatments were applied each year for 3 years. *Cmi* incidence in the untreated plot in Replicate 1 had consistently low incidence across the 4 assessment times, so the whole replicate was removed from the final analysis.

Data were analysed using Analysis of Variance in GenStat Version 10.1 (PC/Windows).

2006 treatments were applied in August 2006. Trial area was grazed in August and irrigated on 23/8/06. The crop and cultivated fallow plots were sprayed with Glyphosate CT 4.0 l/ha + wetter 0.1% on 20/8/06 and rotary hoed to kill the lucerne on 25/8/06. Barley was sown at 80 kg /ha into the cropped plots on 25/8/06. The chemical fallow plots were sprayed with Glyphosate CT 4.0 l/ha + wetter 0.1% on 25/8/06. The control was sprayed with Spray Seed 2.5 l/ha + Diuron 600 g/ha on 30/8/06. Cultivated plots were rotary hoed again on 18/9/06. Chemical fallow plots were sprayed on 28/9/06 with Glyphosate CT 4.0 l/ha + wetter 0.1% to control annual weeds and lucerne seedlings and also to kill any surviving established lucerne. In February 2007 the cultivated and cropped plots were mown and the chemical fallow plots were sprayed with Glyphosate CT 2.0 l/ha + wetter 0.1% to control annual weeds.

2007 treatments were applied on 25 June 2007. The cultivated plots were sprayed with Glyphosate 4.0 l/ha + wetter 0.1% to aid in managing a significant weed population, this was followed by cultivation. The fallow plots were sprayed with Glyphosate 4.0 l/ha + wetter 0.1% as were the

cropped plots which were subsequently sown with wheat @ 80 kg/ha. All treatment plots were sprayed for weed control on 28/1/08 using Glyphosate 2.0 l/ha + wetter 0.1%

2008 treatments were applied on 14 June 2008. It was decided to cultivate all trial plots including the chemical fallow to be more realistic to the general cropping paddock preparation. On 19 June all plots were sprayed (except untreated controls) with Glyphosate 4.0 l/ha + wetter 0.1%. Barley 80 kg/ha was seeded in the crop plots on 14/7/08. The cultivated plots were rotary hoed on 13/10/08 and untreated and chemical fallow plots sprayed with Spray Seed 5.0 l/ha on 20/10/08. Cereal plots were sprayed with Spray Seed 2.5 l/ha on 2/12/08 to control cereal regrowth. On 4/2/09 all plots except controls were sprayed with Glyphosate 2.0 l/ha + wetter 0.1%. All plots were sprayed with Spray Seed 5.0 l/ha on 1/6/09.

1.2.3 Keith

The Keith site was established on 28 September 2006. The chemical fallow plot was sprayed with Glyphosate CT 4 l/ha. A second chemical fallow plot was established in September 2007 and the original plot was resprayed with Glyphosate CT 4.0 l/ha + wetter 0.1%.

1.3 Assessments

1.3.1 *Cmi* assessments

Sample preparation

The live lucerne plants were washed dried and 2 cm of the tap root removed just below the crown. The instruments used were sterilised by alcohol and flaming between plants. The crown segments were stored in separate paper bags and freeze dried. The dry root segment was then homogenised and DNA extracted from 1 g using the SARDI proprietary DNA extraction technique.

Crowns of dead plants were dried at 40°C in a dehydration oven (includes a condenser to remove moisture from the air) before removing the sand. When damp these crowns were very fragile. DNA was extracted from 0.5 g of old crown using the SARDI proprietary DNA extraction technique. If there was less than 0.5 g of old crown available, the buffer ratios were adjusted to maintain a constant ratio.

Soil samples were treated as routine samples by the SARDI molecular diagnostics group. The facilities developed by this group were customised to process soil samples up to 500 g dry weight.

***Cmi* DNA quantification**

Cmi was assessed using quantitative PCR (Marefat *et al.*, 2007) and the results expressed as pg *Cmi* DNA / sample. The sample types included tap roots of live lucerne plants, old tap roots and crowns of treated plants and soil.

1.3.2 *Cmi* distribution in root system

During August 2006, pits were dug at both sites using a backhoe. Intact plants with root systems at least 1.5 metres long were removed, sectioned and distribution of *Cmi* was determined using PCR.

1.3.3 Incidence of *Cmi* in lucerne during growing season

At 5 times between September 2006 and May 2007, 2 tillers were collected from 20 plants in the untreated plots to monitor seasonal changes in the level of *Cmi*. Both tillers were combined for individual plant assessments. DNA was extracted from the tillers and tested by PCR.

Tillers were sampled instead of crowns because of concerns that the sampling intensity would have a significant impact on plant densities.

1.3.4 Field site annual assessments

Time 1: September 2006, 20 lucerne plants were sampled from each plot and level of *Cmi* per gram of tap root determined using quantitative PCR. The plants in the cropped, mechanical and chemical fallow plots had recently been treated.

Time 2: June 2007, after the treatments had been re-applied, the site was re-sampled. Twenty crowns were collected from each untreated plots, and 20 old crowns turned up by cultivations were sampled from the cultivated plots.

November 2007, 20 treated crowns were collected from each chemical fallow plot at Mundulla and Keith sites.

Time 3: June 2008 the Mundulla site was sampled, 20 live crowns were collected from each of the untreated plots at both sites, and 20 treated crowns from each of the cropped, mechanical fallow plots and chemical fallow plots.

The Keith site was sampled on Sept 2008; 20 live crowns were collected from the untreated plots and 20 treated crowns from the plots fallowed in 2006 and 2007. In addition, 20 500 g soil samples were collected adjoining each crown sample.

Time 4: June 2009, 20 live crowns were collected from the untreated plot. By now the old crowns were becoming difficult to find in the Mundulla site, so the old crowns were located by using a shovel to remove the cultivated layer in the cultivated plots and the top 1.5 to 2 cm in the chemical fallow plots. The old root systems were visible as dark brown circles. These were removed from the plot by placing a 2.5 X 10 cm plastic core over the old tap root and hitting it into the ground. The soil cores were dried in a dehydration oven at 40°C to hard the old tap root, which was subsequently carefully removed by hand sorting. DNA was extracted from the old tap root and the surrounding soil sample. *Cmi* levels measured using quantitative PCR and results reported as pg DNA / g sample.

Soil samples (500 g) were also collected from both sites, each sample was a composite of 7 cores; core dimensions were diameter 2.5 cm , length 10 cm. At the Mundulla site 8 soil samples were collected from each plot, while at the Keith site 20 samples were collected per plot.

Chapter 2: Seed producer survey

The purpose of the survey of seed producers was to identify management practices that may either increase or decrease the incidence of *Cmi*. These could then be examined in more detail in replicated trials. The survey question is attached in the appendix.

Each seed producer was selected because they had an infected paddock detected by the phytosanitary survey. They were also asked to include an uninfected paddock that had also been tested by the phytosanitary survey.

The power of the data is constrained by the limited sample size. It is also possible some uninfected paddocks may actually be infected.

While the number of significant differences between infected and uninfected paddocks were limited, lack of difference for some practices was also potentially important. The key findings are summarised below.

Statistically significant findings

- Infected paddocks were all sown before 2000; half the uninfected paddocks were sown since 1999 ($P=0.05$). When the sown before date was set to 1996, 10 years prior to the survey, the probability of the infected and uninfected paddocks being different dropped to $P=0.15$.
- Uninfected crops had a 2 or more year break between lucerne stands ($P=0.05$). The average break was actually the same.
- More uninfected paddocks (5 of 6) used cultivation plus spraying to kill old lucerne stands compared to infected paddocks (1 of 3). Cultivation alone was used in other paddocks. This difference was approaching significance $P=0.13$

Survey results suggest the following factors do not affect infection.

- Previous history of lucerne in the paddock does not appear to impact on the risk of infection, since most (7 of 10) infected crops had no previous history of lucerne, while 6 of 9 uninfected stands were in paddocks that had previous lucerne crops (ns, $P=0.28$).
- When lucerne was sown back to the paddock, old lucerne plants were visible in 1 of 3 infected paddocks and 3 of 6 uninfected paddocks.
- Soil type was similar in infected and uninfected paddocks; however 4 of the infected paddocks were sand compared to 1 uninfected. There were one infected and one uninfected paddocks with clay loam.
- All paddocks were flood irrigated and most were laser levelled.
- Hay cutting and irrigation programs were the same for infected and uninfected paddocks.
- Cultivation was generally used to establish break crops in both infected and uninfected paddocks.
- Annual rainfall of surveyed paddocks varied from 420 to 500 mm pa. There were no differences between infected and uninfected paddocks.

- Sheep are widely used for grazing
- Most paddocks were sown with Basic seed
- 50% of seed producers indicated that having an infected paddock has affected the cost of production and/or marketability of the seed and / or hay

Conclusions:

Cmi had not been detected in any paddocks sown since 1999, 3 infected crops were sown between 1997 and 1998. Limiting stand life to 10 years should reduce, but not eliminate the risk of detecting *Cmi*.

Cultivation plus spraying, rather than cultivation alone, was used to kill old lucerne stands in most uninfected paddocks. The combination of spraying and cultivation would give better control of the old lucerne stand.

Most infected paddocks had no previous history of lucerne, which suggests seed may be the most likely source of infection. While the survey did not reveal a link between any other management practices including irrigation, hay or grazing and infection, they could still play a significant role in spread of *Cmi* in this region.

The incidence of *Cmi* infected plants in the trial paddocks was much higher than that detected in seed. This indicates *Cmi* incidence must increase in the paddock. The means of spread within the paddock could also spread *Cmi* to neighbouring paddocks. Practices such as mowing would have to be a high risk factor because of the wounding and release of sap, but there may be others. Hence it is desirable to minimise the number of infected paddocks.

Chapter 3: Management of *Cmi*

Aims

- Assess impact of crop management on presence of bacterial wilt of lucerne.
- Determine factors affecting decline of bacterial wilt inoculum in an infected lucerne stand

Summary of seasonal rainfall at Keith and Mundulla sites

Seasonal conditions varied during the course of the field trial, 2006 and 2008 had below average rainfall, while 2007 and first half of 2009 had close to average rainfall (Table 1).

Table 1 Monthly rainfall for Bureau of Meteorology Keith and Bordertown sites 2005-2009

Site/Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Keith													
2005	7	11	8	8	8	77	33	72	37	88	45	23	416
2006	33	19	24	43	32	6	28	9	26	2	6	20	247
2007	56	4	11	36	87	14	63	30	37	24	33	32	428
2008	3	2	5	24	49	38	61	70	22	9	11	47	340
2009	0	0	19	46	15	53	100	58					
Bordertown													
2005	8	22	8	8	11	76	22	71	40	84	56	22	429
2006	38	19	19	37	24	8	26	17	30	1	6	11	237
2007	75	1	15	34	76	14	82	31	52	20	40	24	464
2008	6	6	9	14	50	26	62	52	22	11	11	66	335
2009	0	0	29	41	20	44	84	67					

Distribution of *Cmi* in root system

DNA results for Mundulla and Keith sites show *Cmi* can be present at depth in the root system but not necessarily detected in the top 15 cm of the plant (Table 2). The higher incidence of *Cmi* at depth in the root system may have been a response to the severe drought conditions prevailing in 2006.

Table 2 Summary of *Cmi* distribution down the tap roots of plants at both trial sites

Depth	Mundulla	Keith
0-15 cm	0	2
Below 15 cm	2	5
Below 35 cm	0	5
Total Infected	2	5
Total plants	7	9

Incidence of *Cmi* infected lucerne plants during growing season

The incidence of infected plants followed a similar pattern at both sites (Figure 1). The lowest frequency of detection was in January and peaked in May when the tillers were approaching maturity. Both sites were wet in May; the Mundulla site which had a clay loam soil was particularly wet even though it had slightly less rain than the site at Keith (deep sand). This may also have been due to the Mundulla site being irrigated after harvest in April and Keith not being irrigated. Increased incidence in the tillers approaching maturity potentially increases the risk of seed infection.

The results also indicate the summer months should be avoided when assessing incidence of *Cmi* in trial sites or conducting surveys.

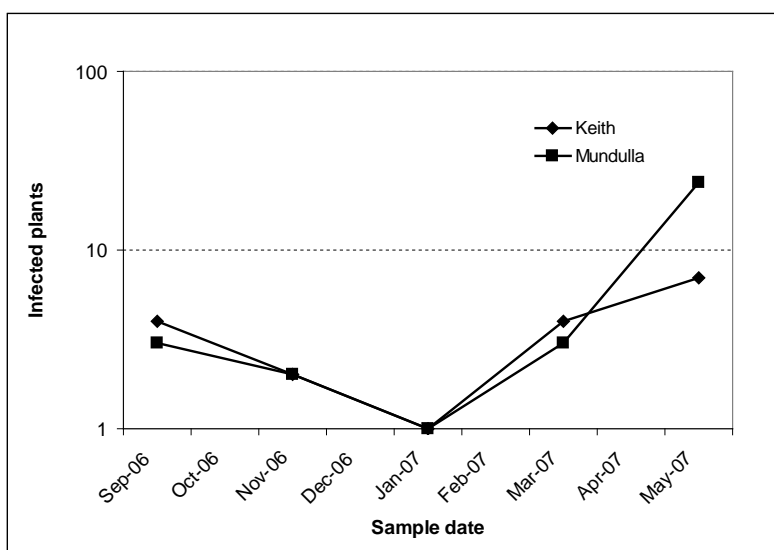


Figure 1 Incidence of *Cmi* infected plants during the growing season

Evaluation of management strategies

Mundulla site

There were no lucerne plants in any of the treated plots in 2009. Plant survival was higher in the cultivated/cropped plots 2 months after the treatments were applied in 2006. Some of these were seedlings (Table 3).

By 2009 *Cmi* levels in the soil samples had declined between 70 to 80% (Figure 2). Although this difference was not statistically significant, the *Cmi* levels in the untreated plot were underestimated because live crowns were avoided when coring the soil. The *Cmi* detected in the cores from untreated plots was probably associated with lateral roots rather than the tap root. Levels in the cultivated/cropped plots were also slightly higher than in the mechanical and chemical fallow plots. This may have been due to the poorer control of lucerne plants in 2006.

Decline in *Cmi* levels in the treated plots does not seem to be associated with decline in concentration of the bacterium in remnant roots (Figure 3). Instead it appears to be associated with degradation and decline in the proportion of root fragments infected with *Cmi* (Figures 4 & 5). Note the significant Year and Treatment effects in Figure 3 are due to high levels of *Cmi* detected in 2008 in the untreated and cropped plots which were probably aberrant.

The proportion of remnant plant residues infected with *Cmi* in the treated plots only changed between 2007 and 2008. This decline coincided with seasonal rainfall in 2007 approaching the district average; 2006 and 2008 had significantly less rainfall.

The decline in the weight of remnant roots recovered from soil is an indication of the degradation occurring. This measurement however is conservative because sampling is biased towards the larger more visible fragments. The remnant root fragments were particularly difficult to recover in 2009, indicating many treated plants had degraded.

Cmi levels detected in the soil in 2009 were much lower than in the root fragments, which suggest the residual *Cmi* population was associated with the old root residues. Encouraging the breakdown of the old root systems should therefore help to reduce *Cmi* levels.

Table 3 Lucerne plant counts at the Mundulla site

Plot	Plants/m ²		
	7/11/2006	24/09/2007	21/07/2009
No treatment	43 (30 – 60)	20 (18 – 22)	19 (15 – 22)
Cultivated/crop	13 (0 -20)	1 (0 - 4)	0
Cultivated only	0	0	0
Chemical fallow	0	0	0

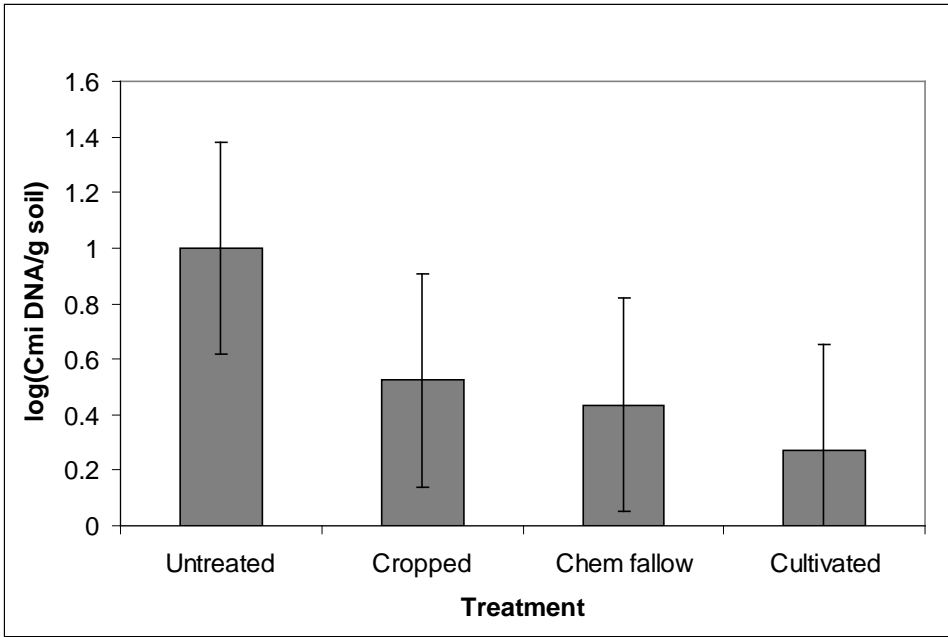


Figure 2 *Cmi* concentration in soil from the Mundulla site

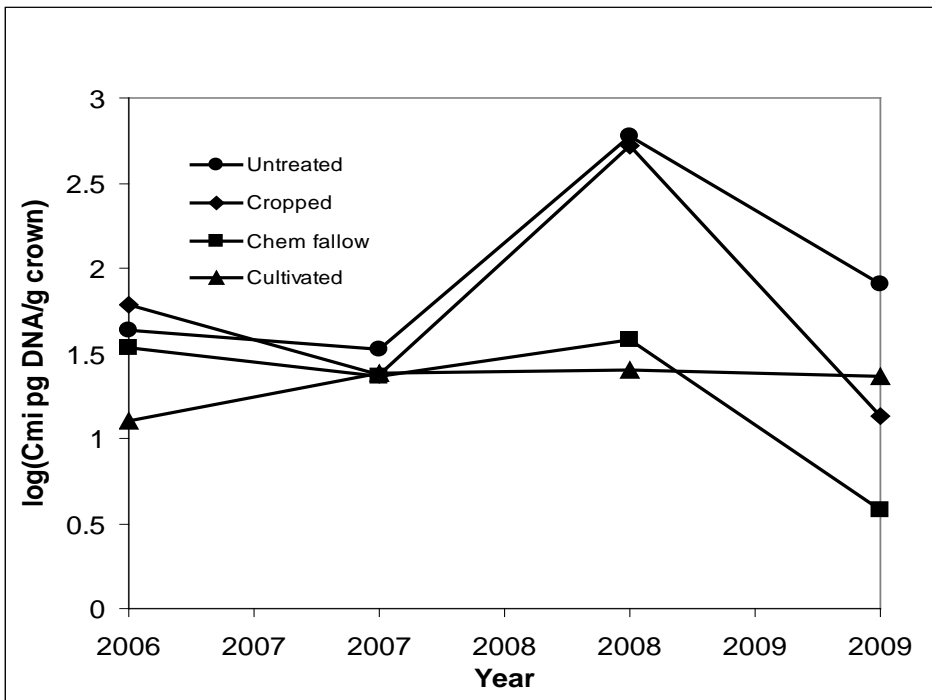


Figure 3 Average concentration of *Cmi* in untreated and treated infected crowns

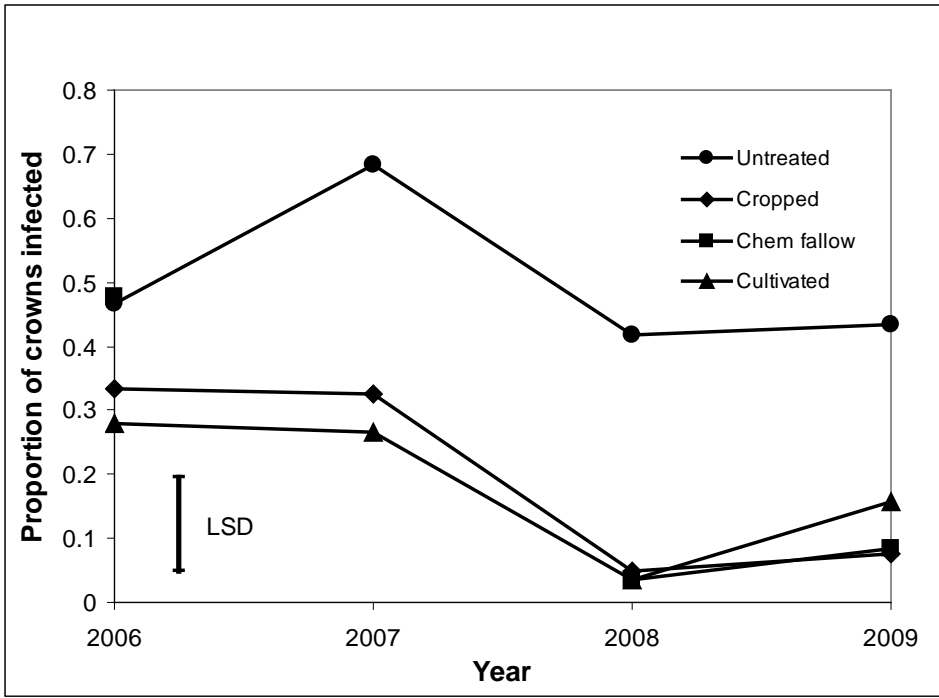


Figure 4 Proportion of crowns infected with *Cmi*.

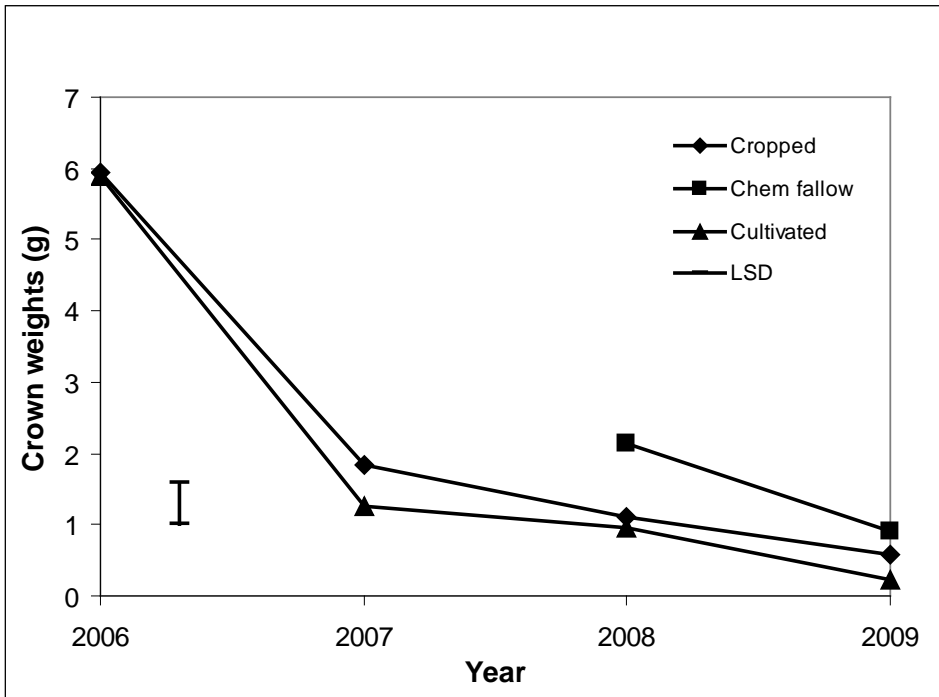


Figure 5 Decline in weight of infected treated crowns.

Keith site

The chemical fallow treatments controlled most established plants at the Keith site. The fallow 2007 treatment was less effective. Soil samples were collected at least 20 cm from live plants in the fallowed plots. The crowns were avoided when sampling soil from the untreated plot.

Cmi concentrations in soil were beginning to decline in 2008 in the fallow 2006 plot. By 2009 the difference was significant and 95% lower than in the untreated plot and significantly lower than in the fallow 2007 plot. The lack of decline in the fallow 2007 plot (sprayed in September) is most likely due to below average rainfall in 2008 (Figure 6), and possibly due to some bacteria detected in the lateral roots of live infected plants.

Cmi levels in the plant residues indicate the decline in the fallow 2006 plot occurred by the 2007 sampling, prior to this the seasonal rainfall had approached district average. The decline appears to be associated with lower concentrations of *Cmi* in the plant residues and possibly a decline in the proportion of remnant plant material infected, though the later was not significantly different from the other plots (Figures 7 & 8). Note also the low concentration of *Cmi* in the untreated plants in 2006 is probably aberrant.

Unlike the Mundulla site, weights of recovered remnant plants did not show a significant decline between 2007 and 2009, and were still easy to find at the 2009 sampling (Figure 9).

The persistence of the plant residues in the treated plots is probably due to lower moisture holding capacity and biological activity in the deep sand. Practices to encourage breakdown of the plant residues would be beneficial. The plant residues were very fragile when the soil was moist; cultivation during this period would be potentially useful.

Table 4 Lucerne plant density and the Keith site in July 2009

Treatment	Lucerne plants/m²
Untreated	20.8 (20 - 22)
Fallow 2007	3.2 (1 – 6)
Fallow 2006	0.5 (0 -2)

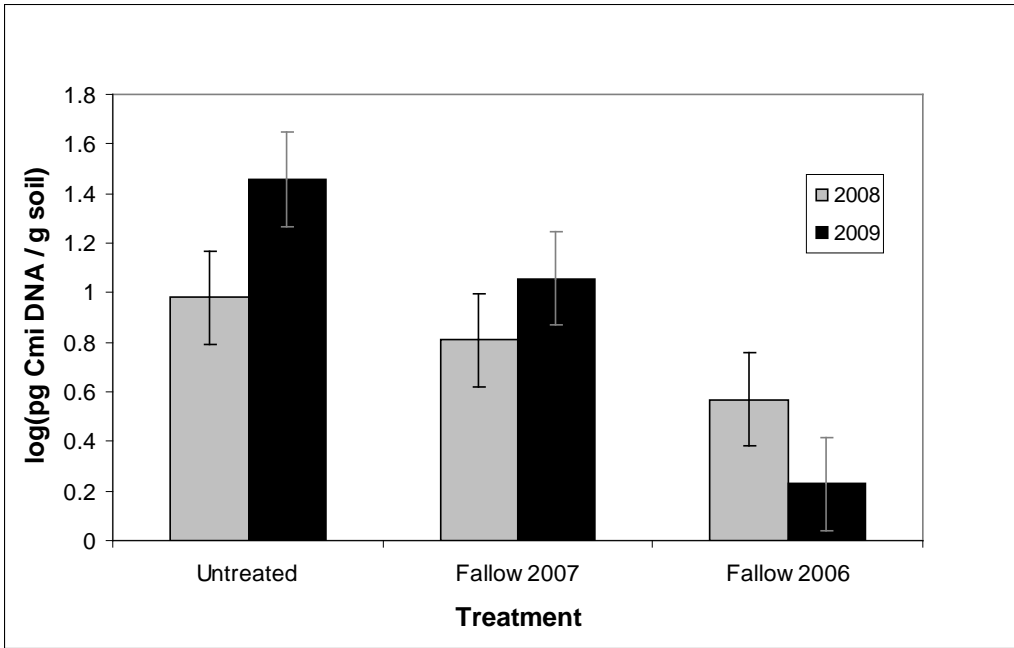


Figure 6 *Cmi* concentrations in soil from at the Keith site

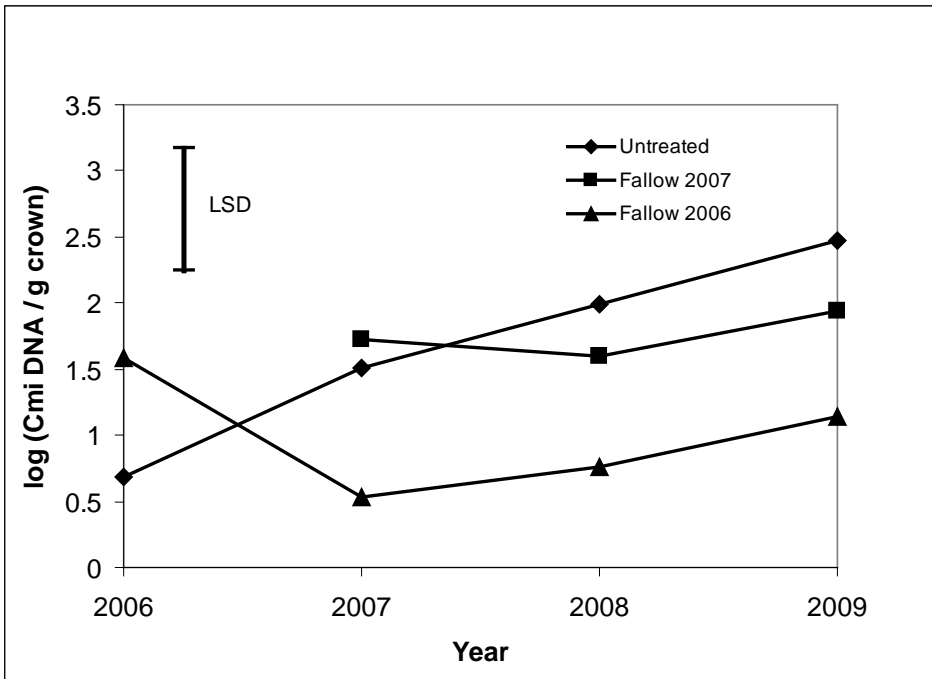


Figure 7 Average concentration of *Cmi* in untreated and treated infected crowns

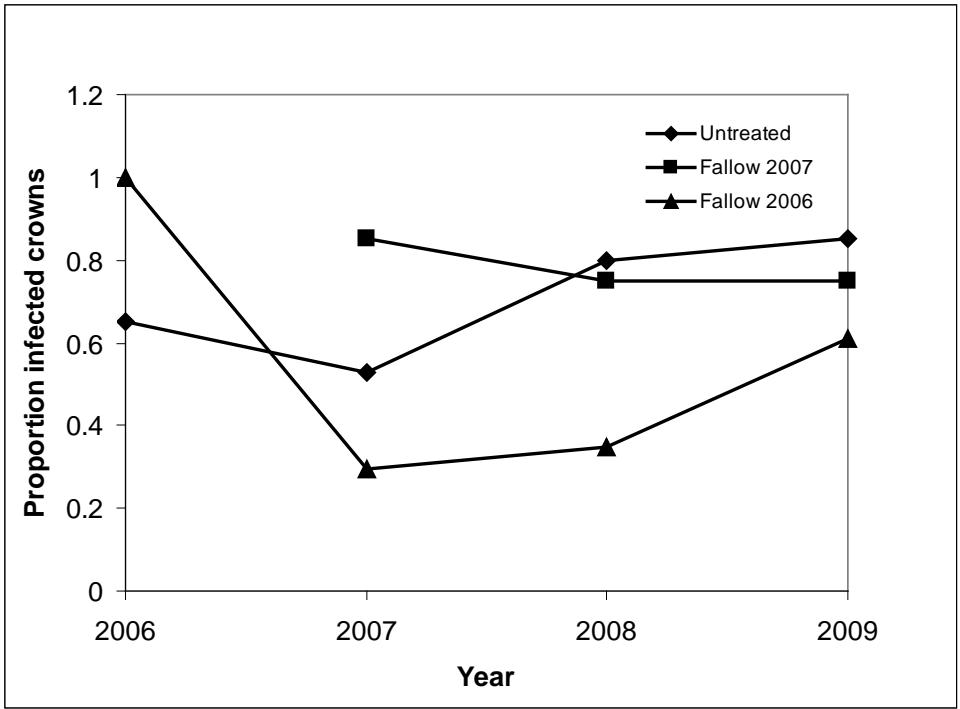


Figure 8 Proportion crown and remnant tap roots infected with *Cmi*

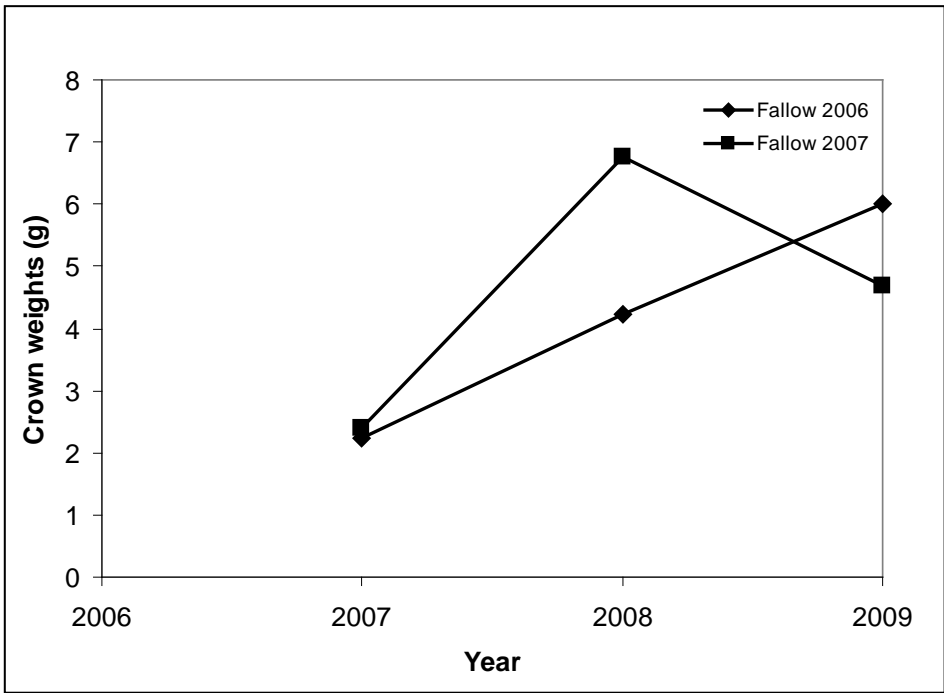


Figure 9 Treated crown weights recovered from the Keith site

Results

This project has generated new information about the survival of *Cmi* in lucerne plants, crowns and soil in the absence of growing lucerne plants.

Results generated from trial sites at both Keith and Mundulla indicate that *Cmi* most likely persists in the old crown residues, because the concentrations in old crowns were much higher than in the soil.

Monitoring at both sites indicate that old crown residues persist longer in sands than in loam soils. This is consistent with the observation that there was a higher incidence of *Cmi* was observed on the sandy site at Keith. Therefore, any crop management practice which encourages decay of the old lucerne crowns will facilitate the decline of *Cmi* at the site.

Cmi levels decrease in host free breaks, but the rate of decline is minimal during droughts.

Low *Cmi* levels could still be detected 3 years after treatments were applied to control the established lucerne plants. This would indicate that *Cmi* can persist for periods greater than 3 years without a growing host, and it would be beneficial to implement practices to increase degradation of the old lucerne crowns and roots.

Cultivated/crop, mechanical and chemical fallow were not significantly different in reducing *Cmi* levels. However the cultivated/crop treatment had more escapes than the other treatments in the first year of application. This is consistent with the grower survey finding that more uninfected lucerne crops were sown to paddocks that used a combination of spraying and cultivation to control the old lucerne stand.

Implications

Implications for seed producers:

Ensure that all plants are controlled in the first year to maximise the benefit of the break. Using a combination of treatments to control the lucerne such as spraying and cultivation is beneficial.

Seed producers should plan for at least a 2 year break, with a high level of control of established plants, between lucerne crops and longer if droughts occur. Eradication of *Cmi* will be difficult; the aim should be to reduce the population to very low levels.

Implications for industry:

The source of *Cmi* infection in the infected paddocks has not been determined. Many infected paddocks had no previous history of lucerne. This suggests seed is a possible source of infection, though spread by other means such as hay equipment is also possible.

Limiting stand life to 10 years will reduce but not eliminate the risk of detecting *Cmi*. Most infected paddocks were older than 10 years, some up to 40 years old.

Seed used to establish seed crops (basic seed) should be tested for *Cmi*.

Further Research:

The primary means of *Cmi* dispersal and establishment of new infections is not fully understood i.e. role of seed versus spread in plant debris. *Cmi* surviving at depth may pose no risk to new crops and the main means of new infection may be via equipment movement.

Recommendations

- The grower factsheet produced in 2003 should be updated to incorporate information on management of bacterial wilt emanating from this research
- Industry to consider recommendations to restrict stand life of Hunter River in order to reduce build-up of pathogens including *Cmi* and discourage maintenance of uncertified crops.
- Basic seed should be tested for *Cmi*. Seed services to monitor the incidence of *Cmi* detected in new lucerne seed crops. If a significant number of new crops become infected, further research will be required to identify the source of infection, e.g. role of equipment in transferring infection.

Appendix 1

Bacterial wilt survey form

This survey forms part of a Rural Industries and Research Development Corporation research project (PSE06-02) titled 'Management of bacterial wilt of lucerne'. The project commences in August 2006 and is scheduled to be completed by July 2009.

The completed survey will remain confidential within the project and will be critical in directing both the field research of the project as well as assisting in further understanding the processes of bacterial wilt survival, spread and control.

The project is managed by Dr. Kathy Ophel Keller of SARDI and also involves Dr. Alan McKay of SARDI. SARDI has key involvement in the screening of plant and seed samples sent to SARDI laboratories for Bacterial Wilt as part of the phytosanitary scheme. James De Barro, of De Barro Agricultural Consulting, is employed by the project to design this survey as well as manage the field trial research in conjunction with SARDI.

This project has the support of Lucerne Australia.

The survey is designed specifically for lucerne seed producers who own and manage lucerne seed paddocks that have tested positive for bacterial wilt. Grower involvement is entirely voluntary and comes highly recommended as a means for both the grower and seed production industry to understand more about bacterial wilt and potential means of managing its presence. The survey aims to focus on two lucerne seed paddocks within one farm, and specifically one paddock with a positive test and one with a negative test. Similar questions will be applied to both paddocks to ascertain if there are any factors peculiar to either paddock that may have influenced test results. For the purpose of this survey it is preferred to select the oldest 'negative test' paddock.

It is recommended that this survey be completed with the assistance of your phytosanitary field inspector or your preferred lucerne seed agronomist advisor and it would be appreciated if the completed survey form could be returned by 31 October 2006.

Thank you for your involvement in this survey and the research project as it evolves. You will be kept informed of the project's progress throughout the following seasons.

GROWER DETAILS	
Name:	<input type="text"/>
Address:	<input type="text"/>

Phone:

Email:

PHYTOSANITARY DETAILS

Phytosanitary Inspection Provider:

Phytosanitary Field Inspector:

Lucerne seed production advisor:

Assisted with survey by:

(This information is requested to assist in obtaining relevant data if/when required. Any specific inspection data required will only be obtained with grower's consent)

*The survey is focussing on two paddocks, **one which has tested positive to bacterial wilt and one which has tested negative** (under the SARDI plant assessment protocol). It is important to choose the oldest negatively tested paddock for this survey as older stands are more prone to bacterial wilt infection. Please do not choose any paddocks that have not been tested or are paddocks that have been rated "grey" and have only had weed inspections in the past one or two seasons.*

Positive Paddock Details	Negative Paddock Details
Name:	Name:
Variety:	Variety:
Where is paddock located? (farm and hundred location) <input data-bbox="236 674 715 797" type="text"/>	Where is paddock located? (farm and hundred location) <input data-bbox="829 674 1308 797" type="text"/>
Year Originally Seeded to current variety: <input data-bbox="336 857 564 931" type="text"/>	Year Originally Seeded to current variety: <input data-bbox="930 857 1158 931" type="text"/>
Has paddock been reseeded since originally planted? <input data-bbox="212 1061 691 1135" type="text"/> If yes, what years? <input data-bbox="212 1193 691 1267" type="text"/>	Has paddock been reseeded since originally planted? <input data-bbox="805 1061 1284 1135" type="text"/> If yes, what years? <input data-bbox="805 1193 1284 1267" type="text"/>
What status of lucerne seed was used (i.e. basic, certified or uncertified)? <input data-bbox="212 1384 715 1458" type="text"/>	What status of lucerne seed was used (i.e. basic, certified or uncertified)? <input data-bbox="805 1384 1308 1458" type="text"/>

Has paddock been sown to lucerne prior to the current variety? <input data-bbox="261 1659 715 1760" type="text"/>	Has paddock been sown to lucerne prior to the current variety? <input data-bbox="855 1659 1308 1760" type="text"/>
What was the variety/varieties? <input data-bbox="261 1845 715 2018" type="text"/>	What was the variety/varieties? <input data-bbox="855 1845 1308 2018" type="text"/>

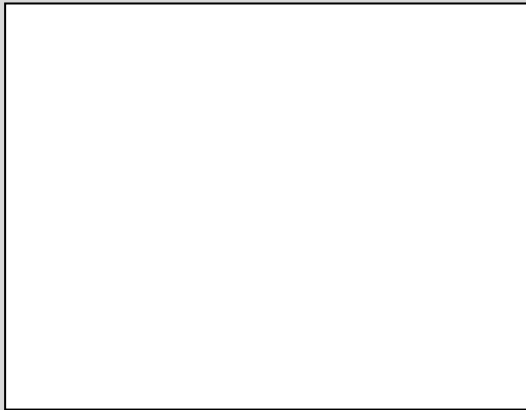
<p>In what years was the paddock sown to this variety/varieties?</p> <div data-bbox="261 295 715 539" style="border: 1px solid black; height: 100px; width: 100%;"></div>	<p>In what years was the paddock sown to this variety/varieties?</p> <div data-bbox="852 295 1305 539" style="border: 1px solid black; height: 100px; width: 100%;"></div>
<p>How many years were there between removing the established lucerne seed crop(s) and seeding a new lucerne seed crop(s)?</p> <div data-bbox="261 712 715 907" style="border: 1px solid black; height: 80px; width: 100%;"></div>	<p>How many years were there between removing the established lucerne seed crop(s) and seeding a new lucerne seed crop(s)?</p> <div data-bbox="852 712 1305 907" style="border: 1px solid black; height: 80px; width: 100%;"></div>

<p>What was paddock history pre lucerne sowing?</p> <div data-bbox="212 1108 715 1283" style="border: 1px solid black; height: 70px; width: 100%;"></div>	<p>What was paddock history pre lucerne sowing?</p> <div data-bbox="802 1108 1305 1283" style="border: 1px solid black; height: 70px; width: 100%;"></div>
<p>Is the paddock irrigated?</p> <div data-bbox="563 1305 738 1379" style="border: 1px solid black; width: 100px; height: 30px;"></div>	<p>Is the paddock irrigated?</p> <div data-bbox="1153 1305 1329 1379" style="border: 1px solid black; width: 100px; height: 30px;"></div>
<p>What type of irrigation (flood or spray)?</p> <div data-bbox="336 1451 715 1525" style="border: 1px solid black; width: 100%; height: 30px;"></div>	<p>What type of irrigation (flood or spray)?</p> <div data-bbox="930 1451 1305 1525" style="border: 1px solid black; width: 100%; height: 30px;"></div>
<p>Was lucerne present prior to development of irrigation?</p> <div data-bbox="236 1619 715 1715" style="border: 1px solid black; width: 100%; height: 40px;"></div>	<p>Was lucerne present prior to development of irrigation?</p> <div data-bbox="831 1619 1305 1715" style="border: 1px solid black; width: 100%; height: 40px;"></div>
<p>When irrigation was developed was lucerne the first irrigated crop?</p> <div data-bbox="236 1821 715 1917" style="border: 1px solid black; width: 100%; height: 40px;"></div>	<p>When irrigation was developed was lucerne the first irrigated crop?</p> <div data-bbox="831 1821 1305 1917" style="border: 1px solid black; width: 100%; height: 40px;"></div>

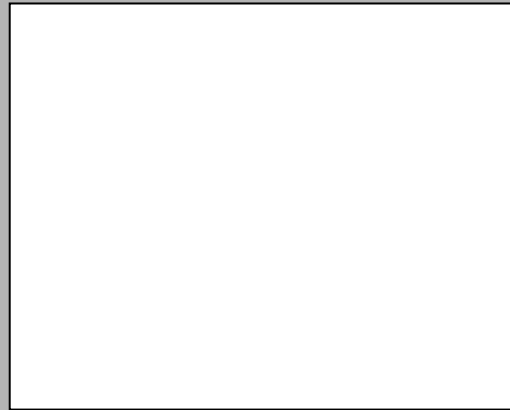
<p>What year was the first irrigated lucerne crop (hay or seed)?</p> <input data-bbox="236 327 715 427" type="text"/>	<p>What year was the first irrigated lucerne crop (hay or seed)?</p> <input data-bbox="833 327 1311 427" type="text"/>
<p>If irrigation was developed and lucerne present prior to you owning the property who was the owner at that time?</p> <input data-bbox="236 580 715 703" type="text"/>	<p>If irrigation was developed and lucerne present prior to you owning the property who was the owner at that time?</p> <input data-bbox="833 580 1311 703" type="text"/>
<p>If lucerne was present prior to you owning the property who was the owner at that time?</p> <input data-bbox="236 844 715 999" type="text"/>	<p>If lucerne was present prior to you owning the property who was the owner at that time?</p> <input data-bbox="833 844 1311 999" type="text"/>
<p>When lucerne was seeded was the paddock cultivated or direct sown?</p> <input data-bbox="212 1140 691 1240" type="text"/>	<p>When lucerne was seeded was the paddock cultivated or direct sown?</p> <input data-bbox="813 1140 1292 1240" type="text"/>
<p>If and when lucerne has been removed has it been cultivated out, sprayed and cultivated or just sprayed out?</p> <input data-bbox="212 1384 691 1503" type="text"/>	<p>If and when lucerne has been removed has it been cultivated out, sprayed and cultivated or just sprayed out?</p> <input data-bbox="813 1384 1292 1503" type="text"/>

<p>In the period(s) when lucerne is not in the paddock was the paddock cultivated annually or direct seeded? If methods have changed over time please outline as applicable.</p> <div data-bbox="212 387 715 775" style="border: 1px solid black; height: 173px; width: 315px;"></div>	<p>In the period(s) when lucerne is not in the paddock was the paddock cultivated annually or direct seeded? If methods have changed over time please outline as applicable.</p> <div data-bbox="810 387 1313 775" style="border: 1px solid black; height: 173px; width: 315px;"></div>
<p>When lucerne has been sown back into the paddock has there been old established lucerne present? Not a lot or easy to see? (If the paddock was certified, your certification provider should have a record of this from the seedling inspections).</p> <div data-bbox="212 1059 715 1317" style="border: 1px solid black; height: 115px; width: 315px;"></div>	<p>When lucerne has been sown back into the paddock has there been old established lucerne present? (If the paddock was certified, your certification provider should have a record of this from the seedling inspections)</p> <div data-bbox="810 1059 1313 1317" style="border: 1px solid black; height: 115px; width: 315px;"></div>
<p>When the paddock has been cultivated what implements have been used, both in the past and current day? (e.g. wideline, offset disc or blade plough)</p> <div data-bbox="212 1518 738 1686" style="border: 1px solid black; height: 75px; width: 330px;"></div>	<p>When the paddock has been cultivated what implements have been used, both in the past and current day? (e.g. wideline, offset disc or blade plough)</p> <div data-bbox="810 1518 1321 1686" style="border: 1px solid black; height: 75px; width: 320px;"></div>
<p>Once established what are the typical grazing patterns for the paddock? Are sheep and/or cattle used?</p> <div data-bbox="212 1888 738 2022" style="border: 1px solid black; height: 60px; width: 330px;"></div>	<p>Once established what are the typical grazing patterns for the paddock? Are sheep and/or cattle used?</p> <div data-bbox="810 1888 1321 2022" style="border: 1px solid black; height: 60px; width: 320px;"></div>

Describe a typical 12 month use pattern for the paddock (i.e. seed, hay and/or grazing). Try to give time periods and associated months.



Describe a typical 12 month use pattern for the paddock (i.e. seed, hay and/or grazing). Try to give time periods and associated months.



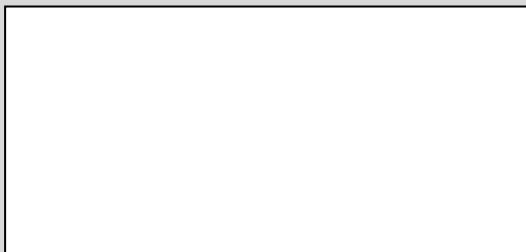
Have you ever over sown existing lucerne with another crop type? (e.g. oats or ryegrass)



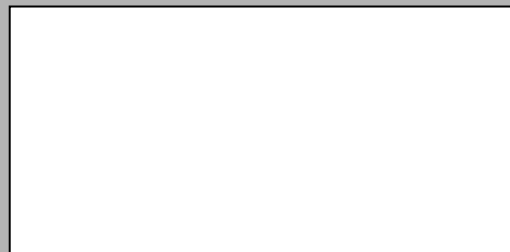
Have you ever over sown existing lucerne with another crop type? (e.g. oats or ryegrass)



When lucerne was removed was it solely due to end of certification period or did the lucerne plant population become too low for optimal seed and/or hay production?



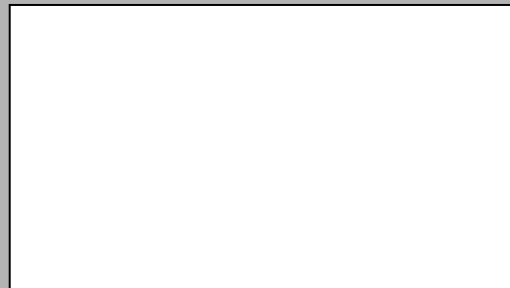
When lucerne was removed was it solely due to end of certification period or did the lucerne plant population become too low for optimal seed and/or hay production?



If the stand became too thin, can you suggest how this happened?



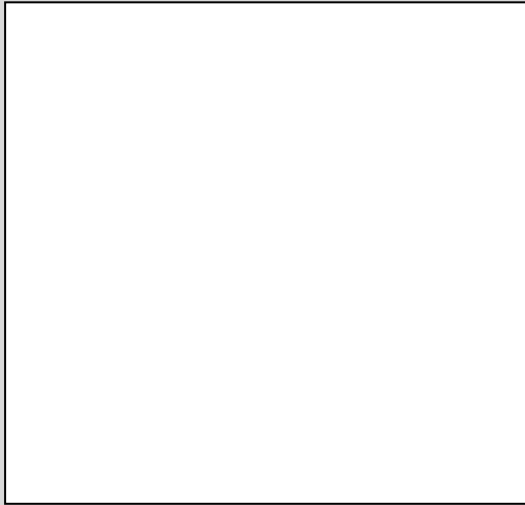
If the stand became too thin, can you suggest how this happened?



<p>What type of mower is used to cut hay? (current and past seasons)</p> <div data-bbox="212 277 738 448" style="border: 1px solid black; height: 76px;"></div>	<p>What type of mower is used to but hay? (current and past seasons)</p> <div data-bbox="809 277 1335 448" style="border: 1px solid black; height: 76px;"></div>
<p>How many hay cuts produced annually? Has this changed over years? Why?</p> <div data-bbox="212 573 738 913" style="border: 1px solid black; height: 152px;"></div>	<p>How many hay cuts produced annually? Has this changed over years? Why?</p> <div data-bbox="809 573 1335 913" style="border: 1px solid black; height: 152px;"></div>
<p>What is the annual rainfall?</p> <div data-bbox="212 981 738 1055" style="border: 1px solid black; height: 33px;"></div>	<p>What is annual rainfall?</p> <div data-bbox="809 981 1335 1055" style="border: 1px solid black; height: 33px;"></div>
<p>Do you irrigate for hay production as well as seed production?</p> <div data-bbox="212 1171 738 1323" style="border: 1px solid black; height: 68px;"></div>	<p>Do you irrigate for hay production as well as seed production?</p> <div data-bbox="809 1171 1335 1323" style="border: 1px solid black; height: 68px;"></div>
<p>What is a typical time period between irrigation and cutting?</p> <div data-bbox="212 1420 738 1516" style="border: 1px solid black; height: 43px;"></div>	<p>What is a typical time period between irrigation and cutting?</p> <div data-bbox="809 1420 1335 1516" style="border: 1px solid black; height: 43px;"></div>
<p>If irrigated, how many irrigations per season? (current and past seasons)</p> <div data-bbox="212 1630 738 1792" style="border: 1px solid black; height: 72px;"></div>	<p>If irrigated, how many irrigations per season? (current and past seasons)</p> <div data-bbox="809 1630 1335 1792" style="border: 1px solid black; height: 72px;"></div>
<p>What is the irrigation water quality (ppm)?</p> <div data-bbox="212 1852 738 1924" style="border: 1px solid black; height: 32px;"></div>	<p>What is the irrigation water quality (ppm)?</p> <div data-bbox="809 1852 1335 1924" style="border: 1px solid black; height: 32px;"></div>

<p>Describe the soil type of the paddock</p> <div data-bbox="210 309 740 412" style="border: 1px solid black; height: 46px;"></div>	<p>Describe the soil type of the paddock?</p> <div data-bbox="807 309 1337 412" style="border: 1px solid black; height: 46px;"></div>
<p>If flood irrigated, how long does it take to irrigate each bay? How large is each bay (ha)?</p> <div data-bbox="210 555 740 698" style="border: 1px solid black; height: 64px;"></div>	<p>If flood irrigated, how long does it take to irrigate each bay? How large is each bay (ha)?</p> <div data-bbox="807 555 1337 698" style="border: 1px solid black; height: 64px;"></div>
<p>Has the paddock ever been burnt?</p> <div data-bbox="210 752 740 851" style="border: 1px solid black; height: 44px;"></div>	<p>Has the paddock ever been burnt?</p> <div data-bbox="807 752 1337 851" style="border: 1px solid black; height: 44px;"></div>
<p>Has the paddock been laser levelled? What year(s)?</p> <div data-bbox="210 940 740 1191" style="border: 1px solid black; height: 112px;"></div>	<p>Has the paddock been laser levelled? What year(s)?</p> <div data-bbox="807 940 1337 1191" style="border: 1px solid black; height: 112px;"></div>
<p>What is the paddocks fertiliser history? Any typical annual applications?</p> <div data-bbox="210 1299 740 1729" style="border: 1px solid black; height: 192px;"></div>	<p>What is the paddocks fertiliser history? Any typical annual applications?</p> <div data-bbox="807 1299 1337 1729" style="border: 1px solid black; height: 192px;"></div>

Is there any typical herbicide usage for this paddock? What is typically used?



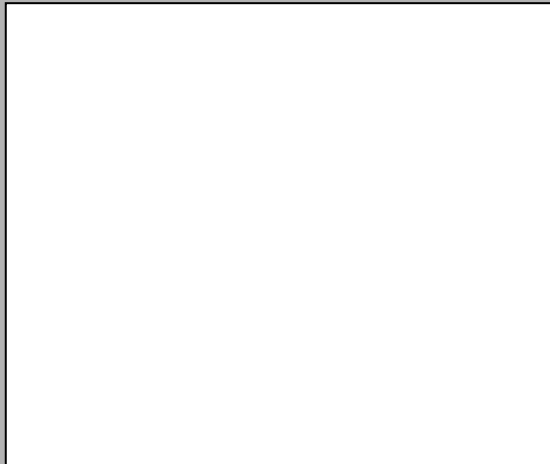
Is there any typical herbicide usage for this paddock? What is typically used?



Has having a positive paddock affected the production, cost of production and/or marketability of the seed and/or hay?



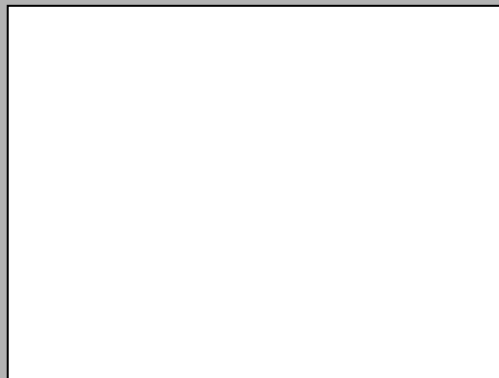
Do you plan to have this paddock phytosanitary inspected this coming season?



Do you have any records as to where the original sowing seed for the paddock was sourced?



Do you have any records as to where the original sowing seed for the paddock was sourced?



Glossary

Cmi - *Clavibacter michiganensis* subsp. *insidiosus*

PCR - Polymerase Chain Reaction, DNA- based diagnostic technology

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Bacterial Wilt of Lucerne

— *Management strategies* —

by Alan McKay, James De Barro and Kathy Ophel Keller

Publication No. 10/009

Lucerne seed exports are worth \$30 million pa to Australia. Overseas markets require phytosanitary declarations for bacterial wilt in lucerne. In addition, Western Australia has a legislative requirement for freedom from bacterial wilt of lucerne seed.

Bacterial wilt of lucerne is caused by the bacterium, *Clavibacter michiganensis* subsp. *insidiosus* (Cmi). The bacterium has been found in lucerne stands in the main lucerne seed production area of South Australia where over 90% of Australia's export lucerne seed is produced.

Although the bacterium causes little apparent yield loss, its presence in seed production areas means that annual phytosanitary inspections and testing are undertaken to ensure that seed crops are free of Cmi.

The aim of this work was to better understand the survival of Cmi so that management strategies can be developed to reduce the inoculum of Cmi to a very low level, to reduce risk of seed infection in subsequent seed crops.

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Cover photo: A healthy lucerne plant (left) and a lucerne plant infected with bacterial wilt (right)

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