Lucerne

EFFECT OF BACTERIAL WILT AND VERTICILLIUM WILT ON SIX LUCERNE CULTIVARS

M.W. DUNBIER, F.R. SANDERSON and T.J. ELLIS

Crop Research Division, DSIR, Christchurch.

SUMMARY

A field trial was carried out over three seasons to test the effect of inoculation with bacterial wilt (*Corynebacterium insidiosum*) and Verticillium wilt (*Verticillium albo-atrum*) singly or in combination, of survival and yield of the lucerne (*Medicago sativa*) cultivars Wairau, Vertus, Maris Kabul, Saranac, Washoe and 520. While all disease inoculation treatments significantly reduced dry matter yields, by up to 26%, only bacterial wilt depressed survival significantly. The effect of bacterial wilt was particularly severe on the susceptible cultivars Vertus, Maris Kabul and Wairau. On these results this isolate of Verticillium wilt is not as severe a pathogen of lucerne as bacterial wilt.

INTRODUCTION

Bacterial wilt and Verticillium wilt are important lucerne diseases in the northern hemisphere (Graham *et al* 1972) and both occur widely in New Zealand (Sanderson 1976). However, there is no published information on their relative effect on lucerne cultivars here.

This experiment using infected and control treatments with resistant and susceptible cultivars in a split-plot design was an attempt to assess the relative significance of these two diseases in the field in New Zealand.

MATERIALS AND METHODS

Seedlings of the six lucerne cultivars Wairau, Vertus, Maris Kabul, Saranac, Washoe and 520 were grown in flats in the glasshouse. When the seedlings were 8 weeks old they were lifted from the soil and the roots washed and trimmed. Seedlings were split into four treatment groups: 0 (control), B (bacterial wilt inoculation), V (Verticillium wilt inoculation), and J (joint inoculation with both bacterial and Verticillium wilts). Pure cultures of local isolates of *C. insidiosum* (grown in nutrient broth and agitated to produce a bacterial suspension) and *V. albo-atrum* (grown for 3 weeks on sterilised wheat grain and washed and diluted to produce a spore concentration of 10^6 spores/ml) were used.

Inoculation was by the root-ball soak technique (Cormack *et al* 1957). Following inoculation seedlings were transplanted into the field on 14 and 15 December 1976. There were 5 rows of 20 plants per plot, with 0.20 m between rows and 0.15 m between plants within rows. This is approximately the equilibrium density of mature disease-free lucerne stands on this site, and is sufficient for maximum production (Palmer and Wynn-Williams 1976).

The field layout was a double randomised block, split-plot design, with the four infection treatments as main plots, cultivars as sub-plots and two replicates i.e. $4 \times 6 \times 2 = 48$ sub-plot of 100 plants. Each treatment block was surrounded by a drilled strip of Washoe 0.91 m wide and the replicates were separated by a 5 m wide drilled strip of Washoe. These strips were designed to minimise cross-infection, which would be caused mainly by harvesting equipment.

The trial was harvested regularly for yield determination. Plant counts were made

Proc. 34th N.Z. Weed and Pest Control Conf.

Lucerne

at intervals, including a final count in the summer of 1980 after yield cuts had stopped because of poor survival of inoculated plants and the consequent heavy weed ingress. The trial was sprayed with insecticide whenever aphids (*Acyrtosiphon kondoi* and *A. pisum*) began to increase.

Production figures are given on a dry matter basis. Plant survival figures at each sampling date were analysed separately using the square-root transformation and results are presented as back-transformed means. The raw survival data are in effect percentages and the means may be read as percentages. The square-root transformation was used rather than the angular transformation because the percentage values did not cover a wide range at any sampling date. Differences between treatment and cultivar means in Tables 1 and 3 were tested using Duncan's new multiple range test (Duncan 1955). Preliminary statistical analysis of plant survival showed the limited replication was insufficient to give significant cultivar x treatment differences. Thus for Table 3 treatments 0 and V, and B and J were pooled. Sums of squares at appropriate levels in the analysis of variance were partitioned into orthogonal sets of single degree of freedom comparisons to test significance.

RESULTS AND DISCUSSION

Tables 1 and 2 show the effects of the treatments on plant survival. The summarised values for treatments over all cultivars are presented in Table 1. Compared with the control treatment bacterial wilt inoculation resulted in a significantly higher death rate after 20 months. For example, 20 months after establishment only 67% as many plants survived in treatment B as in treatment 0, and this proportion declined steadily until after 38 months there were only 31% as many surviving. Treatment V showed no significant decline from treatment 0 at any counting date. Treatment J was intermediate between B and V at all dates. J was significantly below 0 at all dates after 12 months and only significantly higher than the B treatment at 20 months. Froeseiser and Barnes (1976) also showed some inhibitory effect of a fungal pathogen (*Fusarium*) on bacterial wilt in lucerne but in this study it did not persist.

TABLE 1: Effect of disease inoculation treatments on survival of lucerne.

Inoculation treatment		Back-transformed means over all cultivars (percent)			
		Time after inoculation			
		12 months	20 months	26 months	38 months
Nil (Control)	0	93.5 a	84.3 a A	63.2 a A	23.6 a A
Bacterial wilt	B	93.3 a	56.6 c B	32.1 b C	6.9 c A
Verticillium wilt	V	92.5 a	81.2 ab A	53.4 a AB	17.7 ab A
Bacterial plus Verticillium wilt	J	92.0 a	68.7 b AB	39.8 b BC	9.5 bc A

The control treatment also showed severe mortality. This could have been a result of cross-infection, or of the effects of other pathogens, e.g. *Fusarium* spp or *Phytophthora megasperma*, which were almost certainly present. However, unless there was an interaction between these pathogens and those inoculated the comparison between the 0 and the B, V, J treatments should be meaningful.

Table 1 showed that for all except the 23 months sampling date there were significant differences between inoculation treatments. The consistent ranking of severity of treatments and the lack of any significant differences between treatments 0 and V made it logical to pool these treatments and test them against treatments B and J pooled. Single degree of freedom comparisons among individual cultivars or groups of cultivars showed several important practical points (see Table 2).

The most important was the difference in survival between the group of bacterial wilt resistant cultivars (Saranac, Washoe and 520) and the Verticillium wilt resistant group (Vertus and Maris Kabul) in response to inoculation with bacterial wilt. From 20

Lucerne

TABLE 2: Effect of diseases inoculation treatments on survival of lucerne cultivars.

Cultivar	Verticillium wilt	Bacterial wilt	Back-transformed means (percent) Time after establishment					
			20 months		26 months		38 months	
			Mean O & V	Mean B & J	Mean O & V	Mean B & J	Mean O & V	Mean B & J
Wairau	—	S	80.5	54.9	57.2	36.0	18.2	7.4
Vertus	R	S	85.7	51.4	64.8	25.9	25.5	6.1
Maris Kabul	R	SR	82.1	39.7	59.3	25.7	26.3	7.7
Saranac	—	R	85.0	75.7	55.2	41.9	21.6	10.9
Washoe	—	R	88.7	84.5	69.4	49.0	15.1	10.2
520	—	R	80.5	74.6	50.8	39.8	17.9	7.3
Standard error (transformed means) for comparisons within a column			0.20		0.27		0.40	

S = Susceptible, SR = Slight resistance, R = Resistant, — = No reliable information but presumed susceptible.

months onwards, the loss due to inoculation with bacterial wilt was significantly greater in Vertus and Maris Kabul than in the bacterial wilt resistant group. At the 20 month sampling Maris Kabul was significantly more affected by bacterial wilt than Vertus in spite of their reputed resistances. Wairau behaved in a similar fashion to the Verticillium resistant group in the B and J treatments.

Notwithstanding the advantage of the bacterial wilt resistant group, survival of all cultivars was unsatisfactory at 30 months, even in the control treatment. Efforts were made to minimise cross-infection by always cutting treatments in the same order (O, V, B, J) and cutting buffers in between each treatment, but some cross-infection will have taken place. Some other pathogens will have also been present.

In contrast to the differential reaction of the bacterial wilt resistant and Verticillium wilt resistant groups in the presence of bacterial wilt, their reaction to Verticillium wilt inoculation was not significantly different at any harvest. That is, after inoculation with this isolate of Verticillium wilt the U.S.A. bred cultivars were no more severely affected than the European Verticillium resistant cultivars. Also in the presence of this strain of Verticillium wilt the survival of Wairau was not significantly different from that of the other cultivars, resistant and susceptible taken as a group.

Table 3 shows dry matter production from the different treatments. All inoculation treatments reduced yield significantly in both seasons. The 1977/8 yield figures

TABLE 3: Effect of disease inoculation treatments on production of lucerne. Mean of all cultivars.

Inoculation treatment	Relative dry matter production	
	1977/8 (5 cuts)	1978/9 (4 cuts)
Nil (Control)	100 a A	100 a A
Bacterial wilt (B)	85 b AB	80 c B
Verticillium wilt (V)	83 bc B	90 b AB
Bacterial plus Verticillium wilt (J)	74 c B	78 c B
Control mean DM, t/ha	17.6	11.3

Lucerne

probably reflect differences in plant vigour since there were no significant differences in plant numbers at this stage. However, the 1978/79 figures do reflect differences in plant numbers between treatments, [26 month figures in Table 2]. In 1977/8 the B and V treatments were not significantly different from each other and the V treatment not significantly different from J. However in 1978/9 the B and J treatments were not significantly different although both were significantly below the V treatment.

This trial shows that while inoculation with *C. insidiosum* significantly decreases plant survival below that of the control treatment, similar inoculation with the culture of *V. albo-atrum* used in this trial had little influence on plant survival. Jointly inoculating the two pathogens did not have as severe effect on plant survival as *C. insidiosum* inoculation alone. Inoculation with either pathogen, singly or in combination, significantly decreased yield in both seasons. Plant resistance to *C. insidiosum* gave a considerable advantage in survival up to 26 months after inoculation but after that persistence of all cultivars in all treatments, and in the control was poor.

ACKNOWLEDGEMENT

The assistance of A.R. Wallace, Applied Mathematics Division, DSIR with the statistical analysis is gratefully acknowledged.

REFERENCES

- Cormack, M.W., Peake, R.W. and Downey, R.K., 1957. Studies on methods and materials for testing alfalfa for resistance to bacterial wilt. *Canad. J. Plant Sci.* 37: 1-11.
- Duncan, D.B., 1955. Multiple range and multiple F tests. *Biometrics* 11: 1-42.
- Frosheiser, F.I. and Barnes, D.K., 1976. Evaluating alfalfa for reaction to Fusarium wilt-Bacterial wilt combined. *Report 25th Alfalfa Improvement Conf.* ARS-NC-52: 43.
- Graham, J.H., Kreitlow, K.W. and Faulkner, L.R., 1972. Diseases. In Hanson, C.H., Ed. "Alfalfa, Science and Technology" American Society of Agronomy Monograph No. 15.
- Palmer, T.P. and Wynn-Williams, R.B., 1976. Relationships between density and yield in lucerne. *N.Z. J. Exp. Agri.* 4: 71-7.
- Sanderson, F.R., 1976. Lucerne diseases in New Zealand as recognised in the National Lucerne Survey 1975-76. *Proc. 29th N.Z. Weed and Pest Control Conf.*: 38-41.