N.Z. Agronomy Society
Special Publication No. 5

Paper 37

RESISTANCE OF LUCERNE CULTIVARS TO VERTICILLIUM ALBO-ATRUM

B.T. Hawthorne
Plant Diseases Division, DSIR
Auckland, New Zealand

C.M. Triggs
Applied Mathematics Division, DSIR
Auckland, New Zealand

ASTRACT

Six cultivars of lucerne were inoculated with 9 isolates of *Verticillium albo-atrum* Reinke et Berthold and the yield of top growth 6 weeks after inoculation was determined. Cultivars differed widely in yield and in depression of yield caused by different isolates. Statistical methods were used to explore and define the interactions which occurred between particular cultivars and particular isolates. Cultivar reaction was assessed by regressing individual cultivar x isolate yields against the mean yield, over all cultivars, for each isolate. Four cultivars had regression slopes of 1.0 but AS13R and Vertus had lines with slopes significantly different from 1.0 indicating that their reactions were different from the average. Additional information on cultivar reaction was obtained from broken-line regressions which fitted the data better than straight lines for three cultivars, Rere, WL318, and Vertus. We interpret the broken-line regressions as demonstrating the presence, or absence, of genes which substantially modify the overall plant reaction to one, or more, isolates of *Verticillium albo-atrum*. There were several individual cultivar x isolate combinations where the yield was significantly greater than that expected from the overall pattern. We infer that the cultivars involved had one or more genes which increased resistance to that particular isolate. There were no combinations of cultivar and isolate where yield was lower than expected. We conclude that resistance and pathogenicity are both under polygenic control and that there may be a gene-for-gene relationship between the two systems. The comparison between cultivars showed that Vertus had a resistant reaction, especially to the strongly pathogenic isolates; WL318 and Wairau showed slight to moderate resistance; and AS13R, Rere, and Saranac had low to slight resistance.

KEYWORDS

Resistance, gene-for-gene, verticillium wilt.

INTRODUCTION

Verticillium wilt of lucerne causes serious losses of production through reduced growth and stand life. Resistant cultivars are the best way of combating the disease and several such as Maris Kabul (Rogers, 1976) and Vertus (Lundin and Jonsson, 1975), have been developed for use in Great Britain and parts of Europe and Scandinavia. At present in New Zealand there is no commercially available cultivar which has an acceptable level of resistance to verticillium wilt. Although plants resistant to verticillium are uncommon (1 in 1000) in non-selected lucerne cultivars, the frequency of resistant plants (those with no, or very slight symptoms) can be increased to about 70% after seven cycles of recurrent phenotypic selection (Julen, 1980).

Most studies of verticillium wilt of lucerne have used several classes of symptom severity to distinguish different degrees of plant reaction, and although this has been useful in selection programmes, the information is not precise enough for accurate analysis of the nature of the host-fungus interaction.

The genetics of the host-pathogen relationship in verticillium wilt of lucerne is poorly defined because of the complex genetic behaviour of the tetraploid host, and the lack of specific knowledge both of the physiology of the disease process and of resistance mechanisms. The information that is available indicates that resistance is a trait which shows continuous variation and it is produced by the additive effects of a number of genes individually making small contributions (Nielsen and Andreasen, 1975; Panton, 1967a, b). Variation in pathogenicity among isolates of *V. albo-atrum* from lucerne has been detected in some studies (Flood *et al,* 1978; Hawthorne, 1983) and not in others (Christen and French, 1982; Heale and Isaac, 1963) but there has been no attempt to provide a genetic explanation for any of the results.

Our objective in this study was to provide an explanation of host resistance and fungal pathogenicity in

terms of a genetic model. This was based on an experiment in which a number of lucerne cultivars which varied in their resistance to wilt, were inoculated with isolates of *V. albo-atrum* with differing pathogenicity to lucerne, in all combinations.

MATERIALS AND METHODS

Six-week-old plants of six cultivars of lucerne were inoculated with nine isolates of *V. albo-atrum* taken from lucerne, and with water as the uninoculated control. The fungal isolates were classified as mildly pathogenic, pathogenic, or strongly pathogenic according to their effect on cv. Wairau (Hawthorne, 1983); there were three isolates in each group. Plants with about 30% of their roots with cut ends were inoculated by soaking for one hour in a water suspension of spores and chopped mycelium of *V. albo-atrum* grown for three weeks on potato dextrose agar. After inoculation the plants were transplanted singley into pots containing steam-treated potting mix and were held in a glasshouse. Yield of top growth was measured six weeks after inoculation.

The experiment was carried out as a split plot design with cultivars as the main plot factor and isolates as the sub-plot factor. Thus, there were six main plots each of ten sub-plots; the design was replicated twelve times. An analysis of variance was carried out on the six-week yield values to obtain confirmation of cultivar, isolate, and cultivar x isolate effects.

If there were no interactions between cultivars and isolates, each cultivar x isolate mean could be represented, using an additive model, as the sum of the mean of the cultivar (over all isolates) plus the mean of the isolates (over all cultivars) minus the grand mean. To identify any specific interactions between cultivars and isolates a coded table of residuals, or deviations, from the additive model was prepared. If the residual was less than +/− one standard deviation it was coded negative; those between 1 and 2.5 standard deviation in magnitude were coded "a"; and those between 2.5 and 3.5 standard deviations were coded "A". A joint linear regression method developed for study of genotype x environment interactions (Bradshaw *et al.*, 1982; Eberhart and Russell, 1966; Hill, 1975) was used to explore the nature of the host-pathogen interaction. For each cultivar, the individual cultivar x isolate yields were regressed against the mean yield over all cultivars for each isolate (isolate pathogenicity rating). The slope (b value) of the line is a measure of the relative resistance of the cultivar and if the slope is 1.0 the cultivar experiences the same depression of yield due to a particular isolate as the average depression of all cultivars. Cultivars which are relatively unaffected (i.e. resistant) by isolates of differing pathogenicity have regression coefficients (b values) near 0. Comparison of the b values for each cultivar produced a grouping of cultivars in terms of relative reaction. Broken-line linear regressions were also fitted to the data, and where these provided a better fit than the single linear regression, the b values of the part lines were indicative of different host response to one or more isolates of the fungus.

RESULTS

There was a wide variation between the six cultivars in the growth of uninoculated plants, and the individual cultivar x isolate means were expressed as percent of the control (uninoculated) yield for each cultivar to give a comparative assessment of cultivars and of isolates (Table 1). Expressing the mean yield of a cultivar over all isolates as a percent of its uninoculated control yield revealed no major differences between cultivars in terms of overall reaction: Rere (60%), AS13 (62%), Saranac (64%), Vertus (70%), WL318 (73%), and Wairau (74%). However, cultivar reaction against the strongly pathogenic isolates

Table 1. Growth of lucerne plants six weeks after inoculation with *Verticillium albo-atrum*[1].

Isolate of *V. albo-atrum*	Control yield (%)					
	Rere	AS13R	Saranac	WL318	Wairau	Vertus
v10	93.1	83.9	88.9	93.0	91.7	92.7
v20	78.2	88.9	89.3	93.5	87.6	74.9
v174	84.0	76.9	70.5	80.1	105.2	72.4
v164	77.7	70.7	71.7	70.3	79.8	65.6
v7	68.0	71.8	56.6	73.2	83.4	61.1
v16	54.2	50.3	60.9	72.8	65.3	65.2
v13	13.1	30.8	46.6	51.8	37.2	57.1
v18	17.1	29.2	21.0	58.3	43.1	51.9
v15	14.2	16.2	31.5	35.9	44.3	61.5
Approximate s.e.d.	12.8	10.1	10.1	10.7	10.2	8.8
Yield in controls (mg/plant)	604	773	771	725	764	889

[1]Average of 12 replicates.

v13, v15, and v18 was very different: Rere was the least resistant and Vertus most resistant. The analysis of variance for mean yield showed highly significant ($P<0.001$) cultivar and isolate effects, and a significant ($P=0.016$) cultivar x isolate interaction (Table 2). Particular combinations of cultivar and isolate which showed marked deviations from the yield expected, assuming an additive model and no interaction, were WL318 x v18, Wairau x v174, and Vertus x v15, all of which had large, positive residuals (Table 3).

Table 2. Analysis of variance for yield of lucerne cultivars inoculated with isolates of *Verticillium albo-atrum*.

Source of variation	df	MS	VR	F test (P)
Blocks	11	223551		
Blocks x Plots				
Cultivars	5	949199	14.852	0.001
Residual	55	63910		
Blocks x Plots x Subplots				
Isolates	9	2180901	59.987	0.001
Cultivars x isolates	45	55846	1.536	0.016
Cultivar x isolate (linear)	5	152139	4.185	0.001
Deviations	40	43809	1.205	0.185
Residual	594	36356		

Table 3. Coded residuals from additive model of cultivar x isolate interaction[1].

Isolate	Rere	AS13R	Saranac	WL318	Wairau	Vertus
v15	—	A	—	—	a	A
v18	—	—	a	A	—	a
v13	a	a	a	a	a	a
v16	—	a	—	a	—	—
v7	a	—	—	a	—	a
v164	a	—	—	—	A	—
v10	—	a	a	—	—	a
v20	—	—	—	a	a	—

[1]Residuals less than +/− one standard deviation are coded —
Residuals between 1 and 2.5 standard deviations are coded a
Residuals between 2.5 nd 3.5 standard deviations are coded A

The highly significant ($P<0.001$) linear regression of cultivar response to isolates of differing pathogenicity (cultivar x isolate (linear) Table 2) demonstrated that much of the variation in the cultivar x isolate interactions was accounted by for the differences between the slopes of the cultivars fitted regression lines (Fig. 1). Unexplained variation of yield about the regression line was small and not significant. The regression lines fitted well for all cultivars except Vertus which had 72% of the variance in yield explained compared with 88-96.5% for the other cultivars (Table 4, Fig. 1). AS13R and Rere showed relatively greater disease severity with isolates of strong pathogenicity than with mildly pathogenic isolates whereas WL318 and, particularly, Vertus were relatively resistant against the strongly pathogenic isolates. The slope of the regression line for AS13R was significantly greater ($P=0.05$) than 1.0 and the slope for Vertus was significantly ($P=0.05$) less than 1.0 (Table 4). Fitting broken regression lines to the data for each cultivar gave an improved fit for three of the six cultivars, Rere, Vertus, and WL318 (Table 4, Fig. 1B). The slopes of the left-hand and right-hand portions of the broken line regression were tested to determine if they were significantly different from 1.0 and 0 (Table 5). These tests showed that the slopes of all the lines were significantly different from 1.0 and, for Vertus, the left-hand line had a slope which did not differ significantly from 0.

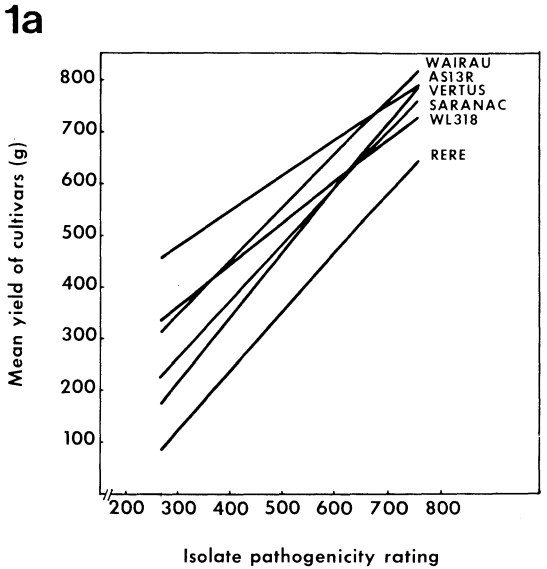

Figure 1. (a) Regression of yield (g) of lucerne cultivars against pathogenicity rating of isolates of *Verticillium albo-atrum* (mean yield of six cultivars). Regression lines for the 6 cultivars.

DISCUSSION

The inoculation method employed in our experiment gave the fungus propagules virtually unrestricted access to the vascular system so factors associated with the pre-vascular phase of the wilt disease can be considered non-operative in determining the outcome of the host:pathogen interaction. Under these conditions disease intensity, revealed by the amount of yield depression, showed continuous variation and none of the six cultivars of lucerne were immune to the pathogen and none of the nine isolates of *V. albo-atrum* were avirulent on the host.

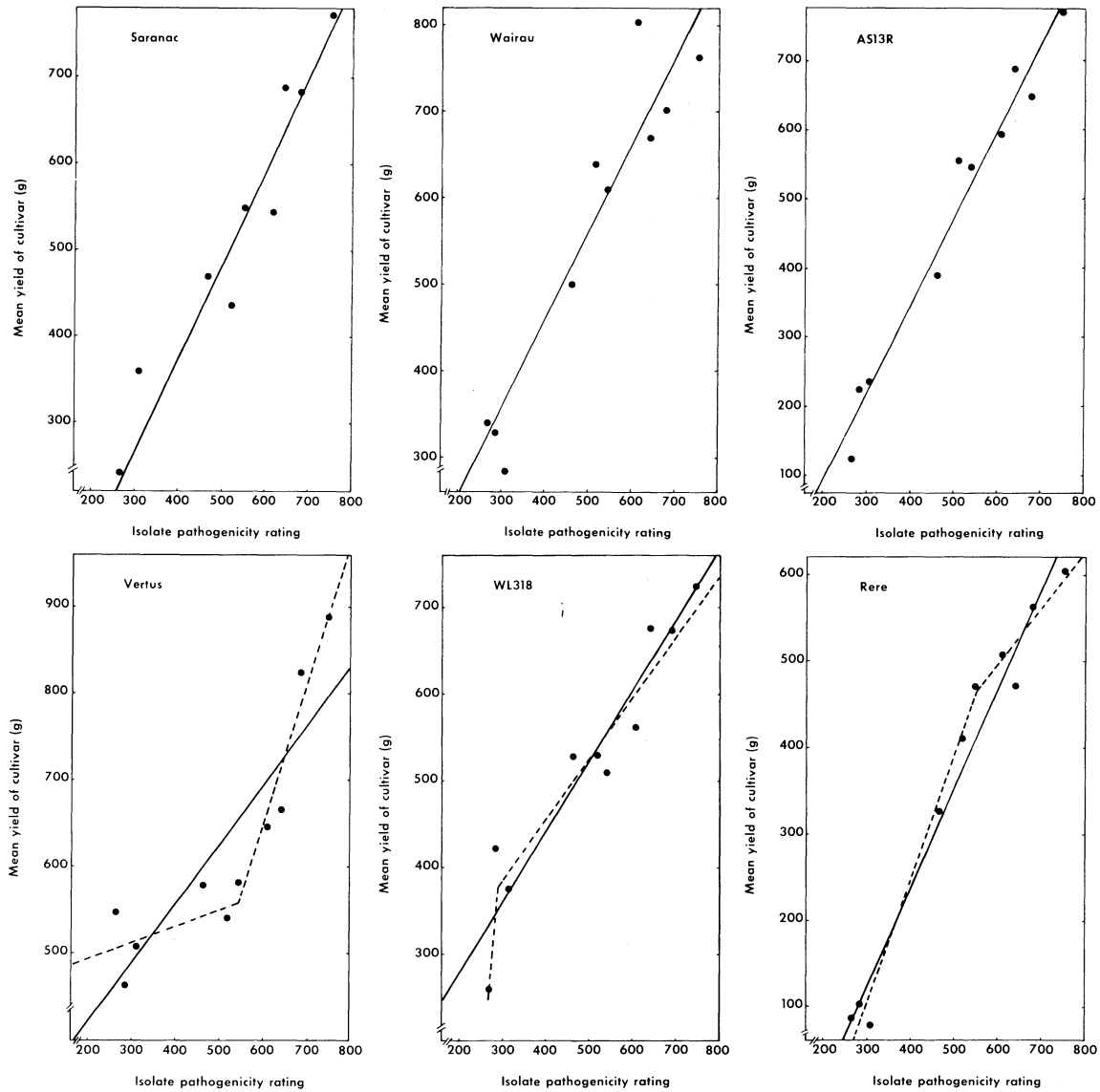

Figure 1. (b) Regression of yield (g) of lucerne cultivars against pathogenicity rating of isolates of *Verticillium albo-atrum* (mean yield of six cultivars). Individual cultivar regressions; the dashed lines are broken-line regressions which provided a better fit than straight lines for three of the six cultivars.

Resistance to verticillium wilt in lucerne is shown in this study to be quantitatively expressed (Table 1) and there is evidence from genetic studies (Nielsen and Andreasen, 1975; Panton, 1967a) that it is determined by the additive effects of a number of genes. Pathogenicity of the fungus is also shown in the results of this study (Table 1) and a previous one (Hawthorne, 1983) to be quantitatively expressed and therefore likely to be under polygenic control. Quantitative expression of pathogenicity has been recognised in *V. albo-atrum* and *V. dahliae* for other hosts and appears to be a characteristic feature of these wilt diseases (Hastie and Heale, 1984).

Table 4. Mean yield and regression statistics for lucerne cultivars inoculated with isolates of *Verticillium albo-atrum*.

Cultivar	Mean yield (g)	Slope of line (b)	t-statistic[2]	Regression statistics: Percent variance explained[1] Straight-line regression	Broken-line regression	F-statistic[3]
Rere	362	1.146	1.59	96.1	98.1	9.43*
AS13R	478	1.248	2.51*	96.5	97.2	2.82
Saranac	491	1.093	0.85	91.5	90.9	0.51
WL318	529	0.801	−2.23	89.9	93.9	6.34*
Wairau	564	1.030	0.25	88.3	91.8	4.48
Vertus	625	0.683	−2.30*	72.2	91.5	19.18**

[1]100 x R adjusted for degrees of freedom.
[2]t-statistic to test if regression coefficients are significantly different from 1; one asterisk indicates significance at P = 0.05.
[3]F-statistic to test if the broken-line gives a better fit than the straight line; one asterisk indicates significance at P = 0.05 and two asterisks indicate significance at P = 0.01.

Table 5. Tests of significance for comparing slopes of the broken-line regressions with 1.0 or 0[1].

Cultivar	Left-hand line Slope b	Left-hand line t-statistics b = 1	Left-hand line t-statistics b = 0	Right-hand line Slope b	Right-hand line t-statistics b = 1	Right-hand line t-statistics b = 0
Rere	1.38	4.09	14.8	0.72	3.54	4.83
WL318	7.0	2.42[2]	2.82	0.70	−3.80	8.86
Vertus	0.21	−5.94	1.58[3]	1.55	6.71	19.40

[1]All t statistics are significant at P = 0.01 except for entries marked 2 and 3.
[2]significant, P = 0.016.
[3]not significant, P = 0.114.

The significant cultivar x isolate interaction which occurred (Tables 2 and 3) is strong evidence of specificity between the host and pathogen genotypes and implies a gene-for-gene relationship (Van der Plank, 1968). We propose as a model of the lucerne:verticillium system, that the resistance genes in the host act additively but interact with genes in the fungus in a gene-for-gene manner. Such a system, called the interactive model, was described by Parlevliet and Zadoks (1977) and found, theoretically, to have advantages for both the host and pathogen as far as stability of their interaction was concerned. The joint linear regression method for examining cultivar x isolate interactions (Eberhart and Russell, 1966; Fairs, 1985; Hill, 1975; Leonard and Moll, 1981) was valuable for distinguishing cultivars and, with the broken-line regressions (Fig. 1, Table 5), it pointed out possible examples of specific genetic differences, e.g. with Vertus the very clear break in the regression line signalled a change in the genetic control of the host:pathogen interaction as well as a possible change in the nature of the interaction.

Specificity in the host:pathogen interaction is particularly difficult to show in quantitatively determined diseases because there is no single, clearly identifiable major event which controls the outcome. Each event is not of the on/off, all or nothing type but rather is controlled as to rate of a process or amount of product. Demonstration of specificity therefore requires that the disease process be resolved into a series of steps which can be quantified and comparisons made for different cultivar x isolate combinations (Zadoks, 1972). Progress has been made in the understanding of wilt diseases generally and detailed models indicate the scheduling of several important events worthy of investigating for evidence of specificity (Beckman, 1984; Talboys, 1964). This type of investigation has not been conducted in any detail for the lucerne:verticillium wilt system but it is known that the speed of movement of the fungus through the plant is retarded in resistant plants (Carr, 1972), increased with strongly pathogenic isolates (Hawthorne, 1983), and depends on the factors affecting sporulation of the fungus within the xylem (Pennypacker and Leath 1983).

Of the six cultivars tested AS13R, Rere, and Saranac showed low to slight resistance; Wairau and WL318 had slight to moderate resistance and only Vertus, which had been selected for resistance (Lundin and Jonsson, 1975), showed a consistent resistant reaction which was especially apparent against the strongly pathogenic isolates. There were also isolated occurrences (v18 x WL318, and v174 x Wairau) of specific interaction for resistance in otherwise slightly resistant cultivars. The demonstrated specificity in the lucerne:verticillium wilt system offers intriguing possibilities, using various combinations of cultivar and isolate for investigating the nature of resistance to wilt in lucerne.

REFERENCES

Beckman, C.H. Modelling the succession of host-parasite interactions in vascular diseases in plants. *Phytopathologia Mediterranea 23:* 109-129.

Bradshaw, J.E., Chapman, J.M., Young, A. 1982. Genotype-environment interactions in fodder kale (*Brassica oleracea* L.). *Journal of Agricultural Science, Cambridge 99:* 433-439.

Carr, A.J.H. 1972. Verticillium wilt of lucerne. *In:* Report of the Welsh Plant Breeding Station for 1971. pp. 58-59.

Christen, A.A., French, R.C. 1982. Growth and pathogenicity of alfalfa strain of *Verticillium albo-atrum. Plant Disease 66:* 416-418.

Eberhart, S.A., Russell, W.A. 1966. Stability parameters for comparing varieties. *Crop Science 6:* 36-40.

Faris, M.A. 1985. Variability and interactin between alfalfa cultivars and isolates of *Phytophthora megasperma. Phytopathology 75:* 390-394.

Flood, J., Isaac, I., Milton, J.M. 1978. Reactions of some cultivars of lucerne to various isolates of *Verticillium albo-atrum. Plant Pathology 27:* 166-169.

Hastie, A.C., Heale, J.B. 1984. Genetics of Verticillium. *Phytopathologia Mediterranea 23:* 130-162.

Hawthorne, B.T. 1983. Variation in pathogenicity among isolates of *Verticillium albo-atrum* from lucerne. *New Zealand Journal of Agricultural Research 26:* 405-408.

Heale, J.B., Isaac, I. 1963. Wilt of lucerne caused by species of Verticillium IV. Pathogenicity of *V. albo-atrum* and *V. dahliae* to lucerne and other crops; spread and survival of *V. albo-atrum* in soil and in weeds; effect upon lucerne production. *Annals of Applied Biology 52:* 439-451.

Hill, J. 1975. Genotype-environment interactions — a challenge for plant breedings. *Journal of Agricultural Science, Cambridge 85:* 477-493.

Julen, G. 1980. Development of high yielding varieties of lucerne resistant to *Verticillium albo-atrum. European and Mediterranean Plant Protection Organization Bulletin 10:* 323-327.

Leonard, K.J., Moll, R.H. 1981. Durability of general resistance: evaluation of cultivar x isolate interactions. *In:* Proceedings of Symposia IX International Congress of Plant Protection Washington, D.C., 1979. Vol. 1 Plant Protection: Fundamental aspects. pp. 190-193.

Lundin, P., Jonsson, H.A. 1975. Weibull's Vertus, a lucerne variety with high resistance to stem nematodes and verticillium wilt. *Agrihortique Genetica 33:* 17-31.

Nielsen, H.M., Andreasen, B. 1975. *Verticillium albo-atrum* in lucerne. II. The effect of selection and the hereditary variation in resistance. *In:* Royal Veterinary and Agricultural University Copenhagen, Yearbook. pp. 79-90.

Panton, C.A. 1967a. The breeding of lucerne, *Medicago sativa* L., for resistance to *Verticillium albo-atrum* Rke. et Berth. II. The quantitative nature of the genetic mechanism controlling resistance in inbred and hybrid generations. *Acta Agriculturae Scandinavica 17:* 43-52.

Panton, C.A. 1967b. The breeding of lucerne, *Medicago sativa* L., for resistance to *Verticillium albo-atrum* Rke. et Berth. III. Transgressive segregation in F3 and the effect on resistance of certain systems of inbreeding. *Hereditas 57:* 115-126.

Parlevliet, J.E., Zadoks, J.C. 1977. The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. *Euphytica 26:* 5-21.

Pennypacker, B.W., Leath, K.T. 1983. Dispersal of *Verticillium albo-atrum* in the xylem of alfalfa. *Plant Disease 67:* 1226-1229.

Rogers, H.H. 1976. Forage legumes. *In:* Report Plant Breeding Station, Cambridge, for 1975. pp. 22-57.

Talboys, P.W. 1964. A concept of the host-parasite relationship in Verticillium wilt diseases. *Nature 202:* 361-364.

Van der Plank, J.E. 1968. Disease resistance in plants. Academic Press, New York. 206 pp.

Zadoks, J.C. 1972. Modern concepts of disease resistance in cereals. *In* The way ahead in plant breeding. F.G.H. Lupton *et al.* (eds). Proceedings 6th Eucarpia Congress, Cambridge 1971. pp. 89-98.

SYMPOSIUM DISCUSSION

Dr M.W. Dunbier, Crop Research Division, DSIR

The conclusions of this paper seem too definite:

1. The method of inoculation probably eliminates several potential defence mechanisms. This is partly explained in the discussion, but not extensively. If gene for gene relationships exist then presumably all mechanisms, not just spread of pathogen through the vascular system, should be considered. Would similar results have been obtained if inoculation had been through spraying onto stubble?

2. It seems to me that this sort of experiment cannot distinguish between additive effects of genes for resistance at different loci in the host with dosage effects of genes for resistance at the same loci in the host e.g.

resistance loci	locus 1		locus 2	locus x
nulliplex	$a_0a_0a_0a_0$		$b_0b_0b_0b_0$	where
simplex	$a_1a_0a_0a_0$	resistance	$b_1b_0b_0b_0$	subscript 0
duplex	$a_1a_1a_0a_0$	increase	$b_1b_1b_0b_0$	= suscept.
triplex	$a_1a_1a_1a_0$		$b_1b_1b_1b_0$	subscript 1
quadriplex	$a_1a_1a_1a_1$		$b_1b_1b_1b_1$	= resist.

In a strictly additive sense each locus could give 5 grades of resistance, but there can be threshold effects, etc.

How would the diploid phase of the fungus cope with this sort of complexity? It would probably be necessary to carry out some controlled crossing and selfing to get some defined genotypic arrays to answer.