Witches' broom Disease of Lucerne

(1) Witches' broom of lucerne in relation to environment.

(2) The identity of witches' broom of lucerne and big bud of tomato in Australia

by

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Witches' broom of lucerne in relation to environment

Contents

(ii) The mortality of diseased plants in relation to severity of symptom expression.

(iii) Comparison of mortality of diseased plants on river terraces and on river flats.

(iv) Comparison of mortalities due to the disease and those due to causes other than the disease.

(v) The effect of two frequencies of watering and two frequencies of cutting on the mortality of diseased plants.

B: Data from the Lockyer Valley, Queensland.

IV. Discussion.

V. Acknowledgments.

VI. References.

Explanation of plates.

(1) Witches' broom of lucerne in relation to environment

Summary

Masking of symptoms of witches' broom infected plants occurred annually in the spring growth in the Lachlan Valley, New South Wales. Symptom expression was shown to be influenced by frequency of cutting and availability of water, and it appeared to be influenced by day length, light intensity and temperature, either directly or indirectly through the effect of winter dormancy on plant growth. Shoots from plants in which symptoms were masked contained virus particles.

The maximum disease incidence of witches' broom was observed in summer or autumn between February and June, the time being influenced by rainfall. At the time of maximum disease incidence, severe symptoms were associated with hot, dry conditions and mild symptoms with high rainfalls.

Variability of symptoms at any one time was partly due to distinct types (strains) of field infected plants. Clonal cuttings developed from two distinctive field infected plants maintained their characteristic features in each of 4 cultural treatments, although symptom expression varied within clones, between treatments. The seasonal pattern of the quantitative data obtained was essentially the same for each treatment. The height and dry weight of regrowth of infected plants was depressed relative to the controls, whereas the water content tended to be increased.

Infected plants produced normal, slightly abnormal or virescent flowers which were fewer in number than those of healthy plants.

Disease incidence and rate of infection tended to be greater in old, thin stands than in young stands where the plant density was high. Disease incidence was also greater on river terraces than on river flats. These aspects of the disease are discussed.

The maximum observed life span of an infected plant was 33 months in the field and 54 months in the greenhouse. In two experiments, in 10 year old stands, the number of lucerne plants was reduced by approximately 12 per cent in 13 months and Witches' Broom was considered to be the most important single factor causing mortality of plants. A high mortality of infected plants was associated with frequent cutting and more mortalities occurred on river terraces than on river flats.

Data on the disease from experimental plots in Queensland are compared with those from New South Wales, and the economic importance of the disease in Australia is discussed.

I. Introduction

Edwards (1936) suggested that environmental factors might strongly influence both distribution and symptom expression of Witches' Broom disease of lucerne in New South Wales. He found that the disease was more prevalent in drier inland areas where lucerne is widely grown for grazing purposes, than in coastal areas where rainfall is higher and lucerne is grown mainly for hay. He also found in inland areas that the disease incidence was lower in irrigated than in non-irrigated fields.

During a survey of lucerne hay growing areas in the Lachlan Valley, New South Wales, Norris and Angell (unpublished report March 1947) similarly observed an apparent association of high incidence of Witches' Broom disease and low water supply. They found that where lucerne fields sloped from a higher level to a river flat, the disease was more prevalent on high ground than on low ground. The difference in elevation between river terraces and river flats was

usually not more than 20 to 30 feet ~~in the summer~~, but lucerne on the high ground was not as luxuriant as on the low ground. On flat land in a bay-irrigated field where the ground had been imperfectly graded as well as inadequately watered, they observed that the disease incidence was two or three times greater on the dry areas than on the well watered areas. From their observations, Norris and Angell suggested that if lucerne plants were adequately supplied with water they might be capable of tolerating the disease and fail to show symptoms. Together with the writer they also observed that lucerne hay crops which were cut regularly and growing vigorously had a lower disease incidence than those which were cut at irregular intervals and growing under harsh dry conditions.

This paper reports field observations and experiments made in the Lachlan and Murrumbidgee Valleys, New South Wales, between 1947 and 1950, and a greenhouse controlled watering and cutting experiment made during the year 1950 to 1951. The studies were designed to obtain detailed information on incidence and symptom expression of the disease in relation to environment of the host plant and on the importance of the disease as a factor in reducing the profitable life of a stand of lucerne. Data on the disease at Lawes, Queensland, which were obtained between 1936-1941 by C.S. Christian are also reported.

To obtain some quantitative information on the effect of the virus on the growth of lucerne, data were obtained on the seasonal effect of two cutting and two watering frequencies on dry weight yield, water content and height of regrowth of diseased plants as compared with healthy plants grown in the greenhouse. The Hunter River variety of lucerne which was grown in fields where experiments were made in New South Wales was also used in the greenhouse studies. Several varieties of lucerne were used in the Queensland experiments.

II. Experiments

The abbreviations, W.B. for "infection with witches' broom disease" and O.C. for "other causes", have been used in the tables and in the discussion of results.

Experiment 1

During the period from October 1947 to September 1948, the percentage infection of a number of lucerne hay crops in the Lachlan Valley was recorded on 7 occasions. This was done by a series of 30 ft. line traverses in which the numbers of healthy and Witches' Broom infected plants were counted. On each occasion at least 5 traverses were made in approximately the same positions in each field. Table 1 gives data that were obtained in 8 of the older stands examined. In 5 of the fields which extended from river terrace to river flat separate counts were made on both ground levels.

Table 1

Disease incidence in relation to age and density of lucerne in Experiment 1

Field No.	Approx. age of lucerne in years	Mean no. of plants per foot when max.% disease incidence observed.			Max. observed % disease incidence.			Min. observed % disease incidence.			No. of 30' traverses made.		
		a	b	c	a	b	c	a	b	c	a	b	c
1	18		0.51	0.53		41.55	24.05		0	0		5	5
2	14	0.75			52.21			0			5		
3	14		0.39	0.62		47.88	27.35		3.80	0		6	6
4	13		0.57	0.73		44.12	12.73		2.35	0		10	5
5	13		0.92	0.62		29.32	9.33		0	0		9	8
6	11		1.00	0.83		12.29	3.03		0.66	0		6	4
7	10	0.99			40.27			0			5		
8	10	1.16			9.77			0			5		
	Observations made on (a) land of uniform elevation (b) high ground (c) low ground												

Experiment 2

This experiment made in 5 properties situated in 5 districts of the Lachlan and Murrumbidgee was begun in November 1948 and continued until April 1950. It gave more detailed information on aspects of the disease observed in Experiment 1.

In each of the lucerne fields which were approximately 10 years old and showed the river terrace - river flat conformation, 12 30 ft. permanent traverses were pegged on the high ground and 12 on the low ground. To prepare the traverses a tape was stretched between each pair of pegs and plants not growing directly under the tape were eradicated. Additional plants were removed in traverses where individual plants could not be easily distinguished and a one foot border was cleared on either side of each traverse. Nine observations, at approximately 6 weekly intervals, were made of the plants in these traverses and the following data were recorded: The number of plants infected with witches' broom disease, the number of healthy plants and the number of dead plants.

A summary of the data obtained is given in Tables 2a and 2b. In Table 2a the total figures for the 5 properties, are given for each of 6 observations when data were obtained for both high and low ground in each property. Data obtained when observations were made in December 1949 and in April 1950 are not recorded because tangled growth and flood damage prevented counts being made in two of the properties. The mortalities recorded in Table 2a are those that occurred during the interval between two successive observations. For example the mortalities recorded in September 1949 represent those that occurred between observations made in July and September 1949 and those recorded in February 1950 represent those that occurred between December 1949 and February 1950. In Tables 2a and 2b, the mortalities recorded as certainly due to W.B. or (W.B. and O.C.) are those that occurred as a result of the disease or as a result of the disease together with additional causes such as flooding. The crown of each dead plant in this class was observed. Mortalities recorded as probably due to W.B. or (W.B. and O.C.) indicate losses of infected plants due to the above causes with the exception that the dead plants were not observed. The number of mortalities in this class is undoubtedly exaggerated at the expense of the previously mentioned class. The loss of a plant in the time interval between two observations was assumed to be due to W.B. or (W.B. & O.C.) if an infected plant had been recorded in the particular traverse at the previous observation, because it was considered

than an infected plant would be more likely to die than an uninfected plant. The mortalities recorded as due to O.C. represent dead plants which had previously been rated as healthy.

(Insert Tables 2a and 2b)

Experiment 3

In Experiment 1 it was found that at any one time there was a range of symptoms of infected plants. It was considered that this might be an expression of either the length of time plants had been infected or of strains of the virus. Therefore, a system of rating was introduced to examine these aspects of the disease as well as to give information on the early diagnosis of infection and the phenomenon of seasonal recovery observed in Experiment 1. The studies of individual plants were made in 4 fields in the Lachlan Valley. The average age of the lucerne was 12 years.

Two methods were used to obtain data. Firstly areas 3 feet square and 6 feet square were pegged and the position of each lucerne plant within the plots was mapped by making use of a quadrat. This method was not entirely satisfactory as the ground area covered by the bases of the plants varied during the year and new shoots sometimes appeared above the ground at a distance of several inches from the crown. Therefore, observations were also made on plants in 3 50 ft. line traverses which were marked as in Experiment 2. In addition the position of each plant was recorded. The results of these observations as from January and September 1948 have been combined and are shown in Tables 3 and 4 respectively.

(Insert Tables 3 and 4)

Data in Lots 1 and 2 (Table 3) were obtained from lucerne grown primarily as hay crops. Lot 1, which was ploughed for resowing in July 1949, was selected because it contained a large number of infected plants growing under harsh conditions. Lot 2 includes data from plants in stands of high quality. Data in Lot 3 of Table 4 were obtained from a stand that was under almost constant grazing by sheep. This made it impracticable to use the same classes of plant ratings as for Lots 1 and 2. In summer the extreme stunting of these plants also made it difficult to distinguish infected from non-infected plants.

Table 2a

Sum of data from five properties at observations made between February 1949 and February 1950 in Experiment 2

	9.ii.49		11.1V.49		7.V1.49		26.V11.49		26.1X.49		14.ii.50	
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
No. of living plants	1050	1160	1045	1151	1018	1145	993	1132	958	1091	889	1036
No. of identifiable W.B.	93	40	124	58	102	41	40	14	12	10	109	41
No. of identifiable W.B. as % of surviving plants	8.86	3.45	11.87	5.04	10.02	3.58	4.03	1.24	1.25	.92	12.26	3.96
Total mortality due to W.B. or (W.B. & O.C.)	0	0	4	2	17	0	20	6	12	6	21	10
Total mortality probably due to O.C.	0	0	1	7	10	6	5	7	23	35	16	26

Table 2b

Totals in each property in Experiment 2 for six observations made between February 1949 and February 1950

No. of property	1		2		3		4		5	
	High	Low	High	Low	High	Low	High	Low	High	Low
No. of initial plants	198	230	197	198	253	285	238	227	164	220
Mortality certainly due to W.B. or (W.B. & O.C.)	15	2	1	0	5	0	1	0	0	0
Mortality probably due to W.B. or (W.B. & O.C.)	14	6	17	5	13	6	2	4	6	1
Mortality probably due to O.C.	2	1	2	3	17	16	22	25	12	36
Total loss due to W.B. or (W.B. & O.C.) as % of initial plants	14.65	3.48	9.14	2.53	7.11	2.11	1.26	1.76	3.66	.455

Table 3

Data from observations of individual plants in Lot 1 and Lot 2 in Experiment 3

			1948						1949						1950	
			7/1	9/3	21/4	22/7	9/9	10/11	10/2	11/4	7/6	28/7*	26/9	8/12	15/2	18/4
a	Total no. of plants living and dead	Lot 1	100	100	100	100	100	96	96	89	87					
		Lot 2	166	166	166	166	164	164	164	162	160	157	153	153	151	150
b	No. of living plants	Lot 1	100	100	100	100	96	96	89	87	86					
		Lot 2	166	166	166	164	164	164	162	160	157	153	153	151	150	137
c	No. of identifiable W.B.	Lot 1	38	47	49	29	4	0	40	47	40					
		Lot 2	13	24	25	13	1	1	9	27	29	17	1	7	33	19
d	No. of identifiable W.B. as % of b	Lot 1	38	47	49	29	4.17	0	44.94	54.02	46.51					
		Lot 2	7.83	14.46	15.06	7.93	0.61	0.61	5.56	16.88	18.47	11.11	0.65	4.64	22.0	13.87
e	No. of W.B. rated X_o as % of b	Lot 1		No distinction made between X_o and X_1 rating.								See g				
		Lot 2	0	1.81	1.81	0.61	0	0	0	2.5	1.27	0	0	0	0	0
f	No. of W.B. rated X_1 as % of b	Lot 1		No distinction made between X_o and X_1 rating.								See g				
		Lot 2	2.41	9.04	9.04	2.44	0	0.61	3.09	14.38	7.64	1.31	0.65	0	6.66	2.19
g	Sum of No. of W.B. rated X_o and X_1 as % of b	Lot 1	11.0	23.0	30.0	16.0	1.04	0	14.61	40.23	37.21					
		Lot 2	2.41	10.84	10.84	3.05	0	0.61	3.09	16.88	8.91	1.31	0.65	0	6.66	2.19
h	No. of W.B. rated X_2 as % of b	Lot 1	27.0	24.0	19.0	13.0	3.13	0	30.34	13.79	9.3					
		Lot 2	5.42	3.61	4.21	4.88	0.61	0	2.47	0	9.55	9.8	0	4.64	15.33	11.68
i	No. of plants rated X ? as % of b	Lot 1	2.0	3.0	3.0	9.0	10.42	0	12.35	14.94	9.3					
		Lot 2	2.41	0.6	1.2	4.88	3.66	1.83	0	1.25	0.64	5.23	3.27	1.32	0	2.19
j	No. of mortalities due to W.B. as % of a	Lot 1	0	0	0	0	3.0	0	7.29	2.25	1.15					
		Lot 2	0	0	0	0.6	0	0	0.61	0.62	1.25	1.27	0	0.65	0	7.33
k	No. of mortalities due to O.C. as % of a	Lot 1	0	0	0	0	1.0	0	0	0	0					
		Lot 2	0	0	0	0.6	0	0	0.61	0.62	0.64	1.27	0	0.65	0.67	1.33
l	No. of new infections as % of b	Lot 1	-	-	-	-	-	-	7.87	5.76	0					
		Lot 2	-	-	-	-	-	-	1.23	3.13	0.64	0	0	0.66	4.67	0.73

* Lot 1 was destroyed when field was ploughed and resown in July.

\- No data recorded. See text for definition of "new infection".

Table 4

Data from observations of individual plants in Lot 3

in Experiment 3

		Date of observation									
		1948		1949						1950	
		9/9	10/11	10/2	11/4	7/6	28/7	26/9	8/12	15/2	18/4
a	Total no. of plants living and dead	64	64	64	59	54	53	53	53	53	52
b	No. of living plants	64	64	59	54	53	53	53	53	52	49
c	No. of identifiable W.B.	13	8	18	44*	42*	32*	11	30	30	33
d	No. of identifiable W.B. as % of b	20.32	12.5	30.51	81.48*	79.25*	60.38*	20.75	56.6	57.68	67.35
e	No. of plants rated x? as % of b	1.56	6.25	35.6	5.56	9.43	24.53	30.19	13.21	9.62	0
f	No. of mortalities due to W.B. as % of a	0	0	6.25	3.39	1.85	0	0	0	0	3.85
g	No. of mortalities due to O.C. as % of a	0	0	1.56	5.08	0	0	0	0	1.89	1.92
h	No. of new infections as % of a	-	-	-	-	-	-	-	0**	1.92**	0**

* All plants were approximately one inch high. This made it impossible to accurately distinguish infected from non-infected plants and these figures give an exaggerated impression of the number of diseased plants.

** These figures are probably too low because of the high percentage infection recorded in 1949.

\- No data recorded. See text for definition of "new infection".

For example, the high percentage of diseased plants recorded between March and June 1949 (Table 4) was shown to have been exaggerated in summer 1950 when the number of diseased plants recorded was considerably lower than in 1949, and the number of mortalities in the interval did not nearly account for the difference.

The basic rating of infected plants used, in decreasing order of severity of symptom expression in Lots 1 and 2, was X_0, X_1 and X_2. An X? rating denoted plants that were not typically diseased, but that were suspected of showing mild symptoms of witches' broom.

X_0 represented extremely stunted diseased plants that were 2 to 4 inches high and frequently chlorotic. The thin rounded stems tended to be procumbent, and to form secondary upright shoots. Leaves were rounded and very small. Flowers were usually absent but occasionally there were a few virescent blooms. Plants of this rating were similar to those shown in Plate 2, Figure 2. X_1 indicated small upright compact plants that were 4 to 6 inches high and often chlorotic. They produced numerous, thin, rounded stems bearing small rounded leaves which were frequently rugose when immature. Occasionally virescent flowers were formed. A plant of this rating photographed in May 1950 is shown in Plate 1, Figure 1. X_2 indicated plants which were vigorous but not as tall as healthy plants in the same stand. Their height ranged from 6 to 24 inches. They were sometimes chlorotic. Stems were numerous, thinner and usually rounded. Many plants of this rating had an extremely compact symmetrical appearance. Leaves were either slightly rounded or almost normal in shape, but they were usually small and sometimes rugose. Normal, slightly abnormal or virescent flowers were formed. Field plants of this rating photographed in May 1950 are shown in Plate 1, Figures 2 and 3. X? was used to indicate plants which were suspected of being infected but which may have been uninfected. They were usually slightly stunted and stems tended to be rounded and thin. Leaves were usually of normal shape and colour, but small and sometimes rugose. Not as many flowers were formed as on

typical healthy plants. They were normal in form but varied in colour from a dark purple to a pale mauve.

This system of rating gave useful information even though the interval between harvesting and time of observation had a considerable effect on symptom expression. Sometimes it was necessary to extend the definition of the classes of rating, one relatively to another, in order to take into account the effect of local environment on symptom expression.

In Table 5 the total figures obtained in Lots 2 and 3 are given for each observation between February 1949 and February 1950 inclusively.

Table 5

Sum of data from Lots 2 and 3 at observations made between February 1949 and February 1950 in Experiment 3

	Feb. 1949	April 1949	June 1949	July 1949	Sept. 1949	Dec. 1949	Feb. 1950
No. of living plants	221	214	210	206	206	204	202
No. of identifiable W.B.	27	71	71	49	12	37	63
No. of identifiable W.B. as % of no. of living plants	12.21	33.18	33.81	23.79	5.83	18.14	31.19
Total mortality due to W.B.or (W.B. & O.C.)	5	3	3	2	0	1	0
Total mortality probably due to O.C.	2	4	1	2	0	1	2

Experiment 4

This experiment was designed to give information on the rate of infection of young stands of lucerne. In January 1948, 12 plots each 6 feet square were pegged in 3 young stands in the Lachlan Valley. Half of the plots in each field were on high ground and half on low ground.

Eight plots which included 571 plants were established in Paddock (1), two plots which included 159 plants in Paddock (2) and two plots which included 100 plants in Paddock (3).

The lucerne in Paddocks (1) and (2) was sown in April 1947, while that in Paddock (3) was sown in April 1945.

As the stands were young, the rows in which the seed had been sown could still be identified. Each plot consisted of 6 rows of plants, around which a one-foot border was cleared. It was not practicable to keep the original number of plants in the plots, because individual plants could not always be clearly distinguished. They were, therefore, thinned out until about 12 remained in each row and as they increased in size during the experiment, the number was still further reduced until approximately 9 remained in each row. The plots were examined for incidence of infected plants on the same dates that observations were made in Experiment 2, but an additional observation was made in October 1950.

Experiment 5

Data from Experiments 1, 2, 3 and 4 had shown an apparent relationship between symptom expression of the host plant and the availability of soil moisture, a seasonal variation in symptom expression of individual plants, and a variation at any one time of the year in the symptom picture of plants within a stand. To obtain further information on these aspects of the disease, an experiment was established in a greenhouse in Canberra. The effects on symptom expression of two frequencies of watering and two frequencies of cutting were studied, and at the same time quantitative data were obtained on height, dry weight and water content of regrowth of infected plants as compared with healthy plants. Two clones of diseased plants, developed from 2 distinctive field infected parent plants, were used to find out if they would respond differently to the same cultural treatments and if they would maintain their distinctive characteristics in all treatments. In a later paper it will be shown that the two clones represented two distinct strains of the virus. One infected clone (clone B) was developed from a plant with severe symptoms (Plate 2 , Figure 2) and the other (clone A) from a plant with milder symptoms (Plate 2 Figure 1). A detailed description of the vegetative and

floral features of these clones is given in another paper. The parent plant of the healthy clone (clone H) is shown in Plate 2, Figure 3.

Soil of 26.5 per cent field capacity was obtained from lucerne fields in the Lachlan Valley and equal quantities were put into each of 104 enamel cans which were 9 inches in diameter and 11 inches in height. The two watering treatments were administered by weighing and watering to field capacity. The following abbreviations have been used in describing the watering and cutting treatments in the experiment.

W = soil moisture maintained between 80 per cent and 100 per cent of field capacity.

D = soil moisture maintained between 32.5 per cent (wilting point) and 100 per cent of field capacity.

I = plants cut at 6 weekly intervals.

II= plants cut at 12 weekly intervals.

Four rooted cuttings of clone H, 11 of clone A and 11 of clone B, were given each of the 4 treatment combinations IW, ID, IIW and IID.

The mean height of the plants at each time of cutting is shown in Table 10, the mean dry weight yield per plant per 6 weeks in Figure 2 and the mean percentage moisture of regrowth per living plant at each cut in Table 9. In Table 9 the water content of the yield is expressed as a percentage of the fresh weight. The number of plants of each clone that were alive at each cut is shown in Table 11.

The cans were randomised on 3 low benches in an insect free greenhouse which was heated during the winter and cooled during the summer. The mean monthly maximum and minimum greenhouse temperatures are shown in Table 6. To minimise the effect of varying daily light intensities on plant growth, each can was moved one position across the greenhouse at each time of weighing.

Table 6

Mean monthly maximum and minimum greenhouse temperatures for Experiment 5

in degrees Fahrenheit

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Maximum	88.35	87.65	77.82	75.23	74.65	75.29	76.28	78.87	82.95	78.71	83.62	91.88	89.5	89.94	88.9	80.26
Minimum	63.94	66.5	64.45	56.0	62.6	66.76	68.0	66.44	64.95	61.76	65.48	67.5	66.9	69.28	67.3	65.68

III. Results

A: Data from the Lachlan and Murrumbidgee Valleys, New South Wales.

(a) Aspects of symptom variability and of the effect of the disease on growth

(i) Symptom expression in relation to season and environment.

In Experiments 1, 2 and 3 there was found to be an annual seasonal variation in the observed incidence of the disease. This variation occurred in all localities and districts where lucerne was examined, and is shown in Tables 2a,3,4 and 5. Data in Table 2a also show that seasonal variation in observed disease incidence occurred on both high and low ground in each of the properties examined in Experiment 2. During the early spring months, from September to November, it was frequently impossible to recognize a diseased plant in a field where the disease had been prevalent in the summer. Each year the maximum infection was usually observed in the late summer or autumn between March and June.

The study of individual plants in Experiments 3 and 4 has shown that this variation in observed disease incidence is due to the seasonal masking of symptoms of diseased plants. Individual plants that showed symptoms during the summer of one year, produced shoots that could not be distinguished from those of uninfected plants in the spring, and again showed symptoms in the following summer. Symptoms which were most readily recognized in growth which followed cutting, sometimes reappeared in the growth made after the first spring cut, but often they did not reappear until later in the summer. Plate 3 illustrates this seasonal masking of symptoms.

The following data obtained from rating individual plants for severity of symptom expression indicate that rainfall and temperature or day length, as well as cutting frequency were factors which influenced seasonal symptom expression. In Tables 3 and 4 the number of plants of ratings X_0, X_1 and X_2 taken as a percentage of the total number of diseased plants observed, and the number of plants of the rating X?, taken as a percentage of the number of living plants observed, are given for Experiment 3. Diseased

plants that appeared healthy in the spring were usually rated X? or X_2 followed by X_1 or X_o during the summer and they were then again rated X_2 or X? again later in the autumn or winter.

The number of diseased plants rated X_2, the sum of plants rated X_2 and X_1 and the sum of plants rated X_2, X_1 and X_o, in Lot 2, considered as a percentage of the number of infected plants observed, is plotted against time in Figure 1. The mean maximum and minimum temperatures and the total monthly rainfall, during the period of the experiment in the district in which these plots were established are also included in the figure, together with the average monthly rainfall figures of the district. The curve that gives the sum of the plants of rating X_2, X_1 and X_o, as a percentage of the number of plants observed represents the total number of identifiable infected plants seen at different times during the experiment. This curve follows the same pattern as the seasonal temperature and day length curves, but there is a time lag which varies from year to year.

The maximum observed infection in Lot 2 tended to occur earlier (and therefore at higher temperatures) in 1950 than in the two previous years. This coincided with an unusually high summer rainfall in the early summer of 1949-50. The amount of rainfall and the time of appearance of maximum observed infection in 1948, tended to be intermediate as compared with 1949 and 1950. The maximum observed infection in Lot 3 (Table 4) also tended to appear earlier in 1950 than in 1949. The high rainfalls in the early summer of 1949-50 caused exceptionally vigorous growth of lucerne in the Lachlan Valley and by the middle of February 1950, 5 cuts had already been made in some crops which included plants under observation in Lot 2. A high summer rainfall with which frequent cutting was also associated, seems therefore to have been an important factor which caused a maximum number of diseased plants to show symptoms in the early summer of 1950 instead of in the late summer or early autumn.

The following data indicate that in Lots 1 and 2 severity of symptom expression was correlated with hot dry growing conditions.

In the hot dry summer of 1949 there was very little growth among plants of Lot 1 that were growing on poor ground. They were darkly pigmented and many did not grow more than 2 to 4 inches in height. This made it impossible to distinguish between the ratings X_o and X_1 and these ratings were therefore grouped together. For comparative purposes, these ratings in Lot 1 were also combined when tabulating data obtained in 1948 and ratings of X_o and X_1 in Lot 2 are presented separately and as a combined rating. Data in Table 3 and Figure 1 show that the maximum percentage of plants of rating X_o and X_1 tended to occur in March or April of each year and that the maximum percentage of plants of these two ratings was greater in 1949 than 1948. In 1950 no plants were rated X_o and only a few were rated X_1. During the experiment more plants were rated X_2 than either X_1 or X_o and the seasonal distribution of X_2 plants was more irregular than that of plants of rating X_1 or X_o. In Lot 2 large numbers of plants rated X_2 tended to occur in the early summer as well as in the late autumn or early winter, but the largest number of plants of this rating occurred in February 1950 when there were no plants of rating X_o. No plants of rating X_2 occurred in April 1949 when there were large numbers of plants of rating X_1.

A comparison of these data with temperature and rainfall data shown in Figure 1 indicate that during the growing season there was an association of hot dry growing conditions and severe symptom expression, and of high rainfall and mild symptom expression.

In Tables 3 and 4 it is shown that there was a considerable variation in the same Lot in the percentage of plants rated X? at different times of the year, and in different Lots at the same time of the year. When the history of individual plants in Lots 1 and 2 which had been rated X? was examined it was found that the rating had been used to represent 5 categories of plants. The data indicate the difficulty in assessing whether or not a field plant is infected. The categories were :

1. Infected plants that showed early seasonal symptoms i.e. the rating preceded a rating of X_o , X_1 or X_2 .

2. Infected plants that showed mild symptoms before complete seasonal masking, i.e. the rating X? followed a rating of X_o, X_1 or X_2.

3. Plants that showed very mild symptoms because they were newly infected but that failed to show definite symptoms until the next summer. For example, a plant in this category was rated X_2, X_1 or X_o in the year following that in which it was rated X?. An extension of this category included some plants which had not previously been rated as infected but which were rated X? during the last summer observations were made. Such plants were considered to show early symptoms although some of them would be more correctly placed in category 5.

4. Some plants were rated X_2, X_1 or X_o in the summer of one year and the following year (probably as a result of different seasonal conditions) they were rated as X?.

5. A number of plants rated X? were afterwards consistently rated as healthy. This suggests that some factor such as insect attack had temporarily produced symptoms resembling those of Witches' Broom. For the purpose of Tables 3 and 4, the 17 plants in this category were considered to be healthy.

No evidence was obtained which showed that a newly infected plant produced less severe symptoms the first season it was recorded infected than the following season. The data suggested that severity of symptom expression was influenced to a greater extent by environment than by the length of time a plant had been infected. For example, in Lots 1 and 2 in the hot dry summer of 1949, 13 newly infected plants were rated X_1 or X_o and 7 were rated X_2. In Lot 2, in the wet summer of 1950, 3 newly infected plants were rated X_1 and 6 were rated X_2.

(ii) Symptoms of mature vegetative growth

The general symptoms of Witches' Broom infected lucerne have been described by Edwards (1936) and Menzies (1946).

The following includes additional information on the flowers and accompanying vegetative symptoms of diseased plants.

Edwards (1936) reported that most diseased plants did not flower, but some produced blooms which were smaller and paler in colour than normal inflorescences and occasionally floral parts were replaced by leafy structures. Helson (1951) also observed green flowers on lucerne in the field. Menzies (1946) did not consider phyllody to be a symptom of the disease in America because this abnormality had been reported on both Witches' Broom and healthy plants.

Field observations in New South Wales showed that flowering of diseased plants was more retarded than flowering of healthy plants and that therefore diseased plants were rarely observed in bloom because the time when field observations were made did not often correspond with the time of cutting. In an experimental plot at Dickson Experiment Station, A.C.T. it was possible to make observations of Witches' Broom diseased plants before cutting.

Forty diseased plants were observed in February, May and November of 1949 and in January, May and November of 1950. In all but one plant partial or complete masking of symptoms occurred in the spring growth harvested in November of each year. During the period of observation, virescent blooms were produced on 12 of the 40 plants. No flowers were formed on 8 plants, 9 formed flowers that were normal except that they were few in number, and the remaining plants formed pale mauve or pink flowers which were usually reduced in size. Green flowers were formed only on the summer growth harvested in January or February. Occasionally both green and pale mauve flowers were together formed on the same plant. In May and November, many plants that had produced green flowers were not flowering at the time of cutting, others formed flowers which were either pale mauve, or which could not be distinguished in colour or shape from flowers of healthy plants.

There was a considerable variation in the vegetative symptoms of diseased plants at the time of flowering. Green flowers were usually accompanied by the formation of small chlorotic rounded

leaves and numerous thin shoots, but the leaves and shoots of some plants with green flowers could scarcely be distinguished from those of healthy plants. Except at the November cut diseased plants were more stunted than healthy plants. The degree of stunting was greater in the dry summer of 1949 than in summer 1950 when considerable rains were recorded. The formation of normal flowers in the mid-summer was usually accompanied by stunted vegetative growth with thin shoots and rounded leaves.

If the apparently normal spring growth of plants which had shown symptoms the previous summer was not cut in the spring symptoms reappeared in the secondary growth. Plants later produced a bushy type of growth by proliferating at the nodes of old stems and in the new shoots at the base of the plant. Uninfected plants that were not cut also produced secondary axillary shoots but these could be readily distinguished from those of diseased plants. Secondary growth formed on a stem of an infected plant that was not harvested in the spring is shown in Plate 4 Figure 1 .

Infected plants with masked symptoms frequently produced normal seed in the spring growth before proliferation occurred, but those that showed symptoms either produced smaller seed pods which contained few fertile seed or showed extreme proliferation of the inflorescences, (Plate 4 Figure 2) and formed no seed. No evidence of seed transmission of the disease was obtained by Edwards (1936) nor by Menzies (1946). These results were confirmed when seed from infected plants in the Lachlan Valley was sown in the greenhouse in Canberra. Forty plants from this seed were observed for 14 months and 150 plants for 6 months.

(iii) Symptom development on cuttings from apparently healthy shoots of diseased plants

In Experiment 3 it was shown that infected plants which showed masking of symptoms in the field during the spring months, again showed symptoms in the following summer. Similar observations were made in the greenhouse.

To determine whether the virus was present in the shoots of plants with masked symptoms, or whether during masking the virus

was temporarily confined to the crown of the plant, a series of cuttings was made from shoots of infected field and greenhouse plants with masked symptoms. Most cuttings were taken from the tops of shoots, but occasionally more than one was obtained from a single shoot. They were rooted in sand in a humidity chamber before being transplanted into pots of soil in the greenhouse.

A total of 96 rooted cuttings were established from infected plants growing in the greenhouse. Symptoms appeared on 27 of these cuttings after two months, 66 showed symptoms within less than 12 months but 3 did not show definite symptoms until after 18, 19 and 20 months. In some, symptoms that occurred in growth made during the summer and early winter months were suppressed in the spring but in others no masking of symptoms occurred after symptoms first appeared. Plate 5 illustrates masking of symptoms in greenhouse plants.

In August and December 1950, 13 cuttings from field plants which had shown symptoms during the two previous summers were established in the greenhouse. Within 4 months all except one showed symptoms. The remaining plant still appeared healthy in July 1952. Forbes and Mills (1943) and other writers have shown that some virus infected plants occasionally produce virus free buds or shoots and it seems probable that some shoots of witches' broom infected lucerne plants may also be found to be virus free. From the results obtained, it is however evident that virus is commonly present in shoots of witches' broom diseased lucerne that show no external symptoms. The long delay in symptom expression of some cuttings, even under environmental conditions suitable for symptom development, suggests that the concentration or the virulence of the virus is considerably reduced in shoots of plants in which symptoms are masked.

(iv) The effect of two frequencies of watering and two frequencies of cutting on symptom expression in the greenhouse

(1) Vegetative growth - The 4 treatments, ID, IW, IID and IIW in Experiment 5, caused greater differences in the vegetative growth of the infected clones than they did in the healthy clone.

This can be seen by comparing Plates 6, 7 and 8, which show representative plants of clones H, A and B respectively as seen before the final harvest. By comparing Plates 6, 7 and 8, with Plate 2, Figures 1, 2 and 3 which show the parent plants of the clones, it is seen that at the end of the experiment both infected clones had retained their distinctive characteristics in all treatments.

The following are some of the more prominent effects on vegetative growth caused by the treatments. In clone B, high water level treatments as compared with low water level treatments resulted in the formation of larger leaves, thicker stems, increased proliferation and little or no purple pigmentation in the stems or petioles. In clone A the treatments caused similar differences with the exceptions that purple pigmentation and proliferation other than from the lower leaf axils occurred rarely. Mature leaves of clone A plants that received a low water level were frequently rugose, whereas leaves of plants that received a high water level were rarely rugose except when juvenile. These differences between the vegetative growth of each clone and treatment occurred at each cut, but in addition there was a seasonal variation in severity of symptom expression within clones and treatments.

Comparatively mild symptoms were produced in growth made between August and November in some clone B plants which received treatment IIW. Complete masking of symptoms did not occur in any plant and the degree of masking of symptoms varied from plant to plant within the treatment. Masking of symptoms was never observed among clone A plants, which, at all other cuts and in all other treatments, were considered to show less severe symptoms than clone B plants. Plate 9 Figure 1 shows clone B plants that received treatments IIW and IID and Plate 9 Figure 2 shows clone H plants that received treatments IIW and IID. Both photographs were taken before the November cut. If a comparison is made of the clone B plants receiving treatment IIW in Plate 9 Figure 1a and in Plate 8 Figure 1a it can be seen that symptoms of plants that received this treatment were more severe in April, 1951 than in November, 1950. In November, stems were thicker, leaves were less rounded, internodes were longer, petioles

were shorter and although the number of stems was considerably greater than in a healthy plant, the amount of secondary proliferation was very much reduced. In contrast to this treatment, in treatment IID there was little difference between the growth of clone B plants at the November and April cut. If clone H plants are compared at the November cut and at the April cut (Plate 9 Figure 2 and Plate 6 Figure 1) it can be seen that although the amount of vegetative growth was greater in April than in November, in treatments IIW and IID, the morphological features of the vegetative growth were similar.

The results in this section confirmed observations referred to in section (a) (1) where it was shown that symptom expression of field plants was strongly influenced by environmental conditions. They also showed that the distinctive characteristics of the two clones were maintained during all treatments and that their response to treatments was not identical. Some seasonal effect on growth was observed but it was not as striking as in the field.

(2) Flowering - The cultural treatments affected the time of flowering and the number of flowers produced on both healthy and infected plants. In clone H, plants that received cut II treatments flowered more profusely than those that received cut I treatments; similarly those that received a high water supply flowered more profusely than those that received the low water supply. Infected plants flowered less frequently than healthy plants and clone B plants flowered less frequently than those of clone A.

In clone H, plants in treatment IIW flowered or were setting fruit at all cuts as from August 18, and those in treatments IID, ID and IW flowered at all cuts as from November 8. In clone B a few plants flowered in treatment IIW on August 16 and in both clone A and clone B a few plants flowered in treatments IIW and IID on January 31. No plants of clone B that received cut I treatments flowered during the experiment. A few plants of clone A that received treatment IW flowered on December 18 and on January 31 and a few clone A plants of treatment ID flowered on January 31.

Flowers of clone A plants were consistently abnormal in form but of normal colour, whereas flowers of

clone B plants were either virescent or pale mauve.

Thus, in the experiment, infected plants tended to produce sparse flowers which were only formed when the day length was near its maximum, whereas healthy plants flowered more prolifically and during a much longer period of the year.

(v) Quantitative data on some effects of the disease on the growth of lucerne in the greenhouse

Virus infections have been shown to affect the physiology of a number of infected hosts. Quantitative data on the effect of the witches' broom virus on growth of lucerne were obtained in Experiment 5.

(1) Dry weight yields - The seasonal variation of dry weight yield of regrowth of two clones of witches' broom infected lucerne as compared with healthy lucerne is shown for 4 treatments ID, IW, IID and IIW, in Figure 2. The figure shows that the variation of the yield with time was essentially the same for each clone and treatment and that with a lag of approximately 6 weeks it tended to follow the annual day length cycle.

(Insert Figure 2)

For all clones and both cutting treatments the yields at each cut were increased by additional watering. At both levels of watering, in clones A and B, the cut II treatments as compared with the cut I treatments increased the yield per 6 weeks, but in clone H the yield from treatment IW was greater than from treatment IIW in January and in April 1951.

The following comparisons have been made with the dry weight yield data.-

1. Dry weight on 24.v.50

This is the last date on which a satisfactory number of plants were living in each of the 4 treatments of each clone. The yields obtained in the cut II treatment have been reduced by 50 per cent. in order to be comparable with the cut I values. Data are shown in Table 7.

Table 7

Mean dry weight production per plant in grams on 24.v.1950.

	Treatment				Mean treatment yield
	ID	IW	IID*	IIW*	
Clone H	3.30	3.25	4.70	6.83	4.52
Clone A	1.09	2.42	2.26	3.74	2.38
Clone B	1.63	1.84	3.15	4.12	2.68
Weighted mean	1.67	2.31	3.03	4.40	
* Half mean yield per plant					
				at 5%	at 1%
Least difference for significance between treatment means				0.58	0.76
Least difference for significance between clones H and A or B in the same treatment				1.18	1.58
Least difference for significance between clone A and clone B in the same treatment				0.88	1.16

The analysis indicates that the responses of clones to the treatments are similar, so that one may compare clone means averaged over the various treatments. The number of plants harvested at each treatment is shown in Table 11. The harmonic mean of the number in the various treatments was 4 for clone H and approximately 10.3 for clones A and B.

2. Total dry weight production

Table 8 gives the mean total production of those plants which have survived to 24.iv.51. An approximate analysis has been made by associating with the means of each treatment of a clone, the harmonic mean of the number of plants in these treatments.

(Insert Table 8)

Due to the very high mortality in treatments ID and IW for clone B, the most suitable comparisons in Table 8 are between all treatments for clones H and A and all clones for treatments IID and IIW.

Table 8

Mean total dry weight production per plant in grams

	Treatment			
	ID	IW	IID	IIW
Clone H	34.48	67.95	41.48	83.30
Clone A	12.22	23.75	19.14	51.95
Clone B	-	-	27.28	66.70

Least difference for significance	at 5%	at 1%
Between treatments in clone H	12.93	17.19
" " " " A	8.02	10.66
" " " " B	9.64	12.82
" " " " H and A	10.76	14.30
" " " " H and B	13.28	17.66
" " " " A and B	8.86	11.79

(a) All treatments for clones H and A only - On both the absolute (actual) and relative (logarithmic) basis there is a significant interaction between clones and treatments. That is, although the treatments are ordered in the same sequence with respect to yield for the two clones, they do not produce changes either absolutely or relatively identical.

(b) All clones for treatments IID and IIW - On this restricted treatment comparison, the effect of varying available water seems to be similar for the different clones. Least differences are given for the comparison of the clones averaged over the two treatments.

The data showed that the disease caused a decrease in the dry weight yield of infected plants which was dependent on the cutting and watering frequencies of the plants. The percentage decrease in the mean total production per plant of clone A relative to clone H was 65 per cent., 61 per cent., 50 per cent. and 38 per cent in treatments ID, IW, IID, and IIW respectively. For clone B it was 34 per cent and 19 per cent for treatments IID and IIW

respectively. As shown in Table 8 the highest total dry weight production per plant in clone H and clone A was obtained in treatment IIW.

Table 7 indicates that on 24.v.50 the mean dry weight yield of plants of clone H was significantly greater than either infected clone except for clone A treatment IW. In Table 8 the difference between the mean of clone H and clones A and B taken over all treatments is highly significant. The mean yield per plant taken over all treatments except treatment IW on 24.v.50 was greater in clone B than clone A though not significantly so and the total production of plants of clone B in treatments IID and IIW was greater than for clone A, the latter difference being significant. However, the effect of mortality has almost certainly been to eliminate the weakest clone B plants so that the comparison of production of surviving plants may give a false impression.

(2) Water content - In Table 9 the mean percentage moisture of the regrowth per living plant at each cut in Experiment 5 is shown to vary in each clone and treatment with the time of year of cutting. It tended to decrease from the first cut of each treatment, to reach a minimum on January 31 and then to increase. This trend was most marked in clone H where the trend for both watering treatments within each cutting treatment was closely parallel. In clones A and B the trend was present in treatments IID and IIW but it was not pronounced in treatments ID and IW.

(Insert Table 9)

The variability in the data obtained in treatments of cut I was particularly noticeable in treatment ID where the amount of growth made in an interval between two cuts was markedly dependent on the length of the time interval between the previous cut and the watering prior to that cut. The irregularities in the data were most apparent when yields were low.

The mean percentage moisture per cut in all clones for both watering treatments was lower for plants cut at 12 weekly intervals than for those cut at 6 weekly intervals. This difference was greater in clone H than in clones A and B. Within each cutting

Table 9

Mean percentage moisture of regrowth per living plant at each cut in Experiment 5

Date of harvest	Treatments											
	ID			IW			IID			IIW		
	Clone H	Clone A	Clone B	Clone H	Clone A	Clone B	Clone H	Clone A	Clone B	Clone H	Clone A	Clone B
12.iv.50	80.7	81.61	83.81	83.13	83.97	84.52						
24. v.50	80.8	74.23	83.49	83.02	82.91	84.47	73.72	74.26	74.46	75.76	76.94	80.19
5.vii.50	80.0	77.85	75.44	80.63	80.28	83.75						
18.viii.50	78.22	75.83	83.99	81.33	79.87	86.05	73.24	70.55	76.34	76.62	74.91	81.95
27. ix.50	74.05	65.62	66.24	73.73	72.59	81.81						
8. xi.50	77.18	72.65	77.44	77.81	78.48	80.64	72.92	72.87	77.05	69.14	76.06	80.68
20. xii.50	69.4	68.25	78.75	73.38	72.88	78.26						
31. i.51	68.61	68.94	75.81	70.18	71.55	60.54	55.46	66.04	69.27	55.15	64.97	69.02
14. iii.51	72.0	76.68	76.6	75.68	77.94	81.94						
24. iv.51	77.5	65.12	76.47	76.35	77.92	84.85	68.13	71.04	81.44	69.55	72.25	75.16
Mean	75.84	72.68	77.8	77.52	77.84	80.68	68.69	70.95	75.71	69.24	73.03	77.40

treatment additional watering gave a slight increase in all clones in the mean percentage moisture per cut.

No consistent trend is evident when the mean percentage moistures of plants of clones H and A are compared in the 4 treatments at different cutting times.

On comparing clones A, B and H receiving cut II treatments, the mean percentage moisture of clone H was significantly lower than that of both clone A and clone B for both watering treatments on January 31. On November 8 same relation held for treatments IIW and on April 24 the mean percentage moisture of plants of clone H was significantly lower than that of plants of clone B in treatment IID.

(3) Height - The mean height of regrowth of each clone and treatment at each cut is shown in Table 10. In clone H the mean height in treatments ID and IW followed the annual day length cycle with a lag of about 6 weeks in the time of reaching the minimum and maximum heights. Correlation with the annual day-length cycle was weaker for treatments IID and not evident for treatment IIW. A minimum was reached in November for treatment IID and in April for treatment IIW.

In clones A and B, the mean height of regrowth in both cutting treatments tended to follow the annual day length cycle but there was a greater lag than in clone H in reaching the minimum mean height. In treatments ID and IW the mean minimum was reached between August and November and in treatments IID and IIW the mean minimum was reached in November.

(Insert Table 10)

Cutting at 12 weekly intervals as compared with cutting at 6 weekly intervals resulted in an increase in the average mean height of regrowth in each clone at both levels of watering. Similarly in each clone, increased watering resulted in an increase in the mean height of regrowth of plants in both cutting treatments.

In all treatments and at each cut the mean height of regrowth of plants of clone H was greater than the mean height of regrowth of plants of either clone A or clone B. ~~The mean height~~

Table 10

Mean height of regrowth in cm. before each cut in Experiment 5

Date of harvest	Treatments											
	ID			IW			IID			IIW		
	Clone H	Clone A	Clone B	Clone H	Clone A	Clone B	Clone H	Clone A	Clone B	Clone H	Clone A	Clone B
12.iv. 50	87	49	42	94	51	37						
24.v.50	57	33	28	63	50	36	90	53	42	87	59	47
5.vii.50	53	24	18	71	48	26						
18.viii. 50	38	26	14	56	27	20	80	45	35	104	67	55
27.ix.50	39	22	11	59	27	23						
8.xi.50	46	24	10	68	39	23	63	33	24	99	51	43
20.xii. 50	47	31	23	104	50	46						
31.i.51	66	32	21	95	61	53	67	41	35	86	84	71
14.iii. 51	64	35	17	95	66	36						
24.iv.51	59	24	13	72	32	25	70	41	29	81	62	54
Mean	55.6	30.0	19.7	77.7	45.1	32.5	74.0	42.6	33.0	91.4	64.6	54.0
Mean % stunting		46.0	64.6		42.0	58.0		42.4	55.4		29.3	40.9

~~of regrowth of plants either clone A or clone B.~~ The mean height of regrowth of clone A plants was consistently greater than that of clone B plants. The mean percentage stunting of both infected clones relative to the healthy clone was greatest in treatment ID and least in treatment IIW (Table 10).

The data in this section indicated that at all times of the year the clone A and clone B viruses caused reduction in yield and stunting of regrowth of plants. The differences in the data obtained from the 4 treatments showed that environmental conditions have a marked effect on lucerne growth. The mean maximum yield and the mean minimum stunting per living plant for each infected

clone occurred in treatment IIW while the mean minimum yield and the mean maximum stunting occurred in treatment ID. The percentage moisture of diseased plants relative to healthy plants was variable but diseased plants tended to have a higher moisture content than healthy plants.

The seasonal pattern of the quantitative data from clone A and clone B was essentially the same and showed some correlation with the day length cycle. Although the data ~~was~~ were variable, plants of clone B tended to have a higher yield and higher percentage moisture than plants of clone A, but the mean height of regrowth was lower in clone B than in clone A. In section (a) (iv) it was shown that partial masking of symptoms occurred in growth of some clone B plants which was made between August and November and which received treatment IIW. At this time the mean dry weight production and the mean height of plants of clone B that received treatment IIW reached a minimum, but among the particular plants in which some symptom suppression was observed, the plant height and the dry weight yield was higher than the mean and the percentage moisture was lower than the mean. For example, the dry weight of the regrowth of the clone B plant shown in Plate 9 Figure 1a was 17.5 g., its height was 66 cm. and the percentage moisture of the regrowth was 77.12. The mean dry weight yield of clone B plants that received treatment IIW, was at this time 8.59 g., the mean height was 43 cms. and the mean percentage moisture was 80.68 (Tables 9 and 10).

(b) Factors affecting the incidence of disease and rate of infection

(i) Disease incidence on river terraces as compared with river flats.

Preliminary observations indicated that there was frequently a variation in disease incidence within a lucerne stand. This appeared to be associated with land contours. The following data indicate that there was a higher disease incidence in the part of the stand on the river terrace (high ground) than in the

part on the river flat (low ground). In 5 of the fields in Experiment 1 (Table 1) random traverses were made on both high and low ground and the percentage infection was consistently greater on the high than on the low ground. In Experiment 2 where the same plants were examined at each observation these results were confirmed. More than 2,000 plants approximately 10 years old, were observed on both high and low ground for periods of from 14 to 17 months. In each property the amount of infection was consistently greater in lucerne growing on river terraces than in lucerne growing 20 to 30 feet lower on river flats. The difference in observed disease incidence between the two aspects, was least in late winter and early spring, and greatest in late summer. The total number of identifiable Witches' Broom infected plants, for each of 6 observations, is shown in Table 2a where these data are also presented as percentages of the numbers of living plants observed.

The incidence of new infections in young stands was recorded in Experiment 4. The data indicate that the infection rate was greater on the high ground than on the low ground, although the figures are too small to carry weight.

(ii) Disease incidence in relation to age and density

Observations in the Lachlan Valley indicated that disease incidence was generally high in fields where the plant density was low, and low where the plant density was high. Old stands had a lower plant density than young stands where the disease was rarely observed in fields less than 4 or 5 years old. In older stands there was a considerable variation in disease incidence even among crops of the same age. For example, in Experiment 2 the maximum observed disease incidence of 5 stands which were approximately 10 years old, ranged from 33.9 per cent. in one property to 4.69 per cent. in another. In Experiment 1 (Table 1) the maximum observed disease incidence in two 10 year old stands was 40.27 per cent. and 9.77 per cent. and that of two 13 year old stands was 44.12 per cent. and 29.32 per cent.

On the western slopes of New South Wales where dryland lucerne is grown primarily for grazing, Edwards (1936) found that

fields 4 or 5 years old usually showed 20 to 25 per cent. infection whereas fields more than 7 or 8 years old commonly showed 60 to 70 per cent infection.

In Table 3 is shown the increase in disease incidence among a number of field plants during 2 and 3 consecutive years. This increase in observed percentage infection with age is partly due to the occurrence of new infections and partly due to a reduction in stand density. The maximum observed disease incidence in 1948 in Lot 1 was 49 per cent. and in 1949 it was 54.02 per cent. In Lot 2 the maximum disease incidence in 1948 was 15.06 per cent, in 1949 it was 18.47 per cent. and in 1950 it was 22.0 per cent. The disease incidence recorded in Lot 3 (Table 4) in 1949 was considerably higher than in 1950, but as stated earlier this was probably due to inaccuracies in identification of diseased plants.

Some information on the plant density of older stands was obtained in Experiment 1 where the mean number of plants per foot was recorded for each series of observations. The figures in Table 1 were obtained when the maximum disease incidence was observed. A comparison of the disease incidence in fields of the same age in Table 1 shows that the disease incidence of many but not all fields was greater where the mean number of plants per foot was lower.

The low plant density that is characteristic of an old stand may be a factor which predisposes lucerne to infection. To what extent, in the Lachlan Valley, the low plant density is correlated with the natural thinning of a stand with age, and to what extent it is a resultant effect of mortality of Witches' Broom diseased plants will be discussed later.

(iii) Rate of infection of young and old stands

Edwards (1936) reported that in New South Wales Witches' Broom disease had not been observed in stands of lucerne less than 15 months old, and numerous field observations together with data from Experiment 4 have confirmed this report. Among 730 plants in Paddocks(1) and (2) of this experiment the first infection observed was in July 1948, 15 months after the crop was sown. Two additional infected plants were recorded in December 1949, 4 in February 1950 and 4 in April 1950.

In Paddock (3) where 72 plants were studied, the first infection recorded was in February 1949, 46 months after the crop was sown. In 1949 two further infections were recorded in April, one in February, and two in December; in 1950, one was recorded in February and one in April.

The infection rate of stands of lucerne of more than 10 years of age was studied in Experiment 3. Because of the phenomenon of masking of symptoms which occurred in the late winter and spring months it was necessary to differentiate plants which showed symptoms in the summer after a period of symptom suppression in the winter and spring, from plants which showed symptoms for the first time following infection. This was done by defining a newly infected plant as one which was showing symptoms at the time when the observation was made, but which did not show symptoms the previous summer. No new infections were therefore recorded during the first year the stands were studied. Most were recorded in February and April of each year, as in Experiment 4, but one was also recorded in December and one in July. Table 3 shows that in Lot 1 where the maximum amount of disease observed in 1949 was 54.02 per cent., there were 7.87 per cent. new infections observed in February 1949 and 5.76 per cent. in April. In Lot 2 the maximum amount of disease recorded in 1949 was 18.47 per cent. and in 1950 it was 22.0 per cent. In 1949 there were 1.23 per cent. new infections recorded in February, 3.13 per cent. in April and 0.64 per cent in June; in 1950 there were 0.66 per cent. new infections recorded in December, 4.67 per cent. in February and 0.73 per cent. in April. In Lot 3 (Table 4) where the maximum amount of disease observed in 1950 was 67.35 per cent, 1.92 per cent. new infections were recorded in February 1950. It is considered that this figure was not a true indication of the new infections that occurred because the number of diseased plants recorded in 1949 was probably exaggerated and therefore, by the definition of a newly infected plant that has been used, this figure is probably lower than it should have been.

Data presented in section (b) (ii) showed that there were more diseased plants in old stands than in young stands and it was

suggested that this may have been associated with a difference in plant density. Data in this section indicate that in addition the rate of infection was greater in old stands than young stands and that this may be correlated with the greater number of diseased plants in old stands. This implies that spread of infection occurs within a crop.

(c) The relationship of the disease to lucerne mortality

(i) The life span of a diseased plant

Individual plants were observed in the field for 33 months. In Lot 2 of Experiment 3, 6 of 9 plants rated as diseased in January 1948, were still alive in October 1951. The 3 mortalities occurred between April 1950 and October 1950. Five of 9 additional plants in Lot 2, observed to be diseased in March 1948, were still alive in October 1950, 2 had been recorded dead in October 1950 and one each in February and April 1949.

Fifteen of thirty newly infected plants studied in Experiment 2 were alive when observations were concluded; the period that they had been known to be infected ranged from 2 to 18 months. The known period of infection of the 15 plants which died, ranged from less than 2 to 10 months.

Two of the 11 young plants which became infected in Paddock (1) of Experiment 4 died during the experiment. One survived 3 months after it first showed symptoms, and the other 15 months. The infection period of surviving plants ranged from 6 to 12 months.

The field data indicate that under the conditions that occur in the Lachlan Valley, mortalities of infected plant s were not confined to one season of the year. They also show that it is not unusual for an infected plant in a 10 year old stand to remain alive for from 31 to 33 months. It seems likely that some survive for a longer period, whereas others die following a period of known infection of less than 2 months.

In May 1947, 10 plants were established in a greenhouse by subdividing several infected plants brought in from the field. Favourable growing conditions were maintained and the plants were

cut back infrequently. One plant survived 53 months and another was still alive after 54 months. This indicates that if given favourable conditions of growth, lucerne is tolerant of the Witches' Broom virus.

(ii) The mortality of diseased plants in relation to severity of symptom expression

The records of rated plants that died were examined, to find out whether field plants with severe symptoms were more likely to die than plants with milder symptoms. In Lots 1 and 2 in Experiment 3, 108 plants showed definite symptoms. Eighty-seven of these plants were rated X_o or X_1 during the experiment and 21 were never rated more severely than X_2. Among those rated X_o or X_1 there were 25 mortalities and among those rated X_2 there were 7 mortalitiew. Some of the mortalities were recorded shortly after a period of masking of symptoms.

In this experiment, therefore, there was no evidence which indicated that field plants with severe symptoms were more likely to die than those with milder symptoms, but the existence of strains is probably a complicating factor. Observations of diseased plants grown in the greenhouse indicated that under favourable conditions infected plants usually showed increased stunting and proliferation before death, even though variations in intensity of symptom expression had previously occurred. Plate 10, Figure 1 illustrates the increase in severity of symptom expression that frequently occurs with age, in the greenhouse, and Plate 10 Figure 2 shows an old plant with severe symptoms which was photographed shortly before it died.

In Experiment 5 (Table 11), plants in the greenhouse with more severe symptoms died out more rapidly than those with milder symptoms; for example, 27 mortalities occurred among plants of clone B as compared with 4 among plants of clone A.

(iii) Comparison of mortalities of diseased plants on river terraces and on river flats

The total mortalities caused by W.B. or (W.B. & O.C.) in the 5 properties in Experiment 2 at each of 6 observations are seen in Table 2a. At each observation more mortalities due to W.B. occurred

on high ground than on low ground. The total number of mortalities in each property certainly caused by W.B. or (W.B. & O.C.) during the period from February 1949 to February 1950 is given in Table 2b. The combined totals of these two classes, presented as a percentage of the original number of plants in each property, indicate that the percentage mortality on the high ground was greater than that on the low ground. In the analysis of the difference between high and low ground from data in Summary Table 2b the proportion p dying from (W.B. & O.C.) was converted to m where $1-p = e^{-m}$. The difference of the logarithms of m values from high and low ground was taken as a criterion of location effect. These differences were weighted inversely proportional to their variances estimated on a random sampling basis. The weighted mean difference proved to be significantly different from zero at the 1 per cent. level using variation between properties for error. The weighted ratio of m values corresponding to this mean difference is 3.42 and is a satisfactory measure of the relative risk of dying from (W.B. & O.C.) on high relative to low ground.

(iv) Comparison of mortalities due to the disease and those due to causes other than the disease

Mortality of Witches' Broom infected plants was not confined to one season of the year, but it was high under hot, dry conditions and also after heavy rainfalls. The chief causes of mortality of plants rated as healthy in Experiments 2 and 3 were water-logging, scarifying, and excessive grazing. No explanation could be given for the loss of a number of plants.

No data ~~is~~ are available on the comparative losses due to W.B. or (W.B. & O.C.) in young stands. In 10 year old stands in Experiment 2 where a maximum of 11.87 per cent. of plants on the high ground and 5.04 per cent. of plants of the low ground were observed to be infected in 1949, the total loss of plants in the period beginning in February 1949 and ending in February 1950, calculated from Tables 2a and 2b, was 12.3 per cent. among 1050 plants on the high ground and 9.05 per cent. among 1160 plants on the low ground. On the high ground 57.4 per cent. of the loss was due to W.B. or (W.B. & O.C.) and 42.6 per cent. was due to O.C. On the low ground 22.9 per cent. of the

loss was due to W.B. or (W.B. and O.C.) and 77.1 per cent. was due to O.C.

Only information obtained from Lots 2 and 3 during the period of February 1949 to February 1950, as shown in Table 5, has been used to compare data of mortalities due to W.B. and O.C. in Experiment 3 with that obtained in Experiment 2, as no information from Lot 1 was obtained during this period. In April 1949, 16.88 per cent. of plants were diseased in Lot 2 and between approximately 57.68 and 81.48 per cent. were diseased in Lot 3. The total loss of plants in Lots 2 and 3 taken as a percentage of the initial number of plants observed in February 1949 was 11.7 per cent. This loss consisted of 53.7 per cent. due to W.B. and 46.2 per cent. due to O.C. The relative losses on high and low ground are therefore more comparable with results obtained on the high ground in Experiment 2 than on the low ground.

In both experiments the number of lucerne plants was reduced by approximately 12 per cent. during a period of 13 months. Except on the low ground in Experiment 2 there was little difference in the percentage of Witches' Broom diseased plants that died and the percentage of apparently healthy plants that died. This indicates that Witches' Broom was the most important single factor that caused mortality of lucerne plants. On the river flat area in Experiment 2, only 5.04 per cent. of plants were infected in April 1949 and 3 times as many plants died due to O.C. as died due to W.B. or (W.B. & O.C.), but this was a direct result of flooding. The unusually heavy rainfalls in the summer of 1950 (Figure 1) resulted in water-logging which caused heavy losses of both healthy and infected plants in Experiments 2 and 3. Losses in stands of similar age in a season of normal rainfall would be much less.

(v) The effect of two frequencies of watering and two frequencies of cutting on the mortality of diseased plants

The length of life of an infected plant in the greenhouse was found to be influenced by cultural treatments. In Experiment 5 (Table 11), a total of 31 of the 88 infected plants died.

With few exceptions plants died after they were cut.

Within the group of plants that received the low water level treatment, there were 11 mortalities among those cut at 6-weekly intervals and 5 among those cut at 12-weekly intervals. Within the group of plants that received a high water level treatment, there were 13 mortalities among those cut at 6-weekly intervals and 2 among those cut at 12-weekly intervals. All the mortalities of plants cut at 12-weekly intervals and 20 of the 27 mortalities of plants cut at 6-weekly intervals occurred among plants of clone B.

The results show that cutting at 6-weekly intervals as compared with cutting at 12-weekly intervals caused a high mortality of infected plants. Neither treatment caused mortalities of healthy plants.

Table 11

Number of plants alive at each cut in Experiment 5

Date of Harvest	Clone H	Clone and treatment							
		Clone A				Clone B			
	All treatments.	ID	IW	IID	IIW	ID	IW	IID	IIW
12.iv .50	4	11	9			10	10		
24.v .50	4	11	9	11	11	10	9	11	11
5.vii .50	4	11	9			9	6		
10.viii.50	4	11	8	11	11	8	1	11	10
27.ix .50	4	11	8			5	1		
8.xi .50	4	11	8	11	11	3	1	10	9
20.xii .50	4	11	8			3	1		
31.i .51	4	11	8	11	11	3	1	9	9
14.iii .51	4	11	8			2	1		
24.iv .51	4	10	8	11	11	1	1	6	9
Total number of mortalities	0	1	3	0	0	10	10	5	2

B. Data from the Lockyer Valley, Queensland

Data in section A gives information on Witches' Broom as a field disease in New South Wales; data in this section indicates that

the disease is of more importance at Lawes in the Lockyer Valley, Queensland, than in the Lachlan Valley, New South Wales, and it suggests that this is primarily due to climate. During some lucerne breeding experiments at Lawes, C. S. Christian made records of all plants that showed symptoms of Witches' Broom disease and the data have been made available for inclusion in this paper. They cover observations of a large number of individual plants and extend the available information on the disease in relation to environment. They are not strictly comparable with those obtained in New South Wales because they were obtained from plants established at a spacing of 3 feet by 2 feet and because many infected plants were dug out when they first showed symptoms.

In July 1936, 974 lucerne plants consisting of a number of local and introduced varieties were established. Plants infected with Witches' Broom disease were recorded at monthly intervals during the first 11 months and afterwards at 2 or 3 monthly intervals. The first 2 infections were recorded 5 months after the lucerne was planted and by the time the crop was 7 months old, 9 plants had become infected; at 19 months, 98 plants had been infected, and at 31 months 262 plants had shown symptoms. Most new infections were recorded between November and April. After 31 months 43.9 per cent of the initial plants were alive and 17.7 per cent. of the losses had been due to W.B. or (W.B. & O.C.).

Another planting of lucerne was made in July 1937 and in December 1938 10.2 per cent. of the 1,161 surviving plants showed symptoms of Witches' Broom. In February 1939, 11.2 per cent. of the living plants were infected, but in April only 9.6 per cent, and in August only 2.8 per cent were infected. The following data indicate that this was due to masking of symptoms. Of the 119 infected plants recorded in February, 75 plants showed definite symptoms in April, 28 plants were dead or nearly dead, 5 appeared healthy and 11 showed very mild symptoms. The loss due to W.B. or (W.B. & O.C.) in the 8 months between December 1938 and August 1939 was equivalent to 35.4 per cent. of the total loss.

In July 1938, 7,776 experimental hybrids were established.

In March 1939, 148 of the 5,229 surviving plants were infected and by April 1940,108 of these were dead and a total of at least 7.7 per cent. of the initial plants had been infected. The loss due to W.B. or (W.B. & O.C.) in the 13 months between March 1939 and April 1940 was equivalent to 14.5 per cent. of the total loss.

The data indicate that the infection rate of young stands was greater at Lawes than in the Lachlan Valley. This may have been partly due to the spacing of the plants at Lawes which may have made them both more susceptible and more readily recognisable as infected, and it may also have been partly because the plants at Lawes consisted of several varieties and experimental hybrids. These factors may also have contributed to the high mortality rate of plants. It is, however, considered that differences in data obtained at Lawes and the Lachlan Valley were primarily due to the effect of climate on the host plant and on the vectors.

Little information is available on the annual distribution of the leaf hoppers in Queensland. Most new infections at Lawes were recorded between November and April, whereas in New South Wales most were recorded between February and April. Helson (1951) showed that at Dickson Experiment Station, A.C.T., Orosius argentatus (EVANS), the only known vector of the disease in Australia, was most abundant in the spring and early summer, although 4 additional species of leaf hoppers reached their population peaks in summer and autumn. It therefore appears probable that in the Lachlan Valley, where the same leaf hopper species occur, (Helson private communication) lucerne would be infected in the spring. If plants were infected in the autumn, symptoms would not appear until the following summer because they are normally masked in the spring. The data on time of appearance of new infections at Lawes suggests that vectors are there prevalent over a longer period of the year, and this, together with the longer growing season, would cause plants to become infected at a younger age and to show symptoms more rapidly following infection. The longer growing season would also tend to increase the mortality rate of plants.

IV. Discussion

(i) Masking of symptoms

Edwards (1936) reported that in New South Wales masking of symptoms of Witches' Broom infected lucerne sometimes occurred in the spring and that there was occasional temporary or permanent masking of symptoms when diseased plants were transplanted from the field to the greenhouse and given favourable growing conditions, but Menzies (1946) stated that this phenomenon had not been observed in America. Results in this paper have shown that in the Lachlan Valley, New South Wales, masking of symptoms normally occurs in the spring, and that the maximum number of diseased plants show symptoms in the mid or late summer. They have also shown that masking of symptoms occurs in diseased plants at Lawes, Queensland, although it is not as general as in New South Wales. Masking of symptoms has been reported in a number of virus infected hosts, but this phenomenon is of particular interest in lucerne as there appears to be no other report of a virus-infected perennial field crop in which there is annually recurring masking of symptoms of individual plants.

Data in Experiment 5 showed that infrequent cutting and a readily available water supply are factors associated with masking of symptoms. Because the partial masking of symptoms in clone B plants occurred at almost the same time of the year that complete masking of symptoms occurs in the field, it is suggested that short day length, low temperature and possibly low light intensity in the growing season are also associated with the phenomenon. Richards *et al.* (1946) noted an association between symptom suppression and short day length and low light intensities in potatoes infected with leaf roll, and Carter (1929) showed that intense light and high temperatures intensified symptoms of curly top of sugar beet.

The occurrence of only partial masking of symptoms in Experiment 5, whereas complete masking of symptoms normally occurs in the field in the spring, may be due to a temperature effect because the greenhouse was heated during the winter. Temperature differences may also have been the main factor which caused masking of symptoms

to be less general in Queensland than in New South Wales.

Low temperatures may induce masking of symptoms either by directly affecting virus activity or by reducing the rate of growth of the host plant. In the field masking of symptoms occurs in the relatively slow growth which follows winter dormancy and symptoms re-appear in the rapid growth that follows either the first spring cut or an early summer cut; symptoms are still slower to re-appear in field infected plants that are not cut. These data suggest that virus activity is dependent on the rate of regrowth of the host or that it is increased by the temporary lowering of resistance of the host following cutting when reserves of protein and carbohydrate are drawn upon to produce new growth. During winter dormancy root reserves of healthy plants are maintained at a high level and respiration is reduced (Willard 1951). This probably also occurs in diseased field plants and it may increase the resistance of the host to the virus. In the greenhouse experiment the continuous growth made during winter (Figure 1) undoubtedly weakened all plants.

It appears therefore that winter dormancy retards the progress of the disease either by increasing the resistance of the host to the virus or by reducing the activity of the virus. Masking of symptoms occurs in the relatively slow growth in the spring, when days are short and light intensity and temperatures are low. At this time of the year infrequent cutting combined with favourable moisture conditions, delay and reduce severity of symptoms.

(ii) Causes of variability of symptom expression between plants

It has been shown that variation in symptom expression of individual plants occurs at different times of the year. Field experiments have also shown that at any one time a considerable variation in symptoms of infected plants exists within a stand. This was particularly noticeable in the late summer or autumn when symptom expression was at its maximum. Variability may be partly an expression of the length of time plants have been infected, but this is not supported by data obtained under field conditions, although it is supported to a certain extent by data obtained in the greenhouse.

Variability may also be due to a difference in varietal response to infection because the Hunter River variety of lucerne grown in New South Wales is not always uniform. Data in Experiment 5 suggest, however, that symptom variability between plants at any one time is largely due to the existence of strains of the virus. In this experiment each of the two infected clones A and B maintained its characteristic identity throughout all treatments, although there was variability, between treatments of each clone at the same time of the year, as well as within treatments of each clone at different times of the year. Variation of symptom expression at one time between plants of a single clone and treatment is considered to have been due to a variation in the virus concentration of the original clonal cuttings.

Both the severity of symptom expression and the total number of mortalities, were considerably greater among plants of clone B than among plants of clone A, and yet partial symptom suppression occurred in some plants of clone B but not in any plants of clone A. In addition, the mean dry weight yield of regrowth and the percentage moisture per living plant tended to be greater in clone B than in clone A, although the mean height of regrowth tended to be lower in clone B than in clone A. In a later paper further evidence will be presented to show that clone A and clone B are infected with distinct strains of the Witches' Broom virus.

(iii) Some physiological effects of the virus on the plant

In the controlled cutting and watering experiment the reduction in the dry weight yield, height and flowering of regrowth of infected plants relative to controls, suggests that the virus reduces the carbohydrate content of lucerne plants. This is supported by the fact that infected plants usually died following cutting when they failed to initiate new growth, and that infected plants cut frequently, showed a greater mortality rate than those cut infrequently. Several writers including Graber et al. (1927), Grandfield (1935) and Hildebrand and Harrison (1939) have shown that frequent harvesting of lucerne at immature stages reduces the productivity and life of lucerne stands and that this is primarily as a result of depleted food reserves in the root. Preliminary microchemical tests have indicated that the

nitrogen content of crowns of infected plants is increased relative to the controls and that the starch reserves are reduced or absent in crowns of infected plants.

(iv) Incidence of the disease on river terraces and river flats

Although field data showed that low incidence of the disease on river flats as compared with river terraces may be due to masking of symptoms of infected plants on ground where the available water supply was more favourable, data obtained in Experiment 5 did not support nor disprove the hypothesis. Partial masking of symptoms occurred among some plants that were cut at 12-weekly intervals and watered frequently, but it only occurred in growth made between August and November. As there was a different response to treatment between clones A and B it is possible that some field infected plants that showed a different type of symptom might show a greater response to treatments than plants of clone A. It is also possible that newly infected plants would respond more effectively than plants that had been infected for some time.

The difference in disease incidence on the two levels of ground may, however, be due to a higher infection rate on the high ground. Direct evidence on this point was obtained from only a small number of plants in a young stand. During a period of 33 months, 10 new infections were recorded on high ground among 279 plants, whereas one occurred among 292 plants on the river flat. In another field 4 new infections were recorded on both levels of ground among a total of 100 plants. Indirect evidence, which showed that the mortality rate of infected plants and the disease incidence on high ground was greater than on low ground, also supports the suggestion that infection rate on high ground was greater than on low ground.

The infection rate of plants on the high ground would be greater than that on the low ground if plant susceptibility was greater, or if the vector population or vector activity was greater on the high ground than on the low ground. In a field where the disease incidence on river flats and on river terraces is compared, the fertility as well as the available water supply is usually greater on the river flat. The observed low incidence of Witches' Broom

in irrigated as compared with non-irrigated lucerne grown in the same area (Edwards 1936) suggests that if disease incidence is related to susceptibility of the host plant, susceptibility is more likely to be associated with the availability of water than with a difference in soil fertility.

Leafhoppers coming into the crop from the hillsides may be more likely to infect lucerne on the river terraces than river flats. They may also show a preference for relatively warm and dry environmental conditions on the terraces. Carter (1930) showed that ~~Eutettix tennelis~~ CIRCULIFER TENELLUS (BAKER) the leafhopper vector of sugar beet curly top favoured relatively open situations with low humidities. Such an influence of microclimate on the population or activity of vectors would provide an explanation for the low incidence of disease on river flats where environmental conditions are shady and humid relative to the high ground where they are warm and dry. It would also explain the observed low disease incidence in irrigated as compared with unirrigated lucerne as well as in young as compared with old crops.

(v) Economic importance of the disease

In Australia where lucerne is grown over a wide climatic range, there has been a considerable variation in the assessed importance of Witches' Broom of lucerne. Data suggest that this is largely due to the phenomenon of seasonal masking of symptoms of infected plants, and indicate that in a temperate climate rating for disease incidence should only be made between mid-summer and early autumn when the maximum number of diseased plants can be recognised. They show that infection is greater on land poorly supplied with water than on land well supplied with water and that frequent cutting induces mortality of diseased plants.

Edwards (1936) found that diseased plants in dryland lucerne cut twice a year gave a mean yield of 37.4 per cent. less green fodder than uninfected plants. Greenhouse data in this paper showed that the mean loss in dry weight yield of one infected clone ranged from 65 per cent. to 38 per cent. according to the cultural treatments used. The reduction in dry weight yield of diseased plants in the

field would not, however, be as great as in the greenhouse as maximum reduction in yield would occur only in the mid-summer or early autumn. In the Lachlan Valley 5 to 7 cuts are made in good seasons, and whereas crops are usually considered to be unprofitable after 10 or 12 years there are many older crops in the district. The disease is rarely seen in stands less than 4 years old but more than 70 per cent. infection has been observed in old crops. Fifteen months is the earliest time after sowing that an infected plant was observed, but it is considered that although fewer plants may become infected in young than old stands because of preference of the vector for more open situations, plants which do become infected are rarely seen because they die out in competition with healthy plants. It is not considered that the disease would cause a significant reduction in yield in crops less than 6 to 8 years old. Up to that time the increased yield from healthy plants as a result of reduced stand density would compensate for mortalities caused by the disease. In a 10 year old stand the loss of plants due to Witches' Broom or the disease together with other causes was approximately equivalent to the losses due to all other causes other than where exceptional flooding caused large losses of uninfected plants.

According to Edwards (1936), the disease is of economic importance at an earlier age in dryland lucerne grown in western New South Wales.

C. S. Christian (private communication) reported that Witches' Broom was probably the most important single factor that caused dryland lucerne in the Lockyer Valley in southern Queensland to become unprofitable 4 or 5 years after sowing. Up to 70 per cent infection was recorded in unirrigated stands 3 to 4 years old, but he considered that the disease was not of economic importance where a 4 or 5 year rotation of dryland crops was practiced. Nevertheless it is evident that the disease becomes a limiting factor in the profitable life of a lucerne stand at an earlier age in Queensland than in New South Wales.

The disease, therefore, is not of economic importance in the southern parts of Australia, but it becomes more severe where

lucerne is grown in northern New South Wales and in southern Queensland. Where experimental stands of lucerne have been grown under tropical conditions in the Northern Territory and in the north of Western Australia, the disease causes severe losses within 2 years of sowing, Durack (1945), and it is considered that if lucerne production were extended in these areas ~~that~~ the disease would be of economic importance.

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Explanation of Plates 1-10

Plate 1.

Witches' broom diseased lucerne plants of ratings X_1 and X_2 photographed in May 1950.

Fig.1. X_1 plant showing extreme stunting and production of small rounded leaves.

Fig.2. X_2 plant with numerous thin stems bearing leaves which are slightly rounded.

Fig.3. X_2 plant which is compact and symmetrical as compared with Fig.2.

Plate 2.

Parent plants of clones used in Experiment 5.

Fig.1. Parent of clone A

Fig.2. Parent of clone B

Fig.3. Parent of clone H

Plate 3

Fig.1. Masking of symptoms in field plants. Plants in Paddock 1 of Experiment 4 seen in May 1950.

(a) Stunted infected plant showing symptoms in young growth at the base of the plant. Older shoots have lost their leaves and most young stems are very fine and have proliferated and produced small rounded leaves. A few stems have formed leaves of normal shape and size. This plant was first recorded as infected in April 1950.

(b) Adjoining healthy plant.

Fig.2. Plants (a) and (b) in Fig.1. as they appeared in November 1950. The photographs were taken at mid-day and the plants wilted when separated from the surrounding growth.

(a) Infected plant with masked symptoms. It is producing vegetative growth that resembles that of a healthy plant.

(b) Healthy plant.

Plate 4

The photograph was taken in January 1948

Fig.1. (a) Top of a stem from a healthy lucerne plant.

(b) Top of an old stem from a lucerne plant that was not harvested in the spring. The stem is of comparable thickness with that of (a) but it shows proliferation. It probably produced leaves of normal shape and size in the spring.

Fig.2. Infected and healthy lucerne plants from a seed crop photographed in February 1948.

Fig.2.(contd)

(a) Infected plant which failed to set seed shows extreme proliferation of the inflorescences.
(b) Healthy plant with normal seed.

Plate 5

Fig.1. Masking of symptoms in greenhouse plants
(a) Plant with masked symptoms photographed in August 1950. It was established in November 1949 from a cutting of an infected plant which was then showing masked symptoms. It first showed symptoms in January 1950.
(b) Uninfected plant established in November 1949 from a cutting of a healthy plant.
(c) Plant established from a cutting obtained from (a) in August 1950, and photographed in December 1950 when (a) was also showing symptoms.

Plate 6

Effects of the 4 treatments in Experiment 5 on plants of clone H on April 23, 1951

Fig.1. (a) Treatment IIW.
(b) Treatment IID.
Fig.2. (a) Treatment IW.
(b) Treatment ID.

Plate 7

Effects of the 4 treatments in Experiment 5 on plants of clone A on April 23, 1951.

Fig.1. (a) Treatment IIW.
(b) Treatment IID.
Fig.2. (a) Treatment IW.
(b) Treatment ID.

Plate 8

Effects of the 4 treatments in Experiment 5 on plants of clone B on April 23, 1951.

Fig.1. (a) Treatment IIW.
(b) Treatment IID.
Fig.2. (a) Treatment IW.
(b) Treatment ID.

Plate 9

Fig.1. Plants of clone B in Experiment 5 on November 10, 1950.
(a) Treatment IIW.
(b) Treatment IID.
Fig.2. Plants of clone H in Experiment 5 on November 10, 1950.
(a) Treatment IIW.
(b) Treatment IID.

Plate 10

Fig.1. Infected plants grown in the greenhouse showing increase in severity of symptom expression with age. The photo-

Fig.1 (contd)

-graph was taken in October 1951.

(a) Plant which was established in November 1949 from a cutting of a parent plant that showed masking of symptoms. It first showed symptoms in ~~July~~ Jan. 1950.

(b) Plant established from (a) in August 1950, when (a) was showing masked symptoms. Plant (b) first showed symptoms in December 1950.

Fig.2. Old infected plant showing extreme proliferation and reduction of leaf size.

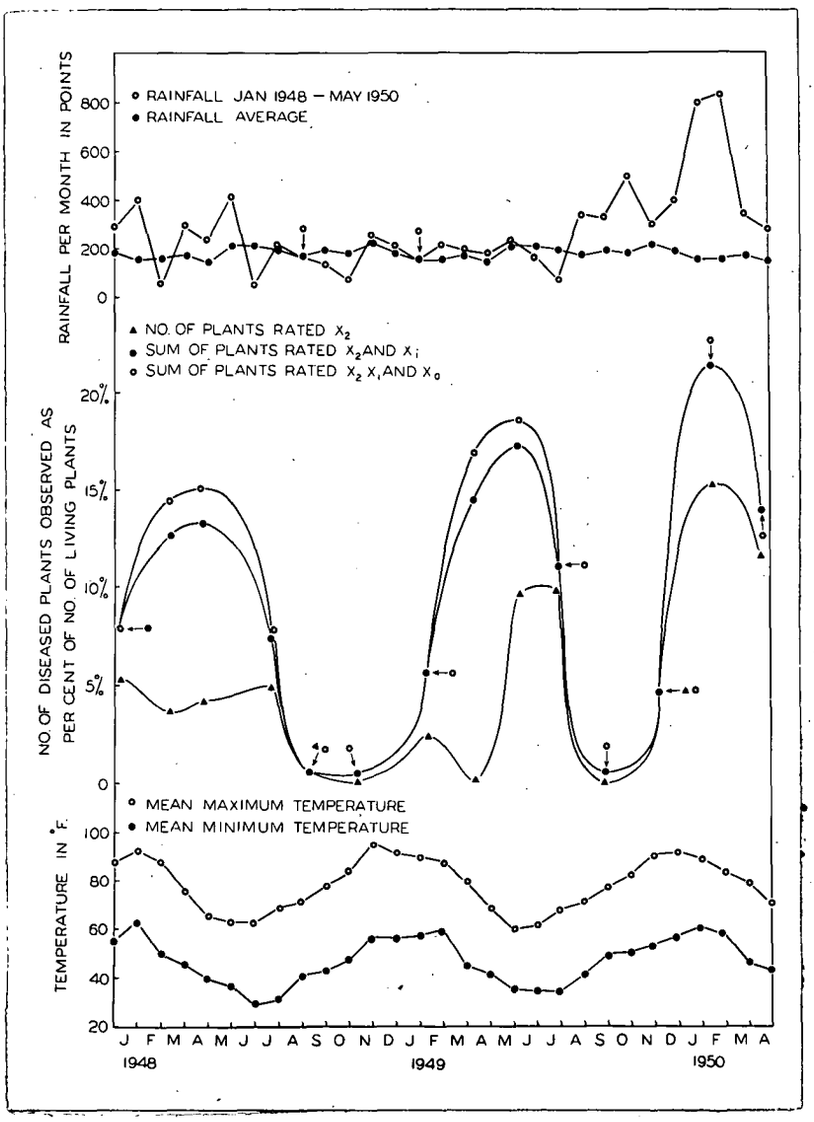

Fig. 1.- Observed incidence and symptom expression of witches' broom of lucerne in the Lachlan Valley, New South Wales, in relation to time of year , temperature and rainfall. Diseased plants were rated x_2 x_1 and x_0 in increasing order of severity of symptom expression and therefore the sum of plants rated x_2 x_1 and x_0 is equivalent to the total number of diseased plants observed.

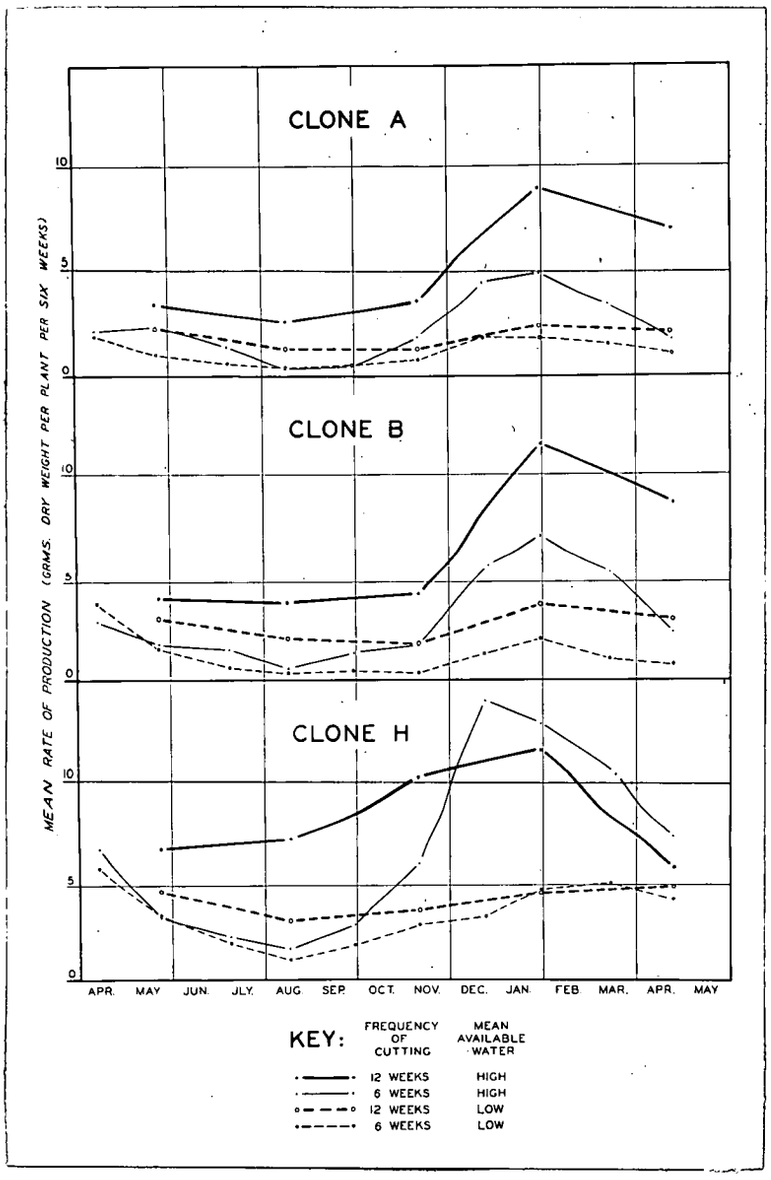

Fig.2.- Effects of 4 treatment combinations of cutting and watering on the mean rate of production of clones A and B (clones infected with witches' broom) and clone H (healthy clone).

PLATE I.

Fig. 1

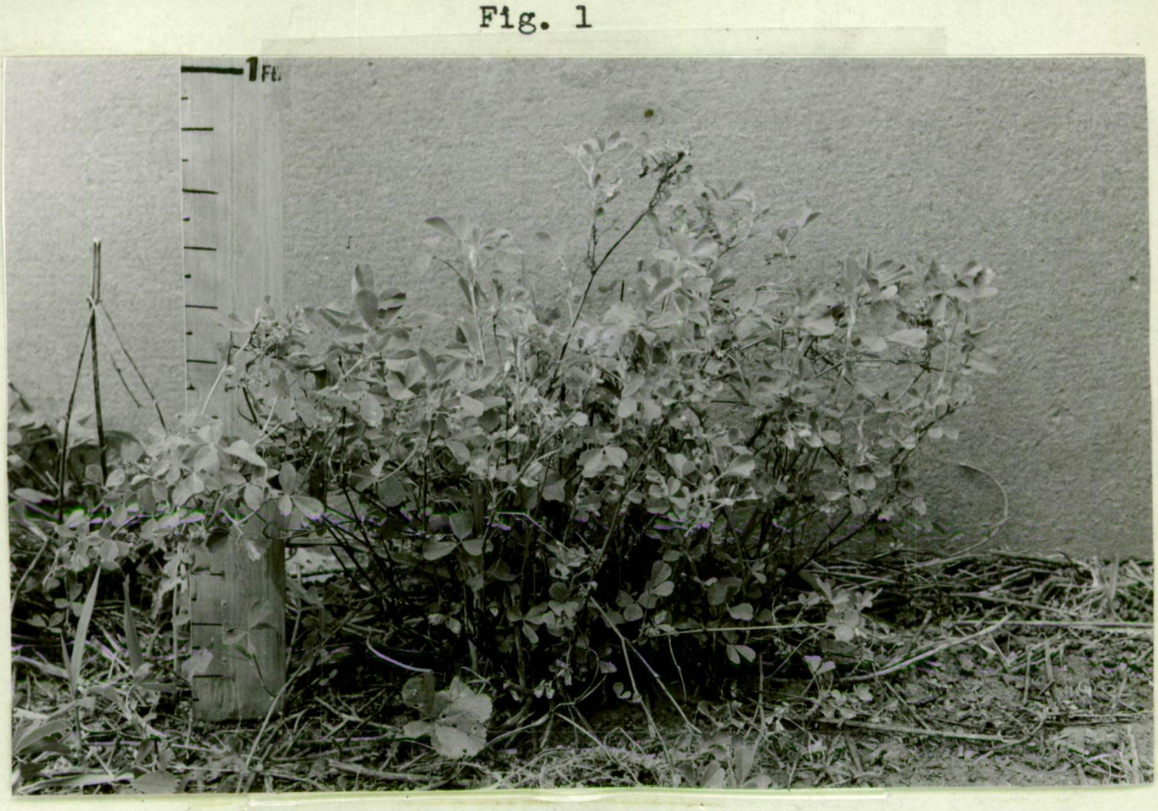

Fig. 2

Fig. 3

Fig. 1

Fig. 2

Fig. 3

PLATE 2.

PLATE 3.

a b

Fig. 1

a b

Fig. 2

PLATE 4.

a b

Fig. 1

a b

Fig. 2

PLATE 5.

a b c

Fig. 1

PLATE 6.

a b

Fig. 1

a b

Fig. 2

PLATE 7.

a b

Fig. 1

a b

Fig. 2

PLATE 8.

a b

Fig. 1

a b

Fig. 2

PLATE 9.

a b

Fig. 1

a b

Fig. 2

PLATE IO.

a b

Fig. 1

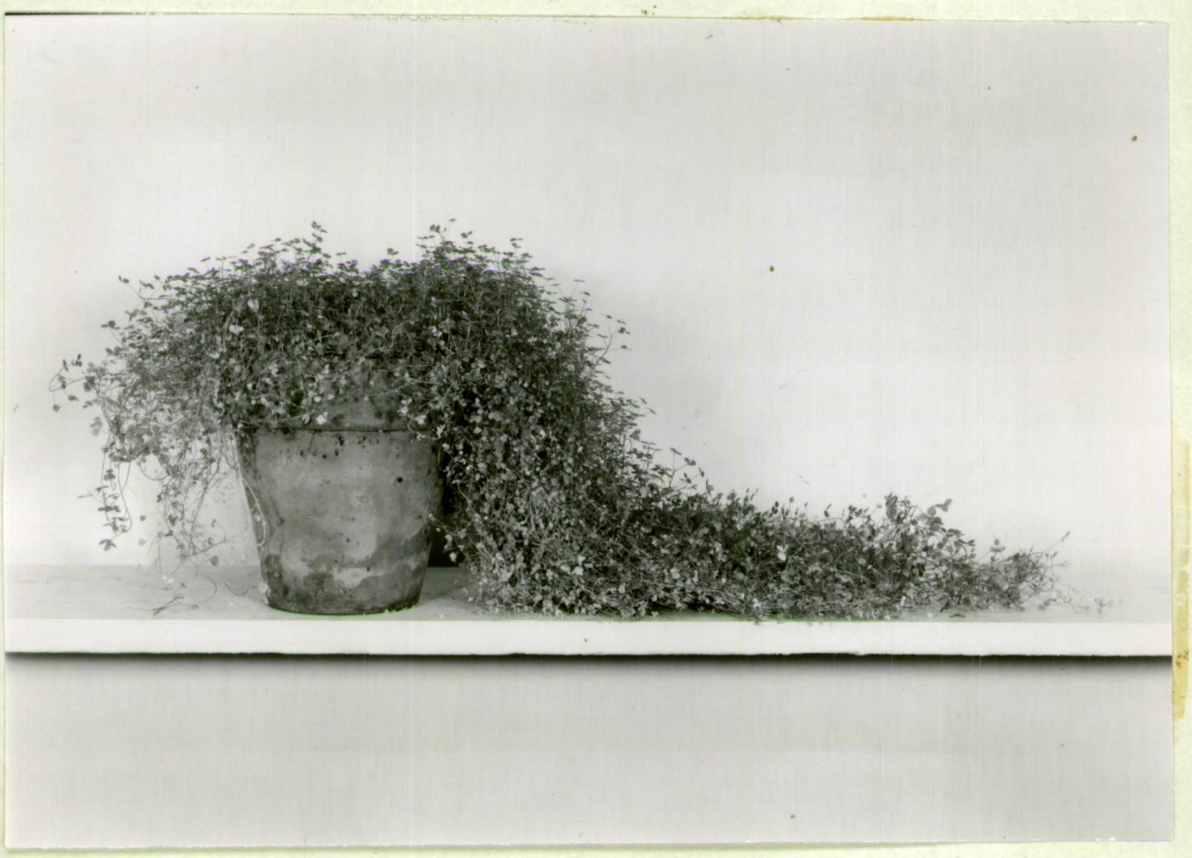

Fig. 2

(2) The identity of witches' broom of lucerne and big bud of tomato in Australia

Summary

The symptoms of witches' broom virus disease of lucerne on 8 new host species are described. Two naturally occurring strains of the disease produced distinctive symptoms on all hosts to which they were transmitted. On 14 host species the more severe of the two strains and similar naturally occurring strains, produced symptoms that were indistinguishable from those of big bud of tomato. Witches' broom and big bud are considered to be caused by the same virus because they are transmitted by the same vector and they have a similar host range on which they produce similar symptoms. Symptoms of witches' broom of lucerne in Australia are compared with symptoms of witches' broom of lucerne in America and some other yellows viruses.

I. Introduction

Witches' broom of lucerne in America was shown by Menzies (1946) to be graft transmissible to Medicago lupulina L. and Medicago hispida Gaert. Orosius argentatus (Evans) was found by Helson (1951) to be a vector of the disease in Australia, and by means of this leafhopper the disease was successfully transmitted to 6 non-leguminous hosts. In America recent leafhopper transmission studies with Scaphytopius (Cloanthus) dubius (Van Duzee) by Klostermeyer and Menzies and dodder transmission studies by Kunkel (1952) have shown that the host range of the virus includes 13 additional legume species and two additional species of non-legumes.

This paper describes results of graft and dodder transmission experiments begun in 1947. They further extend the host range of the virus and indicate that in Australia, witches' broom disease of lucerne and big bud of tomato are caused by the same virus.

II. Methods

All plants used in the experiments were grown in an insect-free greenhouse that was heated during the winter. Lucerne plants developed from clonal cuttings of a plant of the Hunter River variety,

were rooted in sand in a humidity chamber before being transferred to pots. Periwinkle plants (Vinca rosea L.) were propagated by cuttings, potato plants were grown from virus-free tubers and other plants were grown from commercial seed.

(a) Mechanical inoculation

Infected leaf and stem tissues were macerated in water and young plants were dusted with carborundum powder before the inoculum was rubbed on the leaves.

(b) Dodder transmission

Cuscuta campestris Yuncker was used for testing new host plants and for checking infection of these hosts by transmission of the virus to plant species on which symptoms of witches' broom were known. Virus-free dodder was maintained on healthy tomato, tobacco, periwinkle or lucerne plants and infective dodder was grown on field infected lucerne plants. Most of the transmission experiments were done with dodder grown on two clones of witches' broom infected plants, known as clones A and B and identical with those used and illustrated in another experiment (Helms, Paper (1)).

The following is a description of the vegetative features of the two clones:

Clone A. Leaves were small, rounded and either chlorotic or of normal colour. Juvenile leaves were sometimes marginally chlorotic and puckered. Stems were upright, numerous, thin and rounded. Occasionally small axillary shoots developed on old stems but secondary proliferation was not extensive. Sometimes a few abnormal pale mauve flowers were formed (Plate 1, Figure 2).

Clone B. Leaves were very small, rounded, thin and very numerous. They had long petioles and were usually chlorotic. Stems were very thin, rounded and numerous and often purple in colour. Internodes were short. Numerous secondary shoots were formed, especially at the bases of young stems and in the leaf axils of older shoots. These gave the plant a matted procumbent appearance. Flowers were rarely formed and were either pale mauve and almost norma in structure as shown in Plate 1, Figure 3 or virescent with various abnormalities similar to those shown in Plate 2, Figure 1.

Dodder from Clone B was used to a greater extent than dodder from Clone A, because whereas on Clone B, dodder maintained its vegetative growth indefinitely, on Clone A it grew only a short time before setting seed. In the early experiments dodder growing on infected plants was connected to the plant that was to be infected, but, when it was found that detached pieces of dodder were equally successful in transmitting the virus, two or three pieces of stem each 3 to 6 inches long were used for transferring the virus. The dodder was grown on the new host for at least two weeks. In all tests control plants were set up with dodder grown on healthy plants. Dodder was also maintained on a number of tomato plants that were infected with big bud virus from several sources.

(c) Graft transmission

Irrigated scion grafts were used for virus transmission to and from most hosts. Transmission from lucerne to lucerne by shoot grafting was unsuccessful and only crown grafts are recorded in Table 1.

III. Results

(a) Attempts to transmit witches' broom of lucerne mechanically

Between October 1947 and January 1948 juice from witches' broom diseased lucerne was mechanically inoculated to 5 plants of each of 6 species of Medicago (including lucerne), 4 species of Trifolium and 2 species of Melilotus. At the same time, two plants of each species were inoculated with juice from healthy lucerne plants. Twenty months later, when final observations were made, half of the plants had died and none had shown symptoms.

One of the yellows viruses, potato yellow dwarf, was shown to be easily transmitted mechanically to Nicotiana rustica by Black (1938). In January 1951 5 young N. rustica plants were inoculated with juice from witches' broom infected Nicotiana glutinosa and 5 N. rustica seedlings with juice from witches' broom infected N. rustica. Control plants were inoculated with juice from uninfected plants of N. glutinosa and N. rustica. No plant showed symptoms within a period of 6 weeks.

The data showed that the disease was not likely to be

readily transmitted mechanically to alternative hosts. This is in accord with Edwards (1936) and Menzies (1946) who failed to transmit the disease mechanically from lucerne to lucerne.

(b) Transmission of witches' broom of lucerne to lucerne by dodder

Transmission of witches' broom disease to lucerne by means of Cuscuta campestris was not obtained by Menzies (1946) and Kunkel (1952). In Table 1 it is shown that dodder transmission of the disease to lucerne was obtained on seven occasions. These results were obtained in a number of experiments made during a period of four years.

In one of the larger experiments 2 of 20 plants were infected. Dodder which had been growing for a number of months on Clone B lucerne was attached to young rooted lucerne cuttings. It grew vigorously and was removed after 25 days. After 65 days the plants were cut back and 74 days later one showed strongly marked puckering, crinkling and marginal chlorosis of young leaves. Thirty-four days later all plants were again cut back and after six days a second plant showed similar symptoms. The two infected plants continued to show symptoms of the Clone B type but no further plants showed evidence of infection when the plants were again cut back and observed for two more months.

In another experiment one lucerne plant which was infected with Clone A virus, consistently showed symptoms characteristic of Clone A lucerne.

Experimental dodder transmission of both the Clone A and Clone B viruses to lucerne was tested by growing healthy dodder on the infected plants and then infecting N. glutinosa and V. rosea with the virus so obtained.

(c) Symptoms on plants infected with witches' broom of lucerne and big bud of tomato

Both witches' broom of lucerne and big bud of tomato were transmitted to each of the hosts shown in Table 1. Many of the transmission experiments were made in parallel tests so that symptoms could be compared under the same conditions. This was necessary because it was found that the age of plants and time of year were

factors that influenced the incubation period and symptom expression of the virus in the host.

(Insert Table 1)

The following is a description of symptoms on the hosts shown in Table 1.

Calendula officinalis:

Symptoms of the Clone B virus and big bud were similar. Early symptoms were slight chlorosis and vein clearing of young leaves. Plants produced axillary shoots that proliferated and formed narrow leaves which were sometimes slightly rugose. Plants were stunted and flowers were green and abnormal (Plate 15 Figures 1b and 1c).

Crotalaria goreensis:

Symptoms of the Clone B virus and big bud were similar (Plate 3 Figures 1 and 2). Early symptoms were chlorosis, vein clearing and slight rugosity of young leaves. Axillary shoots developed and older leaves showed interveinal chlorosis. Plants became stunted and several months after being infected showed extreme proliferation (Plate 3, Figure 3). Small purple pigmented leaves were formed on axillary shoots. Flowers were green and abnormal.

Datura stramonium and Datura tatula:

Symptoms on Datura stramonium and Datura tatula were essentially the same. Several sources of witches' broom and big bud were used. There was a range of symptoms which was similar for both diseases. Sometimes early symptoms were not well defined, but vein clearing, interveinal chlorosis, slight rugosity and marginal chlorosis tended to appear on young leaves. Proliferation of secondary shoots occurred. Leaves on these shoots were small and narrow, tended to be curled under (Plate 4, Figure 2) and to have plain margins. Old leaves were commonly yellow. Diseased plants were dwarfed. Floral symptoms varied. In some plants the first formed abnormal flowers were streaked with green. In others the calices were enlarged and buds did

not open. Transmission was recorded only when virescent flowers (Plate 4, Figures 1 and 2) were formed either alone or together with twisted white flowers.

Daucus Carota var. Chantenay and Osborne Park.

The first symptoms on plants infected with the Clone B virus were a marginal or general chlorosis of young leaves. The petioles of older leaves of many plants were elongated and twisted and leaves were twisted and often reddened on the margins. Adventitious shoots developed (Plate 5, Figure 1c) and older plants became extremely bushy (Plate 5, Figure 2b). Older leaves tended to drop. Roots (Plate 6, Figures 1 and 2) were reduced in size, pale in colour and produced woolly secondary roots. Those of plants infected at an early age tapered directly from the bushy top. In longitudinal section, the root core extended upwards into a raised crown. In many roots, the vascular bundles tended to be prominent. The xylem tended to be translucent, and in the Osborne Park variety it was enlarged. Some plants died before flowers were formed. Those that formed flowers bloomed earlier than the controls, the flowers being always green and showing proliferation. In several plants the inflorescence stalks were dwarfed but in others, they were comparable with those on big bud infected carrots (Plate 5, Figure 2c).

Symptoms caused by the Clone A virus were very mild (Plate 8, Figure 2). The virus was transmitted from carrot to carrot but not from carrot to other hosts. Young leaves showed some marginal chlorosis, and plants became chlorotic and produced secondary shoots. Petioles of older leaves were often twisted. Roots were reduced in size and showed abnormalities comparable with those of plants infected with the Clone B virus. White flowers were formed but they did not set seed.

There was a range of symptoms on carrots infected with big bud. Some symptoms closely resembled those of the Clone B virus and related strains of the witches' broom virus. Other symptoms were considerably milder but never as mild as Clone A symptoms. In some plants the vegetative growth was erect with few second-

ary shoots. Two distinct types of big bud symptoms are shown in Plate 5, Figures 1a and 1b. Root symptoms were similar to those of witches' broom infected plants (Plate 8, Figure 3). Inflorescences were formed earlier than on control plants. Flowers were green and proliferated extensively (Plate 5, Figure 2c).

Lactuca sativa var. Imperial:

In some plants, the Clone B virus caused necrotic leaf margins or necrotic spots near the leaf margins (Plate 7, Figure 1a). In some plants the heart leaves were completely necrotic. Plants tended to be chlorotic and leaves were twisted and curled under. Older leaves of several plants were almost white. Axillary shoots (Plate 7, Figure 2b) became very spindly at an advanced stage of infection. In some plants symptoms of proliferation occurred without necrosis, whereas in others shoots did not proliferate and leaves were necrotic. Although back transmission tests from plants that produced only necrotic symptoms were unsuccessful, this was not unexpected. Plants that produced only necrotic symptoms formed normal flowers and seed. Those that showed symptoms of proliferation either failed to flower or formed green flowers which did not set seed.

Only one of four plants was successfully infected with the Clone A virus. This plant showed necrotic symptoms.

Leaves on one of two plants infected with big bud showed necrotic spotting, but both showed extreme proliferation of axillary shoots (Plate 7, Figures 1b and 1c). One plant was very stunted (Plate 7, Figure 2c) whereas the other resembled the Clone B infected plant illustrated in Plate 7, Figure 2b.

Lycopersicon esculentum var. Kondine Red:

Plants infected with Clone B witches' broom virus and related strains could not be distinguished from those infected with big bud.

Early symptoms of Clone B infected plants were marginal chlorosis, vein clearing and sometimes slight rugosity of young leaves. In most plants the youngest leaflets at the bases of the young leaves were pinched inwards whereas slightly older leaflets were

twisted and curled under. Plants were stunted and growth was stiff. Axillary shoots ranged from being either few in number and relatively thick with few leaves to consisting of a mass of thin stems with numerous small leaves (Plate 12, Figure 2). Secondary shoots and leaves, as well as leaves at the tip of the plant were often purple in colour. Some infected plants did not flower but most formed flowers with various abnormalities soon after symptoms appeared. Some were normal except that the petals were green, whereas others had enlarged and fused sepals, (Plate 2, Figure 2 and Plate 9, Figures 1 and 2). In some there were reduced floral parts within the fused sepals, but in others there were leafy shoots. In several plants the flowers were similar to those formed in plants infected with Clone A. Plants that had been infected for several months did not produce flowers.

The first symptoms on plants infected with the Clone A virus (Plate 8, Figure 1) were similar to early symptoms on plants infected with the Clone B or big bud viruses. Secondary axillary shoots were formed and the plants became chlorotic, but extreme proliferation did not occur and flowers never showed gigantism. Sepals were free and other floral parts were either reduced in size or absent. No fruit was set after symptoms appeared. Growth was stiff and stunted.

Medicago sativa var. Hunter River:

Symptoms on the one plant which became infected with big bud (Plate 10, Figure 3) closely resembled those of plants infected with the Clone B witches' broom virus (Plate 10, Figures 1 and 2).

Nicotiana glutinosa:

Symptoms on plants infected with the Clone B virus and similar natural strains of the witches' broom virus were indistinguishable from those on plant infected with big bud (Plate 17, Figure 2). The first symptom was vein clearing on young leaves (Plate 11, Figure 1), but this was often transient. Most young leaves were rolled under and some showed marginal chlorosis. Secondary shoots with short internodes were produced in axils of older leaves. The leaves on these shoots were chlorotic, small,

thin and narrow with long petioles. Old infected plants showed extreme proliferation (Plate 15, Figures 2a and 2b). Abnormal flowers (Plate 11, Figure 2) were formed at an early stage of infection, but none was formed when severe symptoms developed. Flowers ranged from being streaked with green though otherwise normal, to showing gigantism and proliferation.

Clone A symptoms were distinct from all other sources of witches' broom virus and big bud. Vein clearing occurred on young leaves, proliferation and chlorosis were not severe, but marginal chlorosis was common. Leaves of old infected plants (Plate 14, Figure 1) were usually less reduced than those of Clone B or big bud infected plants and usually showed vein clearing. Abnormal flowers (Plate 13, Figures 1a and 1b) were formed throughout the life of the plant; floral parts were never leaf-like nor enlarged, and some flowers did not differ greatly from those of healthy plants, except that they were reduced in size, and their corollas were pale pink. Sometimes the floral parts within the calices of old infected plants were absent or very small and pale green.

Nicotiana rustica:

Plants infected with the Clone B strain and similar strains of witches' broom could not be distinguished from plants infected with big bud. Diseased plants tended to be chlorotic and some showed marginal chlorosis and vein clearing of young leaves. Axillary shoots produced small leaves that were often chlorotic. Older leaves were strongly chlorotic and their petioles were frequently twisted. In many plants the first definite symptom was the production of small, pale yellow or green flowers with various abnormalities (Plate 13, Figures c and d). Inflorescences formed numerous axillary shoots (Plate 12, Figure 1).

Plants infected with the Clone A virus showed mild symptoms similar to the above.

Nicotiana tabacum and Nicotiana tabacum var. atropurpurea

Plants infected with Clone B virus and big bud could not be distinguished. Young leaves showed either interveinal chlorosis

or vein clearing, some being slightly rugose. Infected plants became chlorotic and older leaves showed strong interveinal chlorosis. At an advanced stage of infection small rounded leaves were formed on proliferating axillary shoots which had an upright growth habit. Inflorescences proliferated and produced green flowers with various abnormalities. The proliferating inflorescence resulted in some infected plants growing taller than the controls (Plate 12, Figure 3).

Plants infected with the Clone A virus (Plate 14, Figure 2) were stunted. Early symptoms were vein clearing or interveinal chlorosis. Some secondary axillary shoots were formed but these never produced very small leaves as in Clone B infected plants. Older leaves were strongly chlorotic. Flowers were normal in shape and colour but reduced in size (Plate 13, Figure 1g).

Petunia hybrida var. Rosy Morn:

There was variability among witches' broom and big bud infected plants from different sources.

Early symptoms were vein clearing, chlorosis, inward cupping of young leaves and sometimes slight rugosity. Axillary shoots had an upright habit of growth. Leaves on old infected plants were very much reduced (Plate 12, Figure 3). Green flowers appeared at an early stage of symptom expression but no flowers were formed after severe symptoms developed.

Only one plant was infected with the Clone A virus (Plate 18, Figure 1). It showed mild vegetative symptoms and produced small, slightly pale flowers which did not set seed.

Solanum tuberosum vars. Katahdin and Factor:

The varieties Katahdin and Factor showed essentially the same symptoms but the Katahdin variety seemed easier to infect and showed more severe symptoms. There was some variation of symptoms from plant to plant even when the same source of virus was used in one experiment. Many plants failed to show symptoms until new growth developed after they were cut back.

Plants infected with witches' broom could not be distinguished from those infected with big bud. First symptoms were usually

vein clearing and chlorosis of young leaves which were pinched inwards and purple on their lower surfaces towards the petioles (Plate 17, Figure 1d). Older leaves were often inrolled, but sometimes they were curled under. Some shoots wilted and died (Plate 17, Figure 1b) whereas others survived for longer periods than those of control plants. Internodes were short and stem growth was often zig zag in form (Plate 17, Figures 1c and 1d). On some plants nodes were swollen and axillary shoots formed slender purple sprouts or aerial tubers (Plate 17, Figure 1c) which sometimes produced small leaves. Plants were stiff and stunted and when cut back some formed a mass of small secondary shoots which produced small rounded leaves (Plate 15, Figure 2c). One plant infected with the Clone A virus formed leaves which were rolled upwards and very harsh (Plate 16, Figure 1). The shoots had short internodes and later produced small rounded leaves at their bases. Another plant produced numerous shoots from underground stems (Plate 16, Figure 2). Whereas older shoots were only slightly chlorotic and leaves were only slightly cupped.

Six of the 13 plants infected with the Clone B virus in 1950, formed small tubers which were planted the following year. Only one tuber produced a plant that showed symptoms (Plate 15, Figure 2a). The parent of this plant had been infected by means of dodder grown on witches' broom infected periwinkle. Another tuber from the same parent produced a plant that showed no symptoms. The remaining five plants produced tubers which grew into plants that showed no evidence of virus infection. Many formed relatively thin stems which did not produce symptoms when they were grafted to Nicotiana spp.

Tubers from some plants infected with witches' broom disease in the greenhouse in summer 1951, showed no dormancy and produced dwarfed vegetative shoots which proliferated and formed small rounded leaves, similar to those shown in Plate 15, Figure 2b.

Vinca rosea vars. alba and oculata:

A range of symptoms was produced on witches' broom and big bud infected plants. Some big bud infected plants were not as

chlorotic and did not have such small leaves as some witches' broom infected plants but others were similar.

Young leaves were slightly chlorotic and some showed vein clearing, whereas others were slightly rugose. In plants which had been infected for several months (Plate 18, Figure 2) internodes were shorter, young leaves were small and older leaves became yellow and tended to drop. Upright proliferating axillary shoots frequently gave plants a stiff appearance (Plate 18, Figure 3a). Plants were stunted and formed green flowers with various abnormalities which were often the first definite symptom of infection (Plate 13, Figure 1f). Symptoms of plants infected with the Clone A virus were milder than those of plants infected with any other source of witches' broom or big bud virus. Young leaves of newly infected plants showed some marginal chlorosis. Leaves formed later were smaller than those of uninfected plants, but not as reduced as those of plants infected with more severe strains of the virus. Slight proliferation of secondary shoots occurred. Flowers were reduced in size but never green (Plate 13, Figure 1e). Petals of some flowers were pointed.

(d) Some plants which did not become infected with the witches' broom of lucerne in dodder transmission tests

Callistephus chinensis (L) Nees., China Aster var. Giant Grego:

Transmission tests with dodder from witches' broom infected lucerne, periwinkle, N. glutinosa, lettuce, carrot and tomato were made to 57 aster plants but none became infected. Transmission tests were also made with dodder from big bud infected tomato, periwinkle, lettuce and carrot, to 40 aster plants. One aster plant produced two small green flowers but showed no other symptoms. Asters are commonly infected with virescence in Canberra gardens (Hill 1943) and on two occasions dodder grown on diseased asters infected young periwinkle and carrot plants. From these hosts the virus was transmitted to additional periwinkle plants and to N. glutinosa but it was not transmitted to five aster plants. The symptoms on the infected plants were indistinguishable from those produced by big bud or by Clone B and related types of witches'

broom disease. The data indicate that although insect vectors transmit the disease in the field, C. campestris does not readily transmit witches' broom or big bud to asters.

Beta vulgaris, L., Sugar beet:

Helson (1951) showed that Orosius argentatus could transmit witches' broom disease to sugar beet. Hill (1943) showed the same vector could transmit big bud. In dodder transmission tests none of 59 plants were infected with witches' broom disease when lucerne, periwinkle, N. rustica and tomato were used as sources of virus, whereas 9 of 19 plants were infected with big bud when N. glutinosa and tomato were used as sources of the virus.

Apium graveolens, L., Celery var. Golden Self Blanching:

None of 24 celery plants were infected with witches' broom disease, whereas in parallel experiments 13 of 20 celery plants were infected with big bud. The difference in the results may be associated with the fact that in all tests lucerne was the source of the witches' broom virus whereas tomato was the source of the big bud virus.

Most celery plants which became infected with big bud were slightly chlorotic, and petioles of young leaves of some plants were twisted. Others showed no definite symptoms until they formed green flowers which proliferated.

IV. Discussion

(a) General symptoms

The symptoms of plants infected with witches' broom were essentially the same in all hosts although the Clone A virus caused less severe symptoms than the Clone B virus. The range of symptoms produced by the big bud virus was similar to that of the Clone B and related types of witches' broom. The age of the host plant and the time of the year when infection occurred resulted in marked variation in symptom expression. Most host species infected with the severe witches' broom or big bud, were stunted and chlorotic. Vein clearing and marginal chlorosis of leaves were typical early symptoms although in

some hosts they were transient. Young leaves tended to be slightly rugose, and pinched inwards or curled under, and proliferation of axillary shoots was a constant feature. Secondary shoots produced numerous leaves which were reduced in size, and more rounded and thinner than those of normal plants. In a number of hosts, e.g. petunia and periwinkle the secondary shoots tended to assume an upright habit of growth and the internodes were shortened. Other than on potatoes, flowers were produced on at least some plants of each species that was infected. Many plants formed normal flowers at first, but those formed later showed slight to severe abnormalities. It was not unusual to observe mild and severe floral abnormalities on one plant. Many plants that had been infected for several weeks produced leaf-like floral parts, but when extreme proliferation occurred no flowers were formed. In some plants the production of virescent or abnormal flowers was the first symptom that positively identified transmission. Infection usually caused plants to flower earlier than controls.

Symptoms caused by the Clone A virus were less severe than those caused by the Clone B virus. Stunting and proliferation of axillary shoots occurred in all hosts but it was not as extreme as in Clone B infected plants. Leaves were reduced in size and usually showed vein clearing, but they did not differ as much in shape and size from leaves of uninfected plants as did those of plants infected with the Clone B virus. Flowers were always smaller and usually paler in colour than those of uninfected plants but floral parts were never enlarged nor leaf-like. Sometimes the inner floral whorls were very reduced or even absent and flowers frequently dried out and remained attached to the host. Unlike plants infected with the Clone B virus, plants infected with the Clone A virus continued to produce flowers indefinitely, but those formed after the onset of symptoms did not set seed.

(b) Evidence for the existence of strains of witches' broom virus

Helms (Paper (1) ~~in press~~) showed that in the field there was a variation of symptoms of witches' broom infected lucerne. In a greenhouse experiment two clones developed from distinct types of

field infected plants maintained their characteristic identities when given four cultural treatments. Quantitative data indicated that the two viruses had a comparable effect on the growth of lucerne. The host range studies in this paper have shown that the witches' broom virus includes at least two distinct strains and that Clone A is a mild strain and Clone B a severe strain. It is shown that the two strains produce distinctive symptoms on all hosts to which they have been transmitted. They can also be distinguished by their effects on growth of dodder on lucerne. Dodder grown on Clone B lucerne is vigorous, forms innumerable stems and rarely flowers, whereas dodder grown on Clone A lucerne produces little vegetative growth before setting seed.

The data indicate that in Australia there are at least two strains of the witches' broom disease of lucerne or at least two viruses that produce comparable effects on the growth of lucerne. It was shown by Helson (1951) who used a virus type similar to, but slightly milder than, the Clone B virus, that Orosius argentatus was a vector of the witches' broom virus, but the Clone A virus type has not yet been transmitted by an insect vector. Because the host range of the two viruses is similar, and the differences in symptoms on all hosts is essentially a difference in severity of symptoms, it is considered that the two clones represent lucerne plants infected with distinct strains of one virus rather than two distinct viruses.

(c) The relationship of witches' broom of lucerne and big bud of tomato

Helson (1951) suggested that witches' broom and big bud may be caused by the same virus. He showed that witches' broom was transmitted by Orosius argentatus which is the vector that transmits big bud in Australia. Parallel transmission tests of witches' broom and big bud by means of dodder and grafting, described in this paper, indicate that the two diseases are caused by the same virus. Big bud virus obtained from several diseased tomato plants produced a range of symptoms on 14 species of plants (including tomato and lucerne) which could not be distinguished from symptoms produced by the Clone B strain and closely related strains of the witches' broom virus.

Table 1 shows that the time interval between infection and symptom expression was comparable for the two diseases. In the dodder transmission tests the two diseases reacted differently in regard to sugar beet and celery, but further work may show that these differences are not real. For example, whereas big bud, but not the Clone B strain of witches' broom was transmitted to sugar beet by dodder, both viruses were transmitted to sugar beet by Orosius argentatus (Hill 1943 and Helson 1951) and again big bud, but not witches' broom, was transmitted to celery. It is considered that these differences are insufficient to distinguish between the two viruses.

(d) Comparison of the Australian and American witches' broom of lucerne

Kunkel (1952) stated that symptoms he obtained on witches' broom infected tomato, carrot, periwinkle and potato indicated that the disease was distinct from all other yellows type diseases which had been taken to these hosts. The symptoms described by Kunkel differ in some respects from those on the same host species which have been described in this paper and suggest that he may have used more than one source of witches' broom infected lucerne. On tomato and carrot, symptoms of the American disease appear to resemble those of the mild, rather than the severe, strain of the Australian disease, whereas on periwinkle symptoms are relatively severe. On potato symptoms appear to differ in several respects from those caused by the Australian disease.

Differences between symptoms of the American and Australian disease may be partly due to host varietal and environmental differences but they are probably also due to differences in strains of the virus. There is no strong evidence to suggest that the diseases are caused by different viruses, although in addition to differences in symptoms the known vectors of the disease (Scaphytopius (Cloanthus) dubius (Van Duzee) in America) and (Orosius argentatus (Evans) in Australia) are not closely related.

Kunkel (1952) reported that witches' broom disease in periwinkle was cured by heat treatment at several temperatures including a treatment at 42°C. lasting 7 but not 6 days and that plants treated for 6 days showed beneficial effects. In a preliminary test in Canberra in January 1951, a witches' broom infected periwinkle plant kept at

42°C. for 6 days produced normal growth for 3 months, after which it produced green flowers (Plate 18, Figure 3) and gradually showed more severe symptoms. No additional tests were made, but the data suggest that the temperature of inactivation of the Australian and American diseases may be similar.

(e) The relationship of witches' broom of lucerne and other yellows viruses

Hill (1940) and Kunkel (1945) considered that big bud of tomato was identical with, or closely related to cranberry false blossom. The vectors of the two diseases were considered to be closely related and their host range and symptoms were considered to be similar. Data in this paper support their conclusions by showing that periwinkle, N. glutinosa and potato, which are known to be hosts of cranberry false blossom, are also hosts of big bud. The similarity of symptoms of the severe strain of witches' broom, big bud and false blossom on several hosts can be seen by comparing illustrations in this paper with those in Kunkels' papers of 1943 and 1945). The mild strain of witches' broom disease (Clone A) does not produce gigantism on any host and in this respect it differs from typical false blossom described by Kunkel (1943 and 1945). Kunkel (1945) indicated however that several strains of cranberry false blossom exist. Another difference between typical false blossom and witches' broom is that Kunkel (1945), considered lucerne to be immune to false blossom because he was unable to infect it with false blossom by means of dodder. However, both witches' broom and big bud have now been transmitted (though with difficulty), to lucerne.

There is a marked similarity of symptoms of witches' broom and aster yellows on a number of hosts that have been studied. For example, Chantenay carrots infected with witches' broom (Plate 6) resemble Chantenay carrots infected with aster yellows as illustrated by Hervey and Schroeder (1949); lettuce infected with witches' broom (Plate 7) shows symptoms similar to lettuce infected with aster yellows (Severin (1929) Figure 15 and Linn (1940)); potato plants infected with witches' broom resemble potato plants infected with purple top wilt which has been shown to be caused by the aster yellows virus (Younkin

(1943), Leach and Bishop (1946) and Severin and Haasis (1934)).

There are two strains of the aster yellows virus, the New York and the California strain which differ mainly in regard to their host range. Kunkel (1937), also showed that mild strains of the virus were frequently transmitted after insect colonies infected with aster yellows were given heat treatment but he stated that the mild strains had not been reported in the field. Data suggest that the host range of witches' broom disease of lucerne in Australia more nearly corresponds with that of the California strain than the New York strain, although there are several apparent differences. For example, although Frazier and Severin (1945) showed that 3 legumes were hosts of California aster yellows, the disease has not been recorded on lucerne. Kunkel (1931) was unable to infect plants belonging to the family Leguminosae with New York aster yellows.

In making a comparison of the hosts of witches' broom, big bud and aster yellows, it appears that there are several common plants on which further work is needed. Asters are readily infected with aster yellows by means of leafhopper vectors but Folke Johnson (1941) showed that the disease was not readily transmitted to asters by means of C. campestris. They have not yet been experimentally infected with either the mild or the severe strain of witches' broom, by means of C. campestris, although garden plants in Canberra are commonly infected with virescence which is probably caused by the severe strain of witches' broom and this is considered to be the same virus as that which causes big bud. Thus vector transmission studies may show that asters are susceptible to witches' broom. Tomato big bud is known to occur in western America but the writer does not know of any report of its natural occurrence on asters. By means of Cuscuta subinclusa, however, Menzies (1951) succeeded in transmitting a virus from big bud infected tomato to 1 of 6 aster plants in one test and to 2 of 5 aster plants in another test. The asters developed typical yellows type symptoms of axillary shoot growth and greening of flowers. Again, although sugar beet is considered to be immune to New York aster yellows (Kunkel 1943), it was infected with witches' broom by Helson (1951) and it is readily infected with big bud. It is not known whether

attempts have been made to infect sugar beet, N. tabacum or tomato with the California strain of aster yellows. Kunkel (1931) failed to infect N. tabacum and D. stramonium with the New York strain of aster yellows, and the tomatoes he infected did not show symptoms that were identical with either the mild or severe strain of witches' broom. In western America however Frazier and Severin (1945) found a field infected plant of Datura stramonium which they considered showed typical aster yellows symptoms, although they were unable to recover the virus from the plant.

The California strain of aster yellows, witches' broom of lucerne and big bud of tomato occur mainly in western America whereas the New York strain of aster yellows and cranberry false blossom occur mainly in the east. The California and New York strains of aster yellows are considered to be closely related and so also are cranberry false blossom and big bud (Kunkel (1945) and Hill (1940)). In Australia witches' broom of lucerne and big bud are known and data indicate they are caused by the same virus. Both of the aster yellows strains produce purple top wilt of potatoes in America (Leach and Bishop 1946) and Menzies (1951) has indicated that purple top symptoms in America may be caused by the big bud virus. Norris (unpublished data) considers that purple top wilt of potatoes also occurs in the field in Australia. He considers that it is caused by the big bud virus and that it produces symptoms which are identical with those caused by the aster yellows virus.

Studies of symptoms, host range and vectors suggest that aster yellows, cranberry false blossom, big bud of tomato and witches' broom of lucerne are caused by very closely related viruses, if not strains of the same virus. They clearly indicate that definite relationship of these diseases will only be established by more fundamental studies of the virus.

V. Acknowledgments

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Explanation of Plates 1 - 18

Plate 1.

Medicago sativa

Fig. 1 Normal flower from a healthy plant.

Fig. 2 Typical mauve flower from a Clone A plant. The petals are not spread, the standard is wing-like and the keel is the longest floral part.

Fig. 3 Mauve flower from a Clone B plant. The sepals are enlarged and the wings are abnormally incurled.

Plate 2.

Fig. 1 Medicago sativa: Green flowers from field plants infected with witches' broom. A flower from a healthy plant is shown on the left.

Fig. 2 Lycopersicon esculentum.

a. Inflorescence from a healthy plant.

b. Inflorescence with thickened stems and fused sepals from a witches' broom infected plant.

c. Inflorescence from a Clone B infected plant with enlarged, proliferating green flowers.

Plate 3.

Crotalaria goreensis

Fig. 1 a. Healthy plant

b. Big bud infected plant showing proliferation of axillary shoots and interveinal chlorosis of older leaves.

Fig. 2 Clone B infected plant showing symptoms similar to those in Figure 1b.

Fig. 3 Clone B infected plant which shows extreme proliferation 7 months after infection

Plate 4.

Datura stramonium

Fig. 1 a. Normal inflorescence

b. Witches' broom infected plant showing early symptoms of interveinal chlorosis and production of green flowers.

Fig. 2 Witches' broom infected plant showing downrolling and

slight rugosity of leaves, production of axillary shoots and abnormal flowers.

Plate 5.

Daucus Carota

Fig. 1 a. Big bud infected plant (var. Chantenay) with mild proliferation chlorosis and twisting of petioles. It later produced green flowers

b. Big bud infected plant (var. Chantenay) with more severe symptoms than a. It died before producing flowers.

c. Witches' broom infected plant (var. Chantenay) with symptoms similar to b.

Fig. 2 a. Healthy plant (var. Osborne Park)

b. Clone B infected plant (var. Osborne Park) with severe symptoms of extreme proliferation and chlorosis.

c. Big bud infected plant (var. Osborne Park) with a few secondary shoots and a proliferating inflorescence

Plate 6.

Daucus Carota (var. Chantenay)

Fig. 1 a. Root of a healthy plant

b, c. Roots of witches' broom infected plants showing reduction in root size and numerous woolly secondary shoots. The root c, tapers directly from the crown.

Fig. 2 a, b, c. Longitudinal sections of roots in Figure 1.

Plate 7.

Lactuca sativa

Fig. 1 a. Leaves from witches' broom infected plants showing necrotic specking, necrosis of leaf margins and some distortion.

b, c. Chlorotic leaves from a big bud infected plant showing distortion of older leaves and abnormal axillary shoots.

Fig. 2 a. Healthy plant

b. Witches' broom infected plant with secondary shoots

c. Big bud infected plant which is severely stunted and is producing secondary shoots.

Plate 8.

Fig. 1 Lycopersicon esculentum. Clone A infected plant with early symptoms, showing veinclearing and chlorosis and inward cupping of some leaflets whereas others are curled downwards.

Fig. 2 Daucus Carota (var. Chantenay). Clone A infected plant which is chlorotic and has produced a few secondary shoots. The flowers appear normal but failed to set seed.

Fig. 3 Daucus Carota (var. Chantenay). Longitudinal section of a root of a big bud infected plant which shows woolly secondary shoots and upward extension of the crown.

Plate 9.

Lycopersicon esculentum.

Fig. 1 Big bud infected plant with early symptoms of veinclearing, twisting and curling of leaves and fusion of sepals

Fig. 2 Witches' broom infected plant showing symptoms similar to those in Figure 1, although the axillary shoots are further developed.

Plate 10.

Medicago sativa

Fig. 1 Early symptoms of witches' broom diseased plant experimentally infected by means of dodder with the Clone A virus, together with a control plant.

Fig. 2 Old witches' broom plant experimentally infected by means of dodder with the Clone B virus.

Fig. 3 Big bud infected plant experimentally infected by means of dodder.

Plate 11.

Nicotiana glutinosa

Fig. 1 Witches' broom infected plant with early symptoms of veinclearing and down-curling of leaves.

Fig. 2 Abnormal inflorescence of a witches' broom infected plant together with an inflorescence from a healthy plant.

Plate 12.

Fig. 1 Nicotiana rustica. Proliferating inflorescence of a witches' broom infected plant

Fig. 2 Lycopersicon esculentum. Top of a witches' broom infected plant showing proliferation of axillary shoots and formation of small leaves.

Fig. 2 Nicotiana tabacum. Inflorescence of a healthy plant and of a witches' broom infected plant which is producing green flowers and axillary shoots below the inflorescence.

Plate 13.

Fig. 1 a and b. Nicotiana glutinosa. In (a) the Clone A infected flower on the right has a regular corolla, is reduced in size and pale in colour. A healthy flower is on the left. The Clone A infected flower (b) shows greater modification.

c and d. Nicotiana rustica. In (c) a normal flower is shown on the left, one abnormal flower from a Clone B infected plant is on the right and another is shown in (d).

e and f. Vinca rosea. In (e) a flower from a healthy plant is on the left and a flower from a Clone A infected plant is on the right. The green flower (f) is from a Clone B infected plant.

g. Nicotiana tabacum. A flower from a healthy plant is on the left and one from a Clone A infected plant is on the right. It is reduced in size but otherwise appears normal.

Plate 14.

Fig. 1 Nicotiana glutinosa. Clone A infected plant which has shown symptoms for more than one year. It is producing secondary shoots and abnormal pale pink inflorescences.

Fig. 2 Nicotiana tabacum. Clone A infected plant which has shown symptoms for 5 months. The older leaves are chlorotic and a few axillary shoots have been formed

Fig. 3 Petunia hybrida. Clone B infected plant which has been infected for several months and shows extreme symptoms of proliferation.

Plate 15.

Calendula officinalis

Fig. 1
a. Normal inflorescence
b. Proliferating inflorescence of a big bud infected plant
c. Witches' broom infected plant which is producing axillary shoots and a green flower after having flowered normally.

Fig. 2 Solanum tuberosum var. Katahdin
a. Diseased plant grown from a tuber of a parent plant which was experimentally infected with witches' broom by means of infective dodder. It shows spindly growth stunting, production of aerial tubers and proliferation of secondary shoots.
b. Tuber with dwarfed vegetative shoots obtained from a witches' broom infected plant
c. Clone B infected plant showing proliferation of secondary shoots following cutting back.

Plate 16.

Solanum tuberosum var. Katahdin

Fig. 1 Clone A infected plant is on the right and a control plant on the left. Both were cut back after producing normal growth. The infected plant is producing numerous secondary shoots and the leaves are chlorotic and rolling upwards.

Fig. 2 Clone A infected plant which is producing small shoots from underground stems.

Plate 17.

Solanum tuberosum

Fig. 1
a. Shoot from a healthy plant (var. Katahdin)
b. Shoot from a witches' broom infected plant (var. Factor) which is wilting and showing inrolling of leaves.
c. Shoot from a witches' broom infected plant (var. Katahdin). The leaves are slightly inrolled, and aerial tubers and slender sprouts appear in the leaf axils

d. Shoot from a witches' broom infected plant (var. Katahdin) which shows purpling at the bases of the leaflets which are pinched inwards. The internodes are short and the stem is zig zag in form.

Fig. 2 Nicotiana glutinosa

a. Big bud infected plant which has shown symptoms for 2 months

b. Clone B witches' broom infected plant which was infected at the same time as (a).

Plate 18.

Fig. 1 Petunia hybrida. Clone A infected plant showing numerous secondary shoots bearing small marginally chlorotic leaves, and small but otherwise apparently normal flowers which did not set seed.

Fig. 2 Vinca rosea. Control and witches' broom infected plant showing stunting, proliferation, chlorosis and production of small leaves.

Fig. 3 Vinca rosea.

a. Witches' broom infected plant that was not given heat-treatment

b. Witches' broom infected plant which was established by means of a cutting from the same plant as a and was then given a heat-treatment of 42°C for 6 days. It produced normal growth for 3 months and then, as shown in the figure, symptoms reappeared.

PLATE I.

Fig. 3

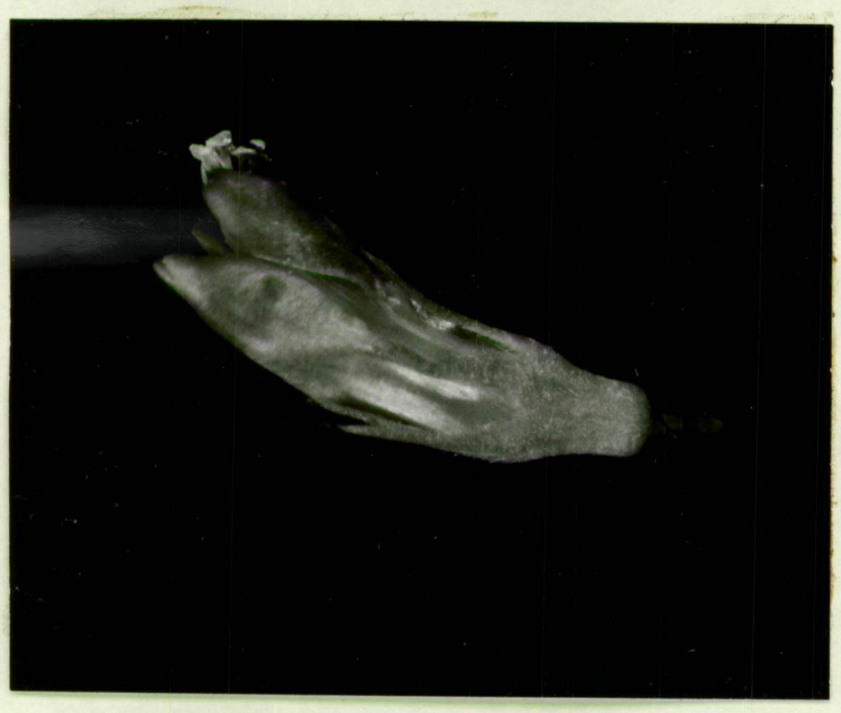


Fig. 2

Fig. 1

PLATE 2.

Fig. 1

a b c

Fig. 2

PLATE 3.

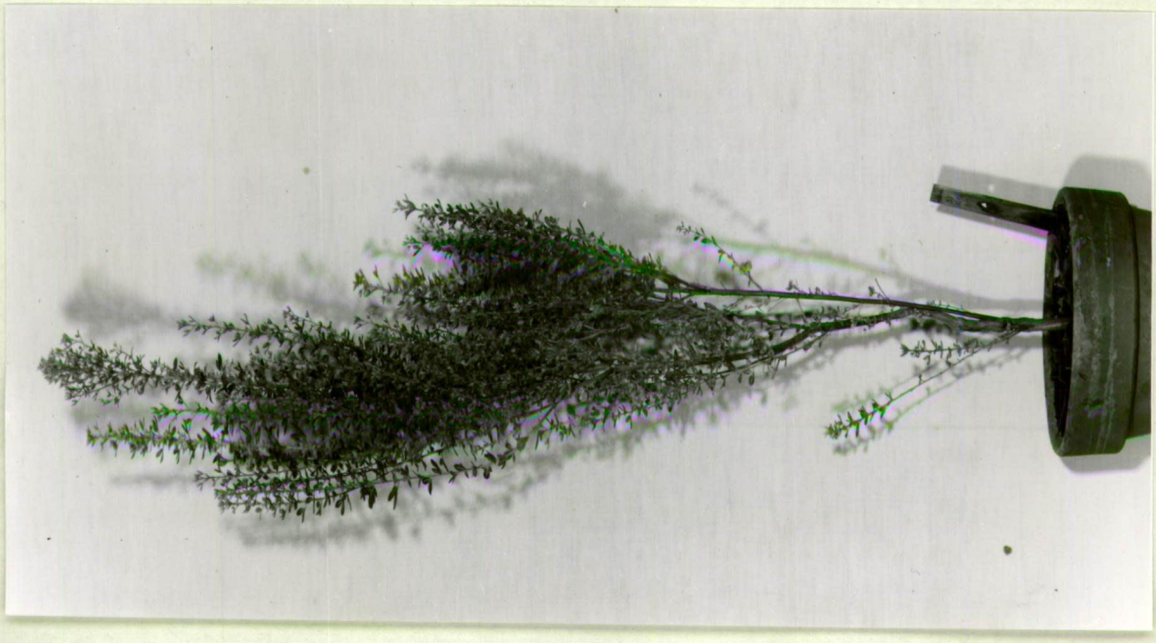


Fig. 3

Fig. 2

a b

Fig. 1

PLATE 4.

a b

Fig. 1

Fig. 2

PLATE 5.

a b c

Fig. 1

a b c

Fig. 2

PLATE 6.

a b c

Fig. 1

a b c

Fig. 2

PLATE 7.

a b c

Fig. 1

a b c

Fig. 2

PLATE 8.

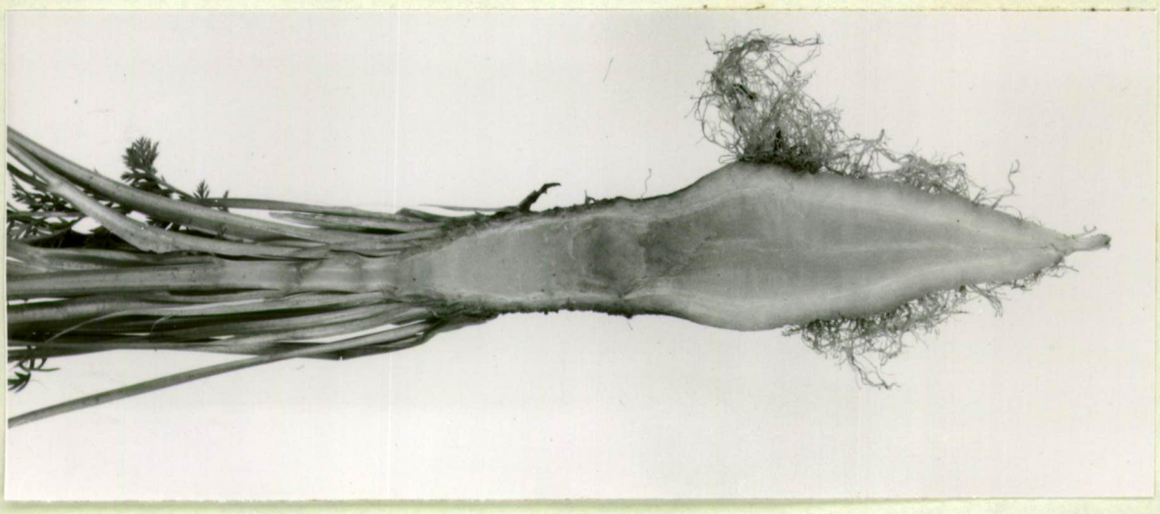


Fig. 3

Fig. 2

Fig. 1

PLATE 9.

Fig. 1

Fig. 2

PLATE 10.

Fig. 1

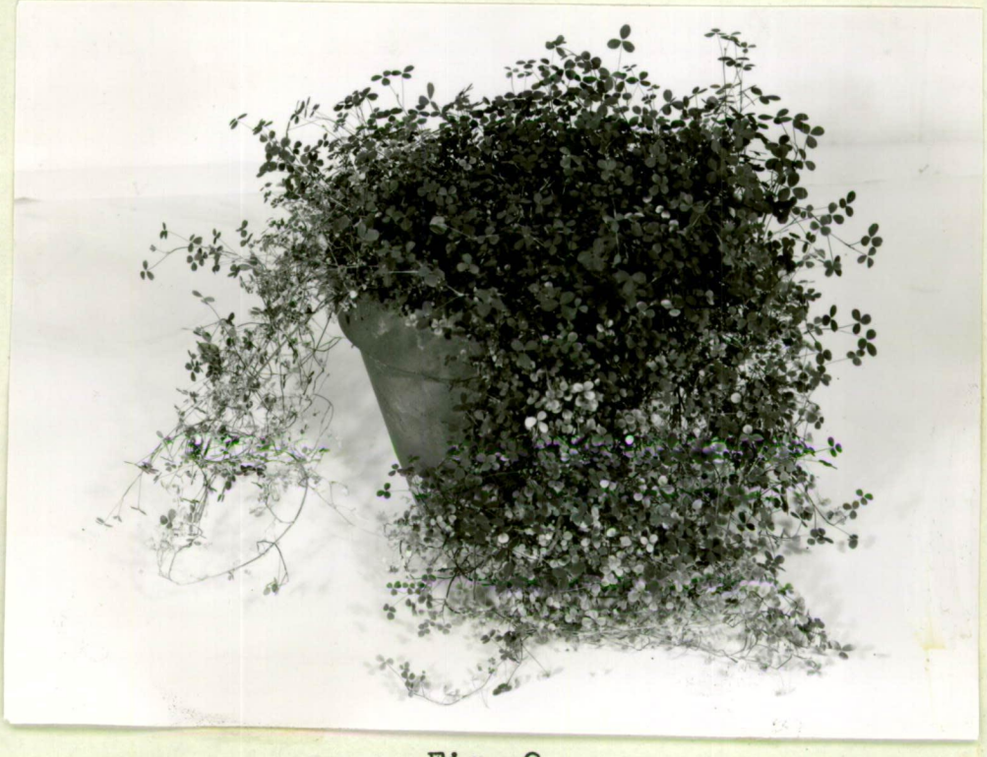


Fig. 2

Fig. 3

PLATE II.

Fig. 1

Fig. 2

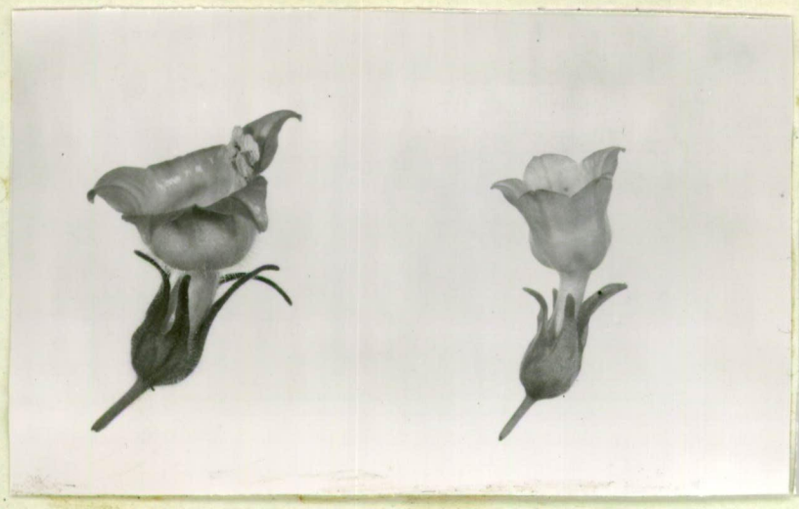
PLATE I2.

Fig. 3

Fig. 2

Fig. 1

PLATE I3.



a

b

c

d

e

f

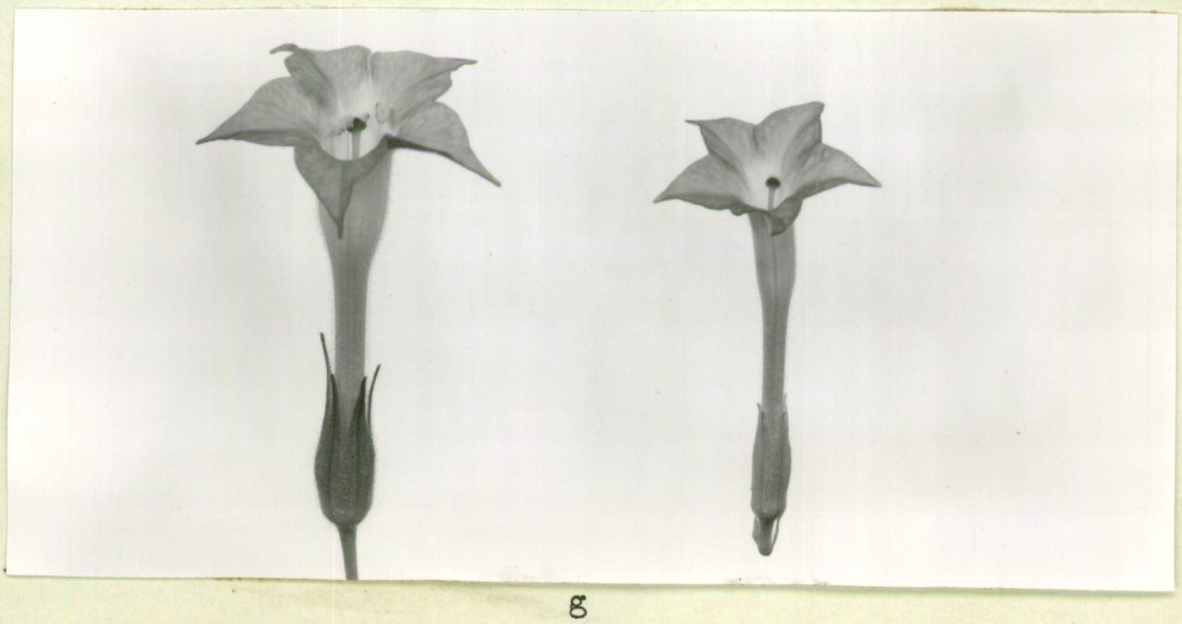


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Fig. 1

PLATE I4.

Fig. 3

Fig. 2

Fig. 1

PLATE I5.

a b c

Fig. 1

a c

Fig. 2

PLATE I6.

Fig. 1

Fig 2.

PLATE I7.

a

b

c

d

Fig. 1

a b

Fig. 2

PLATE I8.

Fig. 1

Fig. 2

a b

Fig. 3