FACTORS AFFECTING THE SUSCEPTIBILITY OF BEAN PLANTS TO INOCULATION WITH BEAN YELLOW MOSAIC VIRUS

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INTRODUCTION

Bean yellow mosaic virus (BYMV), also known as bean virus 2 and Phaseolus virus 2, not only causes an important disease of beans but is also the cause of a virus disease of peas, lupines, clovers and some other legume crops. Bean yellow mosaic virus is transmitted by aphids in a non-persistent manner, according to the Watson and Roberts (1939) classification (37, p. 543).

It has long been known from studies dealing with host susceptibility to parasitic infection that environmental factors, mineral nutrition, temperature, light and humidity, and other factors such as age and varieties of plants influence the host response to infection. In the field of plant-virus investigations several of these factors have been investigated. Most of the work has been done, however, with respect to mechanical inoculation. Very little consideration has been given to the effect of these factors on the transmission of plant viruses by insects. Still less has been done to compare the effect of these factors on transmission by mechanical inoculation and inoculation by insects.

The aim of the present study was, therefore, to compare the effect of such factors on the susceptibility of bean plants to bean yellow mosaic virus infection by mechanical inoculation and inoculation by aphids. Information was also sought on the relation of symptom expression of BYMV in broad bean to virus concentration.

REVIEW OF LITERATURE

Relative Susceptibility of Host Plant Varieties

As early as 1931, Whitehead and Currie (38, p. 518) reported differences in relative susceptibility of several potato varieties to potato leaf roll and mosaic infection, spread by aphids in fields. Shultz *et al*. (22, p. 196-197) compared susceptibility of several potato varieties to the mild mosaic, latent mosaic, vein-banding mosaic, leaf roll and spindle tuber viruses by field exposure tests, as well as by tuber and shoot grafts. They found that some varieties were completely resistant, some escaped infection in the field but became infected by graft, and still others contracted the virus readily both by field exposure and by graft.

Several potato varieties were tested for susceptibility to potato virus Y, both in the field and in glasshouses (4, p. 46). Significant varietal differences in susceptibility to the virus were observed. The relative susceptibility of several varieties in the field was correlated with their susceptibility to virus infection by aphid inoculation in the glasshouse but not to infection by mechanical inoculation.

Several varieties of cruciferous plants were compared for susceptibility to *Brassica nigra* virus. The rutabaga variety of rape, Brassica napus L., was susceptible to the virus by aphid inoculation but not by mechanical inoculation (33, p. 545-546). Varietal differences were reported in sugar beet susceptibility to beet yellows virus by mechanical inoculation (13, p. 235).

Adlerz (1, p. 261-262) tested several bean varieties for susceptibility to BYMV inoculation by green peach aphid and pea aphid. Stringless Blue Lake variety was more susceptible than all others to inoculation by both the aphids. The order of relative susceptibility of several varieties was, however, not the same for the two aphids. Barley varieties differed in susceptibility to stripe mosaic virus infection by mechanical inoculation (25, p. 294).

Effect of Shading on Susceptibility of Host Plant

Several investigators have reported that preinoculation shading alters susceptibility of host plants to virus infection. Samuel and Bald (20, p. 88-89), Samuel et al. (21, p. 512-513), Hougas (15, p. 487-489), Sill and Walker (23, p. 329-330), Ross (19, p. 1-2), Bawden and Roberts (6, p. 295) (7, p. 418), Costa and Bennett (13, p. 235-236), and Yarwood (40, p. 621), working with several viruses and host plants, observed that keeping plants in darkness or in shade for a few hours to several days prior to mechanical inoculation increased their susceptibility to virus manyfold.

Preinoculation shading for 24 hours gave maximum increase in host susceptibility to several viruses by mechanical inoculation (7, p. 418) (23, p. 329-330). Complete darkness was not necessary to bring about this increase in susceptibility (13, p. 236). Sugar beet plants that had been preconditioned in almost complete darkness were compared with plants that had been kept in reduced light under a greenhouse bench. Mechanical inoculation with beet yellows gave a higher number of local lesions on plants kept in reduced light than on those subjected to complete darkness.

On the other hand, Matthews (18, p. 559) found that for most times of inoculation, plants kept in continuous darkness for two days before mechanical inoculation produced fewer local lesions than plants kept in an uncontrolled glasshouse. He further observed (18, p. 564) that increasing exposure of plants to light from dawn to afternoon increased infection from subsequent mechanical inoculation with several viruses. Even a one-minute exposure to 800 foot-candles before inoculation could double the number of local lesions.

Ultra-violet irradiation of French bean leaves kept in light for 24 hours resulted in more lesions from subsequent mechanical inoculation with tobacco mosaic virus than those kept in the dark for a similar period (8, p. 71). Lindner *et al*. (17, p. 84-85)reported that a 24-hour dark period prior to mechanical inoculation did not affect susceptibility of cucumber cotyledons to tobacco mosaic virus.

Wiltshire (39, p. 234-235) investigated the effect of shading on the susceptibility of French bean to tobacco necrosis virus by mechanical inoculation. Out of 31 tests, lesion number was increased twofold or more in 14, decreased to one-half or less in 4, and not affected significantly in 13 by preinoculation shading.

Two to four days of darkness prior to inoculation with henbane mosaic virus increased susceptibility of *Nicotiana rustica* to mechanical inoculation but not to inoculation by aphids (9, p. 93-94). Similarly, 24- to 48-hour preinoculation shading failed to affect susceptibility of mustard plants to *Brassica nigra* virus by aphid inoculation (32, p. 210-211). Susceptibility of lettuce seedlings to lettuce mosaic virus was increased, however, by a 48-hour shading period prior to inoculation by aphids (35, p. 367).

Effect of Age of Test Plant on Host Susceptibility

Carter (11, p. 157) reported that susceptibility of pineapple seedlings to *Commelina nudiflora* L. mosaic virus by aphid inoculation decreased as the seedlings grew older.

Lettuce seedlings varying in age from one to five weeks were compared for susceptibility to lettuce mosaic virus by green peach aphid inoculation (35, p. 368). Older plants were more susceptible than the younger plants. It was not determined whether such results were due to increased susceptibility with age or to a greater probability of aphids feeding on larger plants.

Differences of 15 to 30 days in the age of potato plants did not alter their susceptibility to potato virus Y by aphid inoculation (4, p. 48). Similarly no differences were observed in the susceptibility of one- to five-week-old mustard plants to Brassica nigra virus inoculation by green peach aphid (32, p. 211).

Ross (19, p. 1) reported that Physalis floridana plants at flowering stage gave more distinct and a greater number of local lesions per unit area with mechanical inoculation of potato virus Y than did either younger or senescent plants. Mechanical transmission of beet yellows virus was not achieved when cotyledons of very young seedlings were inoculated. No differences were found, however, between seedlings which had only four or five leaves and older plants (13, p. 235).

Barley plants at different stages of growth were tested for susceptibility to stripe mosaic virus by sap inoculation. Inoculations made one to three weeks before heading, that is, 40 to 50 days after planting, caused most severe symptoms and greatest reduction in yield (14, p. 722) (25, p. 294).

Hougas (15, p. 489-490) found that potato leaves from the middle one-third of test plants were 2 to 12 times more susceptible to potato yellow dwarf by sap transmission than either the younger upper leaves or the older lower leaves, respectively. There was greater variation in susceptibility to Hyoscyamus virus 3 by sap inoculation among tobacco leaves on the same plant than among leaves in corresponding positions on different plants (36, p. 463-465).

Effect of Nitrogen on Host Susceptibility

Spencer (27, p. 188-190) found that there was a definite correlation between nitrogen supplied to *Nicotiana glutinosa*, *N. tabacum*, and *Phaseolus vulgaris* L. and their susceptibility to infection with tobacco mosaic virus by mechanical inoculation. Nitrogen increased susceptibility even when applied in amounts that greatly retarded growth. Susceptibility decreased above a certain level of nitrogen.

Similarly, application of nitrogen increased susceptibility of tobacco and potato plants to sap inoculation with tobacco mosaic and aucuba mosaic viruses (5, p. 56). Greatest increase in susceptibility was obtained, however, with fertilizer treatments that were most favorable to plant growth. Susceptibility did not increase when nitrogen was supplied in amounts which retarded growth. On the other hand, fertilizer treatments that greatly influenced the growth of tobacco and potato plants in pots had little effect on susceptibility to aphid inoculation with potato virus Y.

Application of dung and other nitrogenous fertilizers to potatoes increased the incidence of potato leaf roll and rugose mosaic viruses under natural conditions of spread (10, p. 509). Swenson and Sohi (31, p. 67) reported that nutrient level of bean plants altered their susceptibility to BYMV by green peach aphid inoculation. Nitrogen nutrient solution did not affect significantly the susceptibility of cowpea plants to cucumber mosaic virus 1 by sap inoculation (23, p. 329).

MATERIALS AND METHODS

A. General

All investigations were made in greenhouses at Oregon State University, Corvallis, Oregon, during the summer and fall of 1960. Weather was generally clear and warm, with scattered clouds at times, in summer, and mostly cloudy or intermittently cloudy and sunny during fall. Efforts were made to maintain the greenhouse environment uniform, but still there was much variation in daily temperature and light intensity. Information about temperature and about light intensity in the greenhouse during the period from two days before to two days after inoculating the test plants is summarized in appendix tables 1 and 2.

B. Definitions

Acquisition feeding refers to feeding period of aphids on the diseased plant (24, p. 29) (30, p. 521).

Colony was a number of green peach aphids infesting the same individual plant, which is referred to as a colony plant.

Mechanical inoculation was rubbing of a plant with juice, diluted with a buffer, from a diseased plant. The plant was dusted with carborundum to facilitate infection.

Preliminary starvation denotes the period of starvation after the aphid was removed from a colony plant but before the acquisition feeding (30, p. 522) (34, p. 75-77).

Source plant was the diseased plant used for acquisition feeding of aphids (34, p. 83-84) or for preparation of juice for mechanical inoculation.

Test feeding was the feeding period of the aphid on a healthy plant, used as an indicator plant for the virus (24, p. 29) (30, p. 522).

Test plant was the healthy plant used for test feeding of aphid (24, p. 24) (30, p. 522), or otherwise inoculated as an indicator plant for the virus.

C. Materials

The Virus

The strain of the bean yellow mosaic virus (BYMV) used throughout this study was that isolated and described by Swenson in 1954 from naturally-infected red clover near Geneva, New York (29, p. 1121). This BYMV strain was maintained by means of aphid, *Myzus persicae* (Sulzer), transmission since 1956 in broad bean, *Vicia faba* L., var. major, or Dwarf Horticultural variety of the bean, *Phaseolus vulgaris* L.

The Vector

The green peach aphid, *Myzus persicae* (Sulzer), was used as the vector throughout this study. Aphid colonies were started from colonies maintained by Dr. K. G. Swenson for several years. Frequently during the course of present study colonies were started afresh from single parthenogenetic aphids in order to keep genetic variation in the aphids to a minimum.

The aphid colonies were caged individually and kept in a greenhouse room separate from rooms used for raising source plants or test plants. This was done in order to prevent contamination.

The Plants

Chinese cabbage, *Brassica pekinensis* Rupr., was used for colony plants and broad bean for source plants in all experiments. Both were raised in number 10 cans or six-inch clay pots. Chinese cabbage was first grown in flats, and seedlings were later transferred to cans or pots.

The bean test plants were grown either in five-inch clay pots (three plants per pot), or in number 10 cans (four or five plants per can). *Chenopodium amaranticolor* Coste and Reyn test plants were raised in number 10 cans, with a single plant in a can.

D. Methods

Soil Treatment

The soil used in these experiments was a 3 to 1 mixture of a river-bottom loam and peat moss. For colony plants it was further supplemented with one cup each of ammonium nitrate, 6-10-4, and lime per 22-25 gallons of soil. For source plants and test plants in all except three experiments, where nitrogen level of test plants was an experimental variable, the following fertilizers were added per 10 gallons of soil:

Ammonium nitrate	-	one oz.
6-10-4	-	one oz.
Lime	-	two oz.

Chemical analysis of this soil mixture is given in appendix table 3.

Lime and 6-10-4 were used in nitrogen level trials at the same rate as in other tests. Ammonium nitrate was added to soil to obtain five levels of nitrogen for test plants: 0.06, 0.09, 0.12, 0.15 and 0.17 per cent total nitrogen in the soil. Chemical analysis of soil mixtures with these levels of nitrogen is presented in appendix table 4.

The soil for source plants and test plants was mixed for half an hour in a cement mixer to ensure uniform distribution of fertilizers. All seeds were treated with phygon or spergon fungicides at the time of sowing. In the case of beans, fermocide was also added weekly to the pots or cans in the early stages of growth to prevent root rot and damping off.

Aphid Inoculation

Apterous aphids, other than very young nymphs, were used. In these experiments only aphids from young colonies were used. For any one replication the aphids were used from a single colony. Swenson and Sohi (31, p. 67) reported that aphids from vigorously growing Chinese cabbage leaves transmitted BYMV at a higher rate than aphids from old crowded colonies, and that aphids from young leaves of Chinese cabbage transmitted BYMV at a higher rate than aphids from mature leaves of the same individual plant.

Aphids were removed from the colonies and placed in Erlenmeyer flasks for a preliminary starvation period of 15 minutes or longer. Preliminary starvation increases transmission of nonpersistent viruses (28, p. 262). The maximum effect of this starvation is obtained in 15 minutes with BYMV (30, p. 523-524). The starved aphids were placed on the last fully opened leaf of the source plant and allowed an acquisition feeding of 10 to 45 seconds before they were transferred to test plants. Maximum transmission of BYMV by *M. persicae* occurs with acquisition feedings of 11 to 45 seconds (30, p. 523). Only those aphids which terminated acquisition feeding naturally within 10 to 45 seconds were transferred to test plants, others being discarded. Transmission of some nonpersistent viruses is reduced if acquisition feeding is forcibly terminated (9, p. 81) (34, p. 54).

One aphid was transferred to each test plant. The aphids were handled with a moistened camel's-hair brush. Acquisition feeding was watched with the aid of a 10X hand lens and measured from the time the proboscis of the aphid touched the leaf surface until it was removed. Aphids were allowed a test feeding of 4 to 24 hours, after which they were killed by fumigating the room containing the test plants with TEPP aerosol.

Mechanical Inoculation

Inoculum for mechanical inoculation was obtained from the last fully opened and next half-opened leaves and at times from a part of the second last fully opened leaves of the source plants. Leaf tissue was ground in one per cent potassium dibasic phosphate buffer with a ratio of one-half gram of leaf tissue to 9.5 ml. buffer. The inoculum was sieved through cheesecloth to avoid clogging of pipettes. This gave a stock inoculum of 1/20 concentration which was diluted to the desired strength by adding one per cent potassium dibasic phosphate buffer. Inoculum for each replication was prepared afresh to reduce chances of inactivation *in vitro*.

Test plants were inoculated by rubbing with cheesecloth pad dipped in the inoculum. Carborundum (400-mesh silicon carbide) was dusted on the upper surface of leaves to be inoculated. The leaf being inoculated was supported from below with a piece of stiff cardboard to ensure uniform rubbing. One primary leaf was inoculated on each bean plant, whereas on *Chenopodium* six leaves per plant were inoculated. Each half of a leaf was given one stroke. The cheesecloth pad was dipped in inoculum before each stroke.

Mechanically inoculated test plants were fumigated along with plants inoculated by aphids to keep all the plants uniformly treated in all respects except the methods of inoculation.

Statistical Methods

Since several factors affecting susceptibility of bean plants to BYMV were to be compared under two methods of inoculation (viz., mechanical inoculation and inoculation by aphid), only the factorial design could be used. This design, besides being economical of time, labor and material, makes possible the study of interaction of several factors

(12, p. 152) (16, p. 310) (26, p. 333-334).

Split-plot design in strips was followed in all except one experiment, where randomized blocks were used. The methods of inoculation were assigned to main plots and the factors affecting susceptibility to sub-plots. In the split-plot design the error mean square for the sub-plots and for the interactions is smaller than that for the main plots. This design, therefore, provides a more efficient testing technique for interactions and for differences among sub-plot factors than for the factor assigned to the main plots. This increased efficiency is, however, at the cost of reduced efficiency for the factor in the main plots (12, p. 296-297) (26, p. 366-367). In the present study the effect of factors affecting the susceptibility of plants and their interaction with the methods of inoculation were more important than the methods of inoculation themselves.

Analysis of variance (16, p. 196-208, 309-326), as adapted for split-plot design in strips (12, p. 306-309), was used for all experiments except one, which was a factorial design in randomized blocks and was analysed as such. In three experiments the several error mean squares of split-plot design were not found significantly different by Bartlett's test (3, p. 272-275). These analyses are not included here. The analysis of variance of these three experiments is presented by randomized blocks (tables 4, 5 and 8). The randomized blocks design has an advantage over split-plot design if the several mean squares in the latter are not significantly different. The randomized blocks provide more degrees of freedom for the single error mean square they require (12, p. 296-297).

The data of dilution experiments was further subjected to analysis of covariance (16, p. 344-378) (26, p. 394-412) in order to determine whether the regression of BYMV infection on dilution was linear. Besides, the individual degree of freedom test (12, p. 61-70) (16, p. 226-233) was used to compare any two treatments.

RESULTS

Effect of Method of Inoculation

Transmission of BYMV by mechanical inoculation was compared with inoculation by green peach aphid in nine experiments (tables 2-10). The results are summarized in table 1. Transmission by mechanical inoculation varied from 28.6 to 77.8 per cent and that by aphid inoculation from 30.2 to 53.2 per cent.

Transmission by mechanical inoculation decreased with increasing dilution of inoculum. Dilution of inoculum was changed from experiment to experiment in order to get about 50 per cent infection. Variation in the rate of transmission by aphid inoculation could not be controlled. Similar variations in rate of transmission of BYMV by *M. persicae* have been reported by Swenson (30, p. 521).

Relative Susceptibility of Varieties

Several bean varieties were tested in different combinations in six experiments for susceptibility to BYMV by mechanical inoculation and by inoculation by green peach aphid. Varieties included in these tests were Dwarf Horticultural, Top Crop, Bachicha, Seminole and Ranger. There were large varietal differences in the number of plants infected.

Table 1

Comparison of transmission of BYMV by mechanical inoculation with inoculation by green peach aphid

Experi-ment Number	Aphid		Mechanical		
	Plants inocu-lated	Per cent trans-mission	Dilution of inoculum	Plants inocu-lated	Per cent trans-mission
1	225	30.2	1/100	225	77.8
2	216	34.7	1/300	216	70.3
3	216	38.8	1/700	216	32.4
4	144	47.9	1/200	144	45.8
5	288	33.0	1/400	288	45.8
6	360	35.6	1/600	360	28.6
7	250	53.2	1/600	250	54.8
8	250	42.8	1/700	250	64.4
9	200	47.0	1/700	200	60.0

More plants of the Bachicha variety than of the Seminole variety were infected in three tests. Differences were significant at the one per cent level in two experiments (tables 3 and 6), but not significant in the third (table 2). In a single test, Bachicha was more susceptible than Ranger, the difference being significant at the one per cent level (table 2).

More Dwarf Horticultural plants than Seminole were infected in three tests. Differences were significant at the one per cent level in two experiments (tables 3 and 4), but not significant in the third (table 2). In three tests, more Dwarf Horticultural plants than Ranger were infected. Differences were significant at the one per cent level in one experiment (table 2) but not significant in the other two (tables 4 and 7).

In a single test, more plants of the Top Crop variety than of the Seminole and Ranger varieties were infected (table 2). Differences between Top Crop and Ranger were significant at the one per cent level, but those between Top Crop and Seminole were not significant.

No large differences were found in the number of infected plants among Dwarf Horticultural, Top Crop and Bachicha, or between Ranger and Seminole.

<u>Methods X Varieties Interaction</u>. Varietal differences in the number of infected plants were not the same by mechanical inoculation and by inoculation by aphids. Large differences were obtained by mechanical inoculation, but the corresponding differences by aphid inoculation were much smaller. Separate analysis of variance, not included here, showed the differences by mechanical inoculation to be significant at the one per cent level in three experiments, significant at the five per cent level in another and not significant in two experiments. The differences by aphid inoculation were not significant in any of the experiments.

Table 2

Relative susceptibility of bean varieties to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Rep.[1]	Aphid					Mechanical					Total
	DH[2]	TC	B	R	S	DH	TC	B	R	S	
1	0[3]	1	0	1	2	3	3	3	3	3	19
2	1	0	2	0	1	2	0	3	1	3	13
3	1	1	1	1	1	3	3	3	0	0	14
4	0	1	0	0	1	3	3	3	1	3	15
5	1	1	0	0	1	2	3	3	1	3	15
6	0	0	0	0	1	3	3	3	0	0	10
7	1	1	1	1	3	1	0	3	2	3	16
8	1	1	1	0	0	3	2	2	0	0	10
9	2	3	3	2	1	3	3	3	2	0	22
10	1	0	1	0	0	3	3	3	0	0	11
11	1	1	2	1	2	3	3	3	1	2	19
12	1	1	2	2	1	3	3	3	3	3	22
13	1	0	1	0	2	3	3	3	3	3	19
14	2	1	1	1	0	3	3	3	3	3	20
15	0	1	0	0	2	3	3	3	3	3	18
Total	13	13	15	9	18	41	38	44	23	29	243

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	14	1.72
Methods	1	76.33**
Error (a)	14	1.27
Varieties	4	3.51*
Error (b)	56	0.96
Methods X Varieties	4	2.31**
Error (c)	56	0.38

[1] Rep. = Replication

[2] DH = Dwarf Horticultural, TC = Top Crop, B = Bachicha, R = Ranger, S = Seminole

[3] Number of infected plants out of three inoculated

* = Significant at 5% level, ** = Significant at 1% level

Table 3

Relative susceptibility of bean varieties to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Replications	Aphid			Mechanical			Total
	DH[1]	B	S	DH	B	S	
1	4[2]	2	1	6	6	5	24
2	2	4	2	6	6	3	23
3	4	2	1	5	2	0	14
4	3	3	4	6	5	3	24
5	4	3	1	0	3	2	13
6	1	4	1	6	5	1	18
7	2	1	0	6	6	2	17
8	2	3	4	4	4	1	18
9	1	2	2	6	6	6	23
10	3	2	0	6	6	4	21
11	2	0	0	6	4	1	13
12	2	0	3	6	6	2	19
Total	30	26	19	63	59	30	227

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	11	2.86
Methods	1	82.35**
Error (a)	11	4.38
Varieties	2	22.89**
Error (b)	22	1.39
Methods X Varieties	2	6.72*
Error (c)	22	1.62

[1] DH = Dwarf Horticultural, B = Bachicha, S = Seminole

[2] Number of infected plants out of six inoculated

* = Significant at 5% level, ** = Significant at 1% level

Table 4

Relative susceptibility of bean varieties to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Replications	Aphid			Mechanical			Total
	DH[1]	R	S	DH	R	S	
1	3[2]	2	0	4	3	2	14
2	3	2	3	5	2	3	18
3	2	5	3	5	0	0	15
4	3	3	1	2	1	0	10
5	2	1	1	2	1	2	9
6	0	2	3	6	4	4	19
7	2	3	1	2	1	0	9
8	3	1	3	1	3	3	14
9	3	1	0	2	1	0	7
10	3	5	2	1	3	1	15
11	4	4	0	0	1	0	9
12	4	3	3	3	2	0	15
Total	32	32	20	33	22	15	154

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	11	2.54
Methods	1	2.72
Varieties	2	9.60**
Methods X Varieties	2	1.26
Error	55	1.90

[1] DH = Dwarf Horticultural, R = Ranger, S = Seminole

[2] Number of infected plants out of six inoculated

** = Significant at 1% level

Analysis of variance showed the interaction between methods of inoculation and varieties to be significant at the one per cent level in two experiments (tables 2 and 6), significant at the five per cent level in two (tables 3 and 7), and not significant in the other two (tables 4 and 5). The significance or lack of significance, both in relation to interaction and varieties within methods, was due to the particular combination of varieties compared. There were no varietal differences in the number of infected plants in one of these experiments (table 5). The absence of interaction between methods of inoculation and varieties in this case is not, therefore, unexpected. In the other experiment (table 4), the error mean square was relatively too large to show this interaction.

Effect of Light Intensity

The effect of light intensity on the susceptibility of bean plants to BYMV by mechanical inoculation and inoculation by green peach aphid was studied in three experiments. The light factor was superimposed on varieties.

One-half of the plants were kept in the ordinary fluctuating light intensity in the greenhouse. The others were kept in almost complete darkness by shading with black cotton-cloth. Light intensity in the greenhouse varied between 50 and 4,000 foot candles, and under shaded conditions from zero to three foot candles from 8 a.m. to 6 p.m. Light intensity was measured with a light meter every two hours during this part of the day. Temperature under the shaded conditions was 74-82° F. (day), 68-76° F. (night) and under unshaded conditions 70-86° F. (day) and 68-76° F. (night).

Plants were kept for 45-58 hours in the differential light conditions before being inoculated. All the plants were kept under the fluctuating light conditions in the greenhouse prior to, and after the differential light treatment. The shaded plants looked slightly etiolated and spindly.

Shading did not have much effect on the susceptibility of plants. There were fewer plants infected in the shaded group than in the unshaded group in two experiments (tables 5 and 7). In the third experiment, there were slightly more plants infected in the shaded group than in the unshaded group (table 6). The differences were not, however, significant in any case.

Methods X Light Interaction. Effect of shading, although small, was not the same by mechanical inoculation and inoculation by aphids. Shading slightly increased the number of infected plants by mechanical inoculation in all three tests. Similar shading appreciably decreased, however, the number of infected plants by aphid inoculation in

Table 5

Effect of light intensity on the susceptibility of bean varieties to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Rep-lica-tions	Aphid				Mechanical				Total
	Dwarf Hort.		Top Crop		Dwarf Hort.		Top Crop		
	S[1]	U	S	U	S	U	S	U	
1	0[2]	1	0	2	2	0	2	3	10
2	0	2	0	0	1	1	2	2	8
3	2	1	0	0	3	3	2	1	12
4	1	2	0	2	2	0	2	2	11
5	1	1	1	0	3	3	1	3	13
6	0	0	0	1	3	2	3	3	12
7	0	0	0	2	2	3	1	3	11
8	0	1	1	1	2	1	2	0	8
9	0	2	1	1	3	3	3	3	16
10	1	1	0	1	3	3	2	1	12
11	1	1	1	2	2	2	3	3	15
12	0	1	0	0	1	2	1	2	7
Total	6	13	4	12	27	23	24	26	135

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	11	0.94
Methods	1	44.01**
Varieties	1	0.10
Light	1	1.76
Methods X Varieties	1	0.09
Methods X Light	1	3.02*
Varieties X Light	1	0.51
Methods X Varieties X Light	1	0.25
Error	77	0.64

[1] S = Shaded, U = Unshaded

[2] Number of infected plants out of three inoculated

* = Significant at 5% level, ** = Significant at 1% level

Table 6

Effect of light intensity on the susceptibility of bean varieties to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Replications	Aphid				Mechanical				Total
	Bachicha		Seminole		Bachicha		Seminole		
	S[1]	U	S	U	S	U	S	U	
1	1[2]	2	3	1	6	3	3	0	19
2	2	3	2	0	5	5	2	0	19
3	2	2	3	4	4	5	1	0	21
4	3	5	1	2	6	6	1	2	26
5	2	2	1	2	3	1	0	0	11
6	1	1	1	0	6	4	2	3	18
7	1	2	1	1	6	6	1	0	18
8	2	3	4	2	5	6	0	1	23
9	2	0	0	2	5	6	2	0	17
10	3	1	0	0	6	1	0	3	14
11	5	5	4	3	5	5	0	2	29
12	2	2	3	1	3	1	0	0	12
Total	26	28	23	18	60	49	12	11	227

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	11	3.56
Methods	1	14.26
Error (a)	11	3.74
Varieties	1	102.09**
Light	1	2.34
Varieties X Light	1	0.09
Error (b)	33	1.43
Methods X Varieties	1	55.51**
Methods X Light	1	0.84
Methods X Varieties X Light	1	3.01
Error (c)	33	1.24

[1] S = Shaded, U = Unshaded

2 Number of infected plants out of six inoculated

** = Significant at 1% level

Table 7

Effect of light intensity on the susceptibility of bean varieties to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Replications	Aphid				Mechanical				Total
	Dwarf Hort.		Ranger		Dwarf Hort.		Ranger		
	S[1]	U	S	U	S	U	S	U	
1	1[2]	2	2	1	0	3	1	1	11
2	2	2	3	5	1	1	1	0	15
3	1	3	2	5	3	3	2	0	19
4	2	3	0	4	2	1	0	0	12
5	2	2	3	1	2	1	2	1	14
6	0	2	2	3	4	3	0	1	15
7	3	4	4	3	2	0	0	0	16
8	0	0	0	0	2	2	4	3	11
9	3	3	3	4	0	2	2	2	19
10	4	3	3	3	6	2	4	1	26
11	1	4	1	4	0	2	0	0	12
12	2	3	3	5	2	1	4	2	22
13	2	0	0	0	0	3	4	1	10
14	1	0	1	4	2	4	2	4	18
15	2	1	0	1	1	4	1	1	11
Total	26	32	27	43	27	32	27	17	231

[1] S = Shaded, U = Unshaded

[2] Number of infected plants out of six inoculated

Table 7 (Cont'd)

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	14	2.69
Methods	1	4.54
Error (a)	14	5.10
Varieties	1	0.08
Light	1	2.41
Varieties X Light	1	0.21
Error (b)	42	1.42
Methods X Varieties	1	6.74*
Methods X Light	1	6.74*
Methods X Varieties X Light	1	4.54
Error (c)	42	1.40

* = Significant at 5% level

two experiments, differences being significant at the five per cent level in one experiment (table 5) but not significant in the other (table 7). In the third experiment shading caused a slight increase in the number of infected plants by aphid inoculation (table 6).

Varieties X Light Interaction. The effect of shading was alike on all the varieties included in these tests.

Effect of Age of Test Plant

The effect of age, and nitrogen on the susceptibility of bean plants to infection with BYMV by mechanical inoculation and inoculation by green peach aphid was studied in a three-factorial arrangement in three experiments (tables 8-10). Chemical analysis of the soil samples containing different nitrogen levels tested is presented in appendix table 4.

More nine-day-old plants than seven-day-old plants were infected in one experiment. The difference was significant at the one per cent level (table 8). No large differences were present, however, in the number of infected plants between eight-day-old and 11-day-old plants (table 9) or between nine-day and 12-day-old plants (table 10).

Methods X Age Interaction. The effect of age on the number of infected plants was not the same by mechanical inoculation and aphid inoculation. There was practically no effect of age on the number of plants infected by aphid inoculation. Such an effect was present in the case of mechanical inoculation in two experiments (tables 8 and 10), though there was no effect in the third experiment (table 9).

Analysis of variance showed the interaction between methods of inoculation and age of test plants to be significant at the one per cent level in one experiment (table 8), significant at the five per cent level in another (table 10), but not significant in the third (table 9).

Effect of Nitrogen

An increase in the total nitrogen content of soil up to 0.1 to 0.15 per cent increased the number of infected plants in all the three experiments. The increase was not, however, statistically significant (tables 8-10). The number of infected plants was decreased if nitrogen content was higher than 0.15 per cent.

Methods X Nitrogen Interaction. The effect of nitrogen was the same on the number of plants infected by mechanical inoculation and aphid inoculation.

Table 8

Effect of age and nitrogen on the susceptibility of Dwarf Horticultural bean to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Treatments			Replications					Total
Method	Age of T.P.[1]	N	1	2	3	4	5	
Aphid	7 days	.06	2[2]	4	4	1	2	13
		.09	3	5	2	3	0	13
		.12	2	5	3	4	0	14
		.15	2	4	3	3	2	14
		.17	3	4	3	2	0	12
	9 days	.06	3	3	1	2	1	10
		.09	3	2	4	2	1	12
		.12	3	5	3	3	2	16
		.15	3	4	3	2	2	14
		.17	4	3	3	4	1	15
Mechanical	7 days	.06	1	2	1	4	0	8
		.09	1	4	1	2	1	9
		.12	2	2	2	3	1	10
		.15	2	3	4	3	1	13
		.17	1	4	4	3	0	12
	9 days	.06	3	3	5	2	1	14
		.09	4	2	4	1	4	15
		.12	5	4	3	1	4	17
		.15	5	4	5	3	4	21
		.17	4	5	4	2	3	18
Total			56	72	62	50	30	270

1 T.P. = Test Plants, N = Per cent total nitrogen in soil

2 Number of infected plants out of five inoculated

Table 8 (Cont'd)

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	4	12.30**
Methods	1	0.16
Age	1	11.56**
Nitrogen	4	2.35
Age X Nitrogen	4	0.36
Methods X Age	1	10.24**
Methods X Nitrogen	4	0.66
Methods X Age X Nitrogen	4	0.29
Error	76	1.20

** = Significant at 1% level

Table 9

Effect of age and nitrogen on the susceptibility of Dwarf Horticultural bean to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Treatments			Replications					Total
Method	Age of T.P.[1]	N	1	2	3	4	5	
Aphid	8 days	.06	2[2]	4	1	0	3	10
		.09	0	1	1	2	2	6
		.12	4	2	3	1	4	14
		.15	3	2	4	1	5	15
		.17	1	2	3	2	0	8
	11 days	.06	3	3	3	1	1	11
		.09	3	1	2	0	3	9
		.12	4	3	3	0	3	13
		.15	2	0	3	2	2	9
		.17	3	3	3	1	2	12
Mechanical	8 days	.06	4	2	4	3	1	14
		.09	2	1	5	5	4	17
		.12	4	1	3	4	2	14
		.15	2	2	4	5	4	17
		.17	2	1	5	5	4	17
	11 days	.06	3	2	3	3	3	14
		.09	3	3	3	5	3	17
		.12	5	3	4	5	4	21
		.15	4	2	3	4	1	14
		.17	3	3	5	4	1	16
Total			57	41	65	53	52	268

1 T.P. = Test Plants, N = Per cent total nitrogen in soil
2 Number of infected plants out of five inoculated

Table 9 (Cont'd)

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	4	3.79
Methods	1	29.76
Error (a)	4	9.11
Age	1	0.16
Nitrogen	4	1.44
Age X Nitrogen	4	1.66
Error (b)	36	1.33
Methods X Age	1	0.04
Methods X Nitrogen	4	1.36
Methods X Age X Nitrogen	4	1.34
Error (c)	36	0.93

Table 10

Effect of age and nitrogen on the susceptibility of Dwarf Horticultural bean to BYMV by mechanical inoculation and to inoculation by green peach aphid

A. Data

Treatments			Replications					Total
Method	Age of T.P.[1]	N	1	2	3	4	5	
Aphid	9 days	.06	3[2]	0	3	2	3	11
		.09	2	1	2	2	1	8
		.12	2	1	4	2	1	10
		.15	3	0	2	0	2	7
		.17	2	0	3	0	2	7
	12 days	.06	1	0	3	1	1	6
		.09	4	3	3	0	2	12
		.12	1	3	3	2	1	10
		.15	2	1	4	3	3	13
		.17	3	1	1	2	3	10
Mechanical	9 days	.06	0	3	2	4	3	12
		.09	2	3	3	4	2	14
		.12	3	2	3	3	4	15
		.15	2	3	3	3	4	15
		.17	1	3	1	3	4	12
	12 days	.06	1	1	0	4	2	8
		.09	3	1	3	4	3	14
		.12	1	2	1	3	3	10
		.15	2	2	1	4	1	10
		.17	2	0	0	4	4	10
Total			40	30	45	50	49	214

[1] T.P. = Test Plants, N = Per cent total nitrogen in soil

[2] Number of infected plants out of four inoculated

Table 10 (Cont'd)

B. Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square
Replications	4	3.34
Methods	1	6.76
Error (a)	4	9.09
Age	1	0.64
Nitrogen	4	1.06
Age X Nitrogen	4	1.39
Error (b)	36	1.01
Methods X Age	1	5.76*
Methods X Nitrogen	4	0.16
Methods X Age X Nitrogen	4	0.91
Error (c)	36	0.91

* = Significant at 5% level

Relation of Symptom Expression of BYMV in Broad Bean to Virus Concentration

The relation of symptom expression of BYMV in broad bean to virus concentration was studied by mechanical inoculation in a series of three experiments. Test plant in one of the experiments was Dwarf Horticultural variety of the bean, *P. vulgaris* L., and in the other two, the local lesion host *Chenopodium amaranticolor*.

The source plants were divided into two groups on the basis of symptom expression of BYMV in them:

1. Plants with mild symptoms
2. Plants with severe symptoms

The two types of source plants were compared at several dilutions of inoculum. Bean plants were inoculated just before trifoliate stage. In the case of *Chenopodium*, six leaves, serially numbered from top to bottom after excluding the 4-5 top, purple leaves, were inoculated.

Symptom Expression. The relation of symptom expression in broad bean to virus concentration is shown in figures 1-3. In all three experiments source plants with severe symptoms gave higher infection than those with mild symptoms. The differences were significant at the one per cent level in one experiment (table 11) but not significant in the other two (tables 12 and 13).

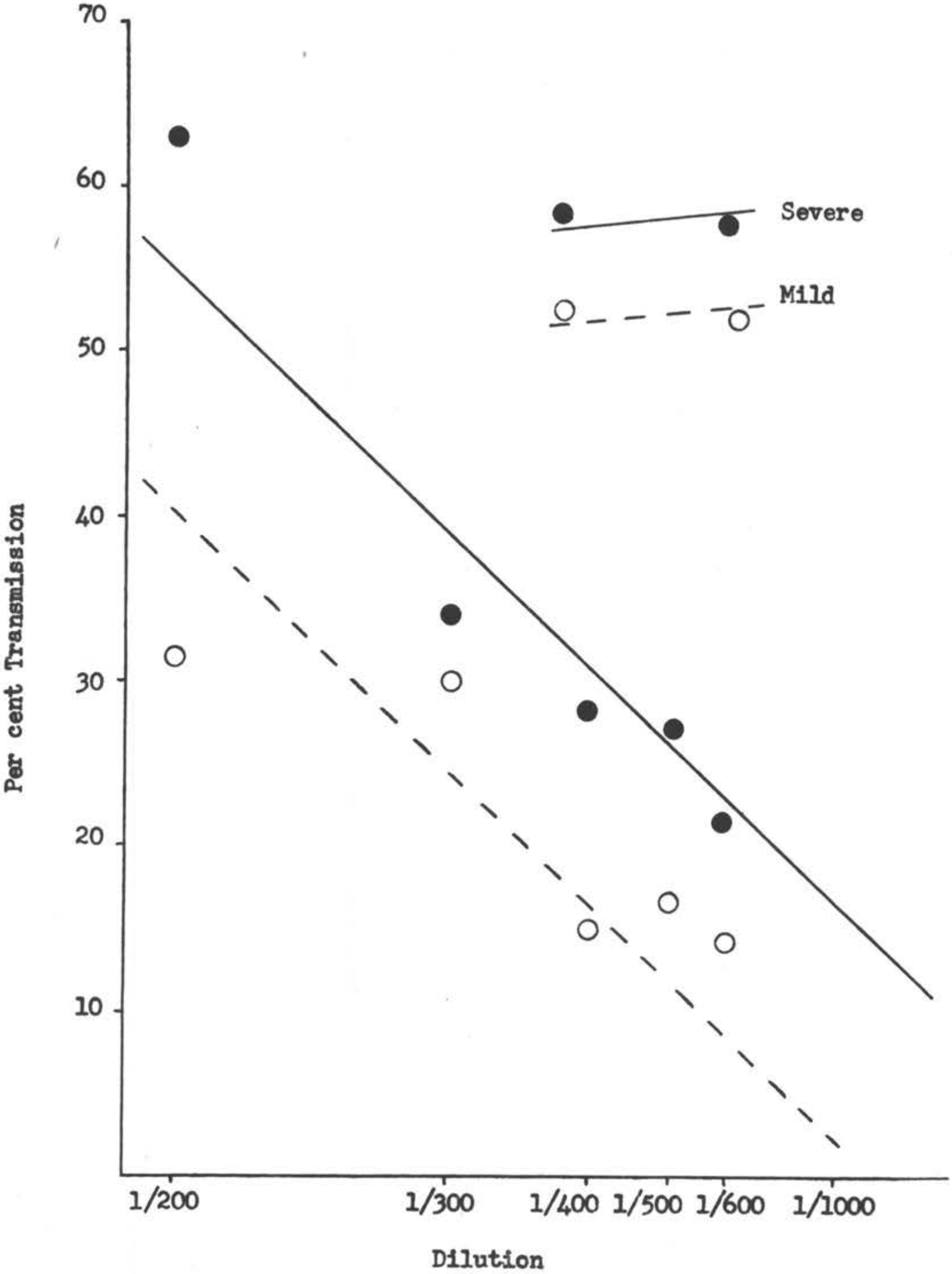

Figure 1. Relation of symptom expression of BYMV in broad bean to virus concentration.

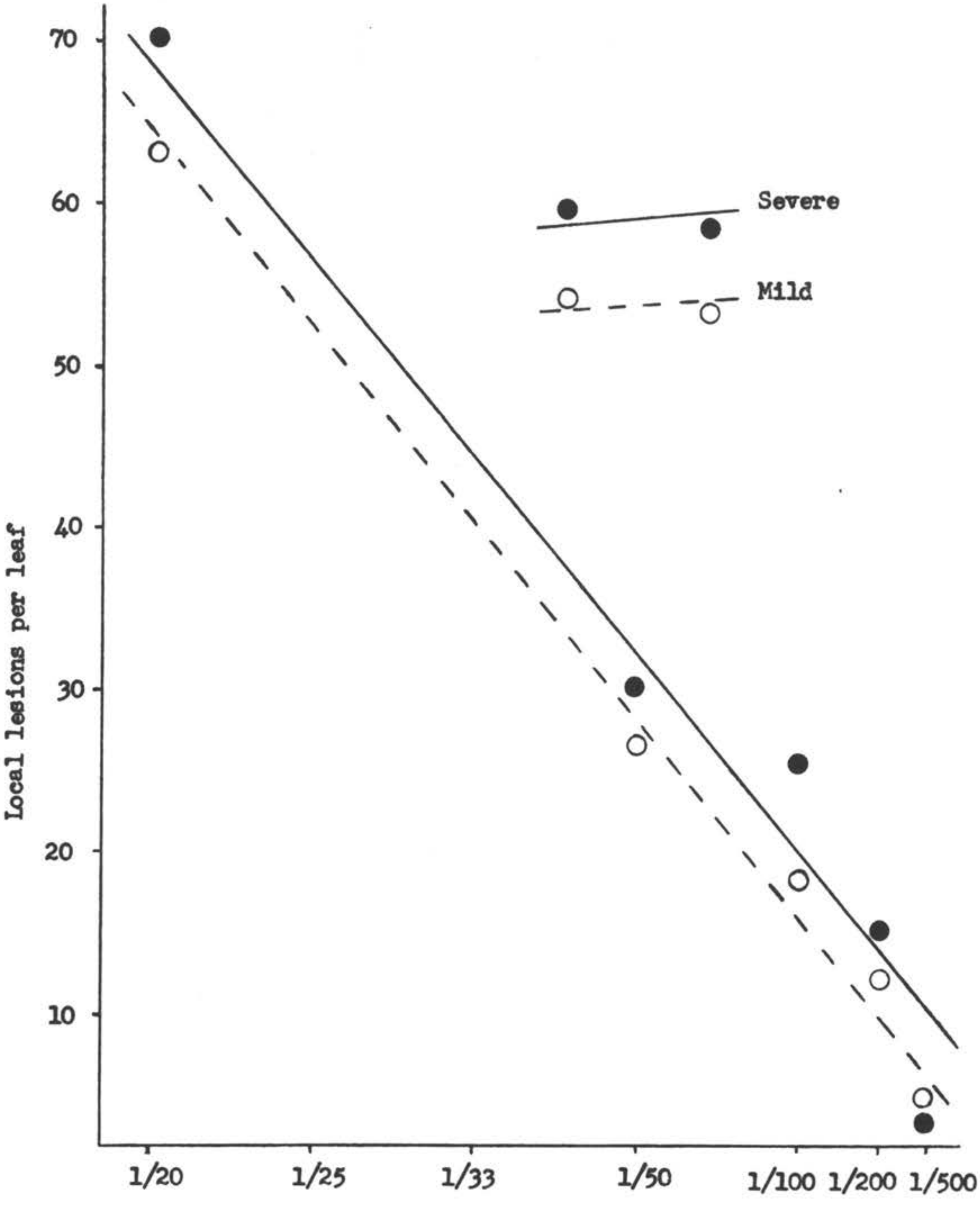

Figure 2. Relation of symptom expression of BYMV in broad bean to virus concentration.

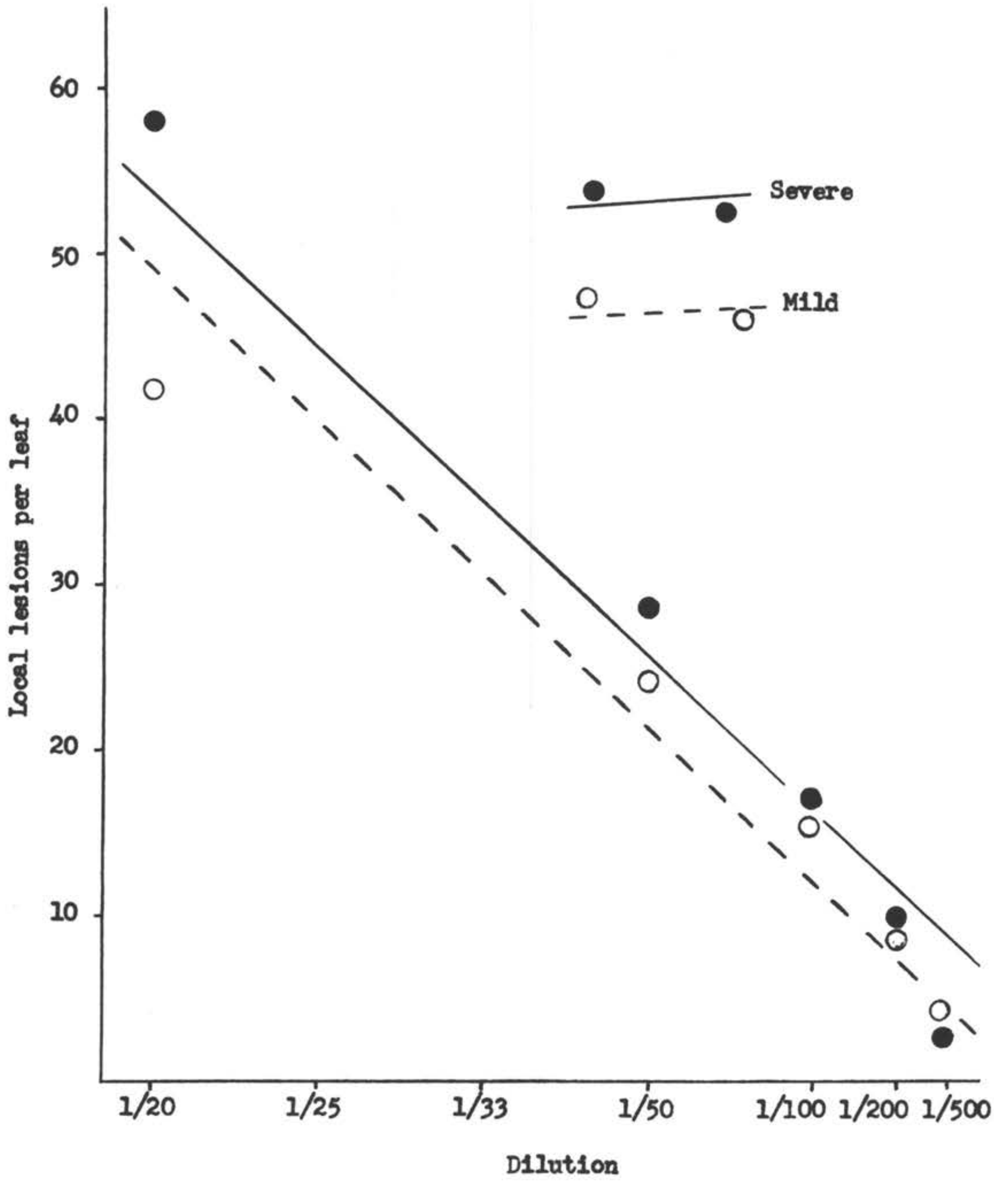

Figure 3. Relation of symptom expression of BYMV in broad bean to virus concentration.

Dilutions. Infection decreased with an increase in dilution of inoculum (figures 1-5). The decrease was significant at the one per cent level in all the three experiments. The regression of infection on dilution of inoculum was linear (tables 11-13).

Symptoms X Dilutions Interaction. More plants were infected from source plants with severe symptoms than the plants with mild symptoms at all the dilutions tried except at a dilution of 1/500 in two experiments with Chenopodium test plants (figures 1-3). The analysis of covariance showed no interaction between symptom expression of the virus in source plants and dilutions in any of the three experiments (tables 11-13). The lines of regression for symptoms are parallel to each other, showing absence of interaction (figures 1-3).

Age of Chenopodium Leaves. Examination of data revealed differences in the susceptibility of leaves of the same Chenopodium plant. The data were, therefore, analysed for the relative susceptibility of leaves. The younger, upper leaves gave greater numbers of local lesions than the older, lower leaves (tables 12 and 13, and figures 4 and 5). The differences were significant at the one per cent level in both experiments.

Dilutions X Leaves Interaction. The rate of fall in number of local lesions per leaf with a unit increase in

Table 11

Relation of symptom expression of BYMV
in broad bean to virus concentration

A. Data

Rep.[1]	Severe Symptoms					Mild Symptoms					Total
	1/200[2]	1/300	1/400	1/500	1/600	1/200	1/300	1/400	1/500	1/600	
1	3[3]	1	0	0	2	10	7	0	1	1	25
2	1	1	2	1	2	1	3	0	1	0	12
3	6	5	2	1	1	0	1	3	0	0	19
4	7	0	1	0	2	0	0	0	1	0	11
5	12	10	3	2	5	10	10	4	5	2	63
6	12	9	6	10	3	3	2	5	1	0	51
7	10	1	7	5	0	4	6	2	7	0	42
8	4	0	6	4	3	2	2	0	0	0	21
9	12	4	4	1	5	2	0	4	2	3	37
10	9	10	3	8	3	6	5	0	2	2	48
Total	76	41	34	32	26	38	36	18	20	8	329

1 Rep. = Replication

2 Dilution of inoculum

3 Number of infected Dwarf Horticultural bean plants out of 12 inoculated

Table 11 (Cont'd)

B. Analysis of Covariance

Source of Variation	Degrees of Freedom	Mean Square
Replications	9	31.05**
Symptoms	1	79.21**
Dilutions	4	47.50**
Linear Regression	1	187.94**
Deviation from Linearity	3	0.68
Symptoms X Dilutions	4	8.12
Variation among R.C.'s[4]	1	12.78
Deviation from Linearity	3	6.57
Error	81	6.46

[4] R.C.'s = Regression Coefficients

** = Significant at 1% level

Table 12

Relation of symptom expression of BYMV in broad bean to virus concentration

A. Data

Treatments			Replications						Total
Symptoms	Dil.[1]	Leaf	1	2	3	4	5	6	
Severe	1/20	1[2]	51[3]	68	70	204	105	135	633
		2	45	54	75	171	25	61	431
		3	28	107	69	141	25	60	430
		4	28	49	81	109	28	67	362
		5	41	61	49	111	43	82	387
		6	18	49	49	51	54	69	290
	1/50	1	63	37	58	50	7	64	279
		2	36	19	28	53	0	74	210
		3	17	15	30	37	2	87	188
		4	32	18	22	31	3	69	175
		5	19	23	24	25	5	24	120
		6	15	5	16	25	1	55	117
	1/100	1	16	12	24	62	29	62	205
		2	27	17	18	24	23	69	178
		3	6	11	12	44	14	56	143
		4	7	19	15	14	23	28	106
		5	5	16	22	39	41	46	169
		6	10	9	12	34	39	27	131
	1/200	1	22	13	23	40	23	44	165
		2	5	6	7	27	5	26	76
		3	4	13	18	10	3	32	79
		4	2	8	16	23	4	43	96
		5	3	6	9	21	1	32	72
		6	7	8	14	11	6	27	73
	1/500	1	5	6	7	9	6	11	44
		2	6	6	1	4	3	9	29
		3	3	5	6	6	5	4	29
		4	1	3	2	3	1	5	15
		5	3	1	4	4	6	10	28
		6	0	0	3	3	1	0	7

1 Dil. = Dilution of inoculum
2 Leaves are numbered from top to bottom
3 Local lesions per *Chenopodium* leaf

Table 12 (Cont'd)

Treatments			Replications						Total
Symptoms	Dil.	Leaf	1	2	3	4	5	6	
Mild	1/20	1	38	136	51	119	115	146	605
		2	37	48	47	81	81	178	472
		3	18	59	58	51	52	97	335
		4	26	95	24	46	63	125	379
		5	19	82	17	50	51	40	259
		6	17	38	28	62	31	61	237
	1/50	1	9	37	26	49	81	45	247
		2	4	26	7	20	33	65	155
		3	2	25	4	18	36	65	150
		4	1	14	6	38	43	25	127
		5	2	38	4	21	55	38	158
		6	3	28	5	18	48	32	134
	1/100	1	31	13	31	36	52	31	194
		2	15	19	9	25	32	25	125
		3	5	20	15	15	23	20	98
		4	16	38	9	12	26	12	113
		5	7	18	14	9	14	18	80
		6	7	10	16	20	5	13	71
	1/200	1	10	25	13	14	37	16	115
		2	8	24	7	13	20	47	119
		3	0	15	5	7	8	15	51
		4	7	12	4	14	10	22	69
		5	0	3	6	12	11	12	44
		6	3	6	2	7	24	20	62
	1/500	1	7	1	3	9	9	13	42
		2	2	6	4	3	7	13	35
		3	4	10	0	8	20	1	43
		4	0	1	1	1	8	2	13
		5	1	2	0	5	20	1	29
		6	0	2	1	6	7	0	16
Total			824	1515	1201	2175	1553	2576	9844

Table 12 (Cont'd)

B. Symptoms X Dilutions

Dilutions	Symptoms		Mean
	Severe	Mild	
1/20	70.36[4]	63.53	66.94
1/50	32.25	26.97	28.61
1/100	25.89	18.92	22.40
1/200	15.61	12.75	14.18
1/500	4.22	4.94	4.83
Mean	29.27	25.42	27.34

C. Dilutions X Leaves

Leaves	Dilutions					Mean
	1/20	1/50	1/100	1/200	1/500	
1	103.17[5]	43.83	33.25	23.33	7.17	42.15
2	75.25	30.42	25.25	16.25	5.33	30.50
3	63.75	28.17	20.08	10.83	6.00	25.77
4	61.75	25.17	18.25	13.75	2.33	24.25
5	53.83	23.17	20.75	9.67	4.75	22.43
6	43.92	20.92	16.83	11.25	1.92	18.97
Mean	66.94	28.61	22.40	14.18	4.83	27.34

[4] Average number of local lesions per Chenopodium leaf, based on 36 leaves.

[5] Average number of local lesions per Chenopodium leaf, based on 12 leaves.

Table 12 (Cont'd)

D. Analysis of Covariance

Source of Variation	Degrees of Freedom	Mean Square
Replications	5	6,813.63
Symptoms	1	1,330.18
Replications X Symptoms	5	2,569.37
Dilutions	4	41,139.73**
Linear Regression	1	161,679.58**
Deviation from Linearity	3	959.78
Replications X Dilutions	20	1,303.53
Leaves	5	4,026.40**
Replications X Leaves	25	256.80
Symptoms X Dilutions	4	183.87
Variation among R.C.'s[6]	1	286.63
Deviation from linearity	3	149.61
Replications X Symptoms X Dilutions	20	996.01
Symptoms X Leaves	5	91.18
Replications X Symptoms X Leaves	25	182.01
Dilutions X Leaves	20	666.37**
Variation among R.C.'s[6]	5	2,540.49**
Deviation from linearity	15	41.66
Replications X Dilutions X Leaves	100	148.87
Symptoms X Dilutions X Leaves	20	141.07
Replications X Symptoms X Dilutions X Leaves	100	176.93

[6] R.C.'s = Regression Coefficients

** = Significant at 1% level.

Table 13

Relation of symptom expression of BYMV in broad bean to virus concentration

A. Data

Treatments			Replications					
Symptoms	Dil.[1]	Leaf	1	2	3	4	5	Total
Severe	1/20	1[2]	91[3]	40	98	49	36	314
		2	70	39	76	50	80	315
		3	105	28	88	28	93	342
		4	109	23	69	45	35	281
		5	52	27	92	30	74	275
		6	56	25	44	36	62	223
	1/50	1	42	9	52	27	40	170
		3	81	25	44	28	42	220
		3	16	3	30	40	44	133
		4	18	11	24	38	27	118
		5	23	9	30	30	28	120
		6	17	12	30	24	25	108
	1/100	1	15	8	40	13	27	103
		2	18	17	31	20	12	98
		3	13	7	17	15	22	74
		4	16	9	20	38	20	103
		5	9	6	18	11	14	58
		6	11	8	10	26	15	70
	1/200	1	21	9	14	6	21	71
		2	7	4	9	5	19	44
		3	15	7	8	5	24	59
		4	9	4	5	4	16	38
		5	10	3	11	3	10	37
		6	12	9	5	5	18	49
	1/500	1	3	2	3	4	6	18
		2	9	2	2	2	5	20
		3	6	3	2	2	4	17
		4	12	1	3	2	4	22
		5	0	3	3	2	9	17
		6	4	0	1	1	7	13

[1] Dil. = Dilution of inoculum

[2] Leaves have been numbered from top to bottom

[3] Local lesions per Chenopodium leaf

Table 13 (Cont'd)

Treatments			Replications					Total
Symptoms	Dil.	Leaf	1	2	3	4	5	
Mild	1/20	1	28	50	107	8	68	261
		2	17	29	119	8	56	229
		3	11	42	104	8	40	205
		4	21	28	110	6	65	230
		5	16	30	68	7	46	167
		6	5	24	75	14	50	168
	1/50	1	14	48	72	11	9	154
		2	26	45	73	11	11	166
		3	13	45	47	4	14	123
		4	12	21	58	3	16	110
		5	8	28	36	2	19	93
		6	16	3	42	2	17	80
	1/100	1	20	28	24	8	4	84
		2	15	35	47	7	8	112
		3	18	51	12	6	8	95
		4	15	20	12	3	5	55
		5	6	25	24	4	7	66
		6	8	18	15	6	10	57
	1/200	1	5	8	21	7	10	51
		2	10	19	9	6	11	55
		3	7	21	16	4	7	55
		4	8	9	12	2	7	38
		5	8	9	6	2	7	32
		6	10	8	6	3	6	33
	1/500	1	6	11	9	0	4	30
		2	12	12	4	1	0	29
		3	2	5	10	1	1	19
		4	2	4	5	2	3	16
		5	2	6	2	1	1	12
		6	5	1	1	1	3	11
Total			1216	1036	2025	737	1352	6366

Table 13 (Cont'd)

B. Symptoms X Dilutions

Dilutions	Symptoms		Mean
	Severe	Mild	
1/20	58.33[4]	42.00	50.17
1/50	28.97	24.20	26.58
1/100	16.87	15.63	16.25
1/200	9.93	8.80	9.37
1/500	3.57	3.90	3.73
Mean	23.53	18.91	21.22

C. Dilutions X Leaves

Leaves	Dilutions					Mean
	1/20	1/50	1/100	1/200	1/500	
1	57.5[5]	32.4	18.7	12.2	4.8	25.12
2	54.4	38.6	21.0	9.9	4.9	25.76
3	54.7	25.6	16.9	11.4	3.6	22.44
4	51.1	22.8	15.8	7.6	3.8	20.22
5	44.2	21.4	12.4	6.9	2.9	17.57
6	39.1	18.8	12.7	8.2	2.4	16.24
Mean	50.17	26.58	16.25	9.37	3.73	21.22

4 Average number of local lesions per Chenopodium leaf, based on 30 leaves.

5 Average number of local lesions per Chenopodium leaf, based on 10 leaves.

Table 13 (Cont'd)

D. Analysis of Covariance

Source of Variation	Degrees of Freedom	Mean Square
Replications	4	3,826.85
Symptoms	1	1,606.10
Replications X Symptoms	4	2,746.48
Dilutions	4	20,064.93**
Linear Regression	1	78,799.90**
Deviation from linearity	3	486.60
Replications X Dilutions	16	1,324.56
Leaves	5	766.66**
Replications X Leaves	20	118.37
Symptoms X Dilutions	4	695.03
Variation among R.C.'s[6]	1	2,747.50
Deviation from linearity	3	10.87
Replications X Symptoms X Dilutions	16	672.73
Symptoms X Leaves	5	4.14
Replications X Symptoms X Leaves	20	134.41
Dilutions X Leaves	20	117.25*
Variation among R.C.'s[6]	5	241.04**
Deviation from linearity	15	75.98
Replications X Dilutions X Leaves	80	63.15
Symptoms X Dilutions X Leaves	20	58.46
Replications X Symptoms X Dilutions X Leaves	80	84.78

[6] R.C.'s = Regression Coefficients
* = Significant at 5% level.
** = Significant at 1% level.

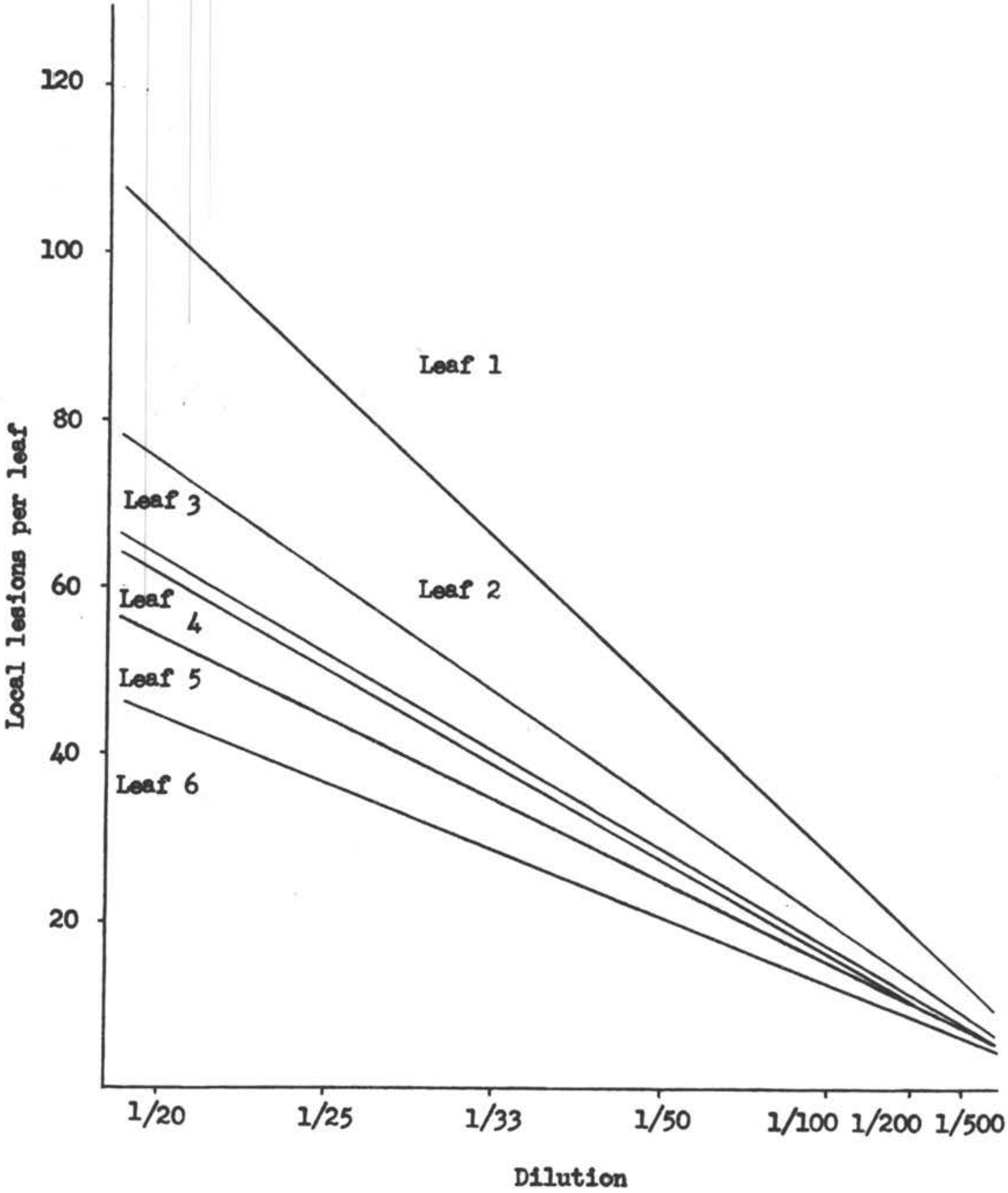

Figure 4. Relative susceptibility of Chenopodium leaves to mechanical inoculation with BYMV.

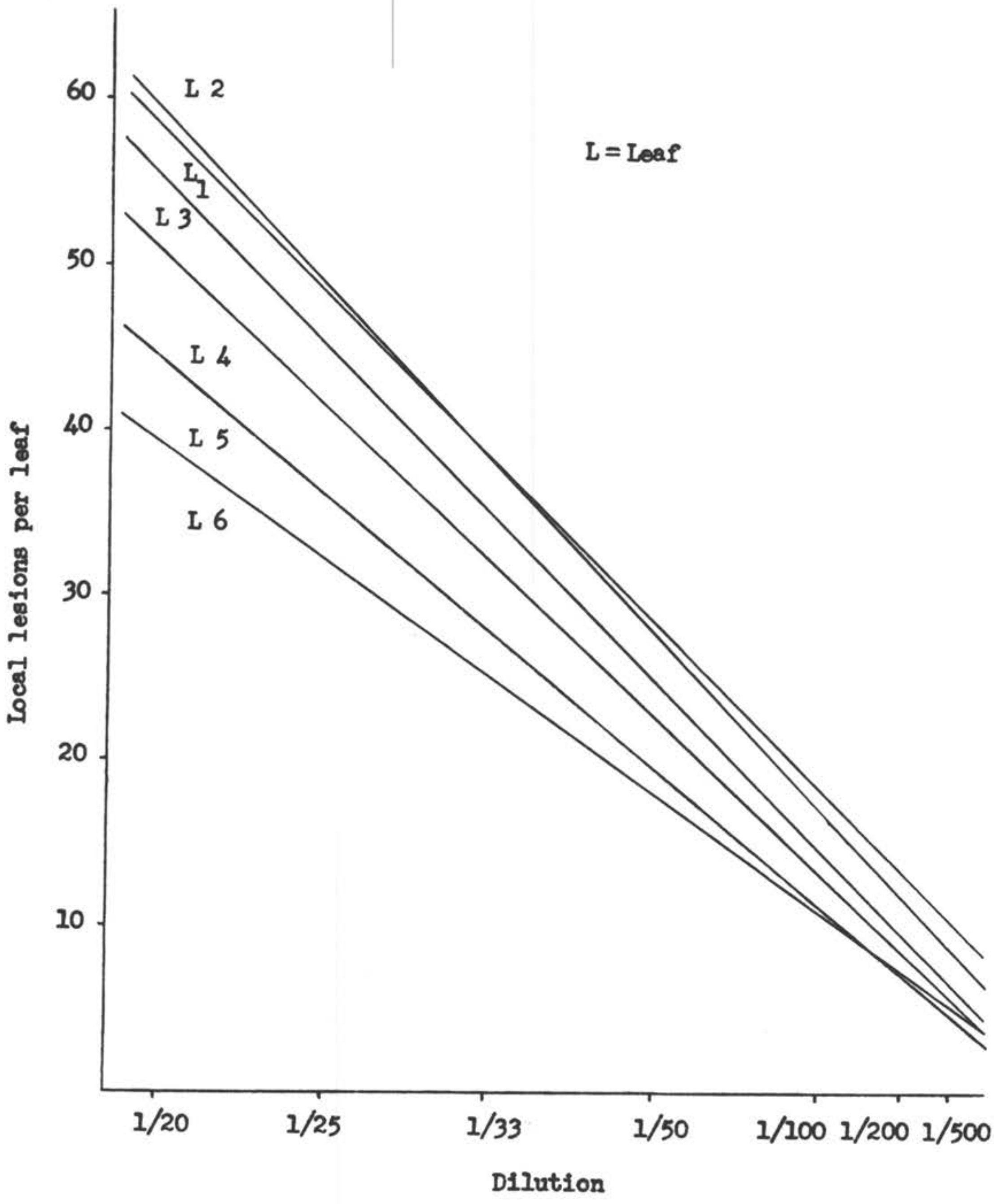

Figure 5. Relative susceptibility of Chenopodium leaves to mechanical inoculation with BYMV.

dilution was not the same for the different leaves of the same individual *Chenopodium* plant. This rate was higher for the younger, upper leaves than for the older, lower leaves. This is illustrated in figures 4 and 5. The lines of regression for the different leaves converge at higher dilutions, and in some cases even cross each other, showing that their slopes are not the same.

The analysis of covariance also showed the interaction between dilutions and leaves to be significant at the one per cent level in both the experiments (tables 12 and 13).

DISCUSSION

One of the problems encountered during the course of the present study was the inconsistent results in some of the experiments. Preinoculation shading slightly increased the number of bean plants infected with BYMV by mechanical inoculation in all three tests. Similar shading appreciably decreased the number of infected bean plants by aphid inoculation in two experiments but slightly increased their number in the third. Consequently, there was an interaction between methods of inoculation and light intensity in two experiments but no such interaction in the third.

Again in experiments dealing with the effect of age on the susceptibility of bean plants to BYMV, there was an interaction between methods of inoculation and age of test plants in two experiments but not in the third. Also there were large variations from one replication to another in most of the experiments.

Instances of such variable results are not uncommon in the literature. Swenson (30, p. 521) and Adlerz (2, p. 33-34) have reported similar variations in their results of BYMV transmission by aphids.

Wiltshire (39, p. 234-235) obtained quite variable and contradictory results in preinoculation shading trials. Out of his 31 tests, lesion number was increased by twofold or more in 14, decreased to one-half or less in four, and not affected significantly in 13, by shading French bean prior to mechanical inoculation with tobacco necrosis virus.

Much needs to be done to locate and eliminate the causes of such variations in transmission of plant viruses, especially in the case of insect transmission.

SUMMARY

Transmission by mechanical inoculation varied from 28.6 to 77.8 per cent and that by aphid inoculation from 30.2 to 53.2 per cent in nine experiments.

There were large varietal differences in bean susceptibility to infection with BYMV by mechanical inoculation but not to aphid inoculation. Dwarf Horticultural, Top Crop and Bachicha were more susceptible than Ranger and Seminole. There were, however, no large differences among Dwarf Horticultural, Top Crop and Bachicha, or between Ranger and Seminole.

Preinoculation shading for 45-58 hours did not have much effect on the number of bean plants infected with BYMV by sap inoculation in three tests. The same degree of shading appreciably decreased the number of bean plants infected by aphid inoculation in two experiments, though it did not have much effect in the third.

There was practically no effect of age on the number of plants infected by aphid inoculation. Such an effect was observed, however, in the case of mechanical inoculation in two experiments, though there was no effect in the third.

Nitrogen did not have any significant effect on the susceptibility of bean plants to infection with BYMV either by mechanical inoculation or by aphid inoculation.

Inoculum obtained from source plants with severe symptoms caused more infection than that from plants with mild symptoms. The regression of infection on dilution was linear. There was no interaction between symptoms and dilutions.

There was a great variation in the susceptibility of leaves of the same individual plant of *Chenopodium amaranticolor* to BYMV. The drop in infection with an increase in dilution was steeper in the younger leaves than in the older leaves.

The results of these experiments indicated that effects of environmental and other factors on the susceptibility of plants to virus infection by sap inoculation may not be the same for insect transmission of viruses. The relative resistance of candidate varieties to virus infection by mechanical inoculation may not be the same in the field where virus is spread by insects and not by sap inoculation. The extrapolation of results of transmission of plant viruses by sap inoculation to inoculation by insects should, therefore, be made with caution and reservation.

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APPENDIX

Appendix Table 1

Temperature and light intensity data for source plants for the period from two days before until two days after inoculation of test plants in 12 experiments

Experiment No.	Date of Experiment	Temperature (°F.)		Light Intensity[1] (Foot Candles)	Weather
		Day	Night		
1	June 1960	-	-	-	-
2	August 1960	76-96	70-74	350-2,800	Partially cloudy
3	September 1960	73-79	72-76	350-2,000	Partially cloudy
4	July 1960	-	-	140-2,800	-
5	September 1960	74-94	72-74	250-4,000	Clear sky
6	September 1960	70-86	66-72	50-3,000	Mostly cloudy
7	September 1960	70-86	66-72	50-3,000	Mostly cloudy
8	October 1960	70-80	68-76	140-3,000	Mostly cloudy
9	October 1960	72-87	72-74	80-2,000	Mostly cloudy
10	August 1960	74-90	72-74	400-3,000	Clear sky
11	October 1960	72-80	74-78	50-3,000	Mostly cloudy
12	November 1960	62-81	68-78	20-2,500	Mostly cloudy

[1] Light intensity was measured with a light meter every two hours between 8 a.m. and 6 p.m. during June to September, and between 8 a.m. and 4 p.m. during October and November.

- Information was not recorded.

Appendix Table 2

Temperature and light intensity data for test plants for the period from two days before until two days after inoculation of test plants in 12 experiments

Experiment No.	Date of Experiment	Temperature (°F.)		Light Intensity[1] (Foot Candles)	Weather
		Day	Night		
1	June 1960	-	-	-	-
2	August 1960	78-98	72-76	200-2,600	Partially cloudy
3	September 1960	76-86	67-72	250-2,600	Partially cloudy
4	July 1960	75-96	64-76	250-2,800	-
5	September 1960	72-87	72-76	150-4,000	Clear sky
6	September 1960	70-90	68-76	50-2,800	Mostly cloudy
7	September 1960	72-86	68-72	20-3,000	Mostly cloudy
8	October 1960	72-90	68-74	60-3,000	Mostly cloudy
9	October 1960	70-85	70-73	50-3,000	Mostly cloudy
10	August 1960	75-94	68-76	600-2,500	Clear sky
11	October 1960	72-84	70-80	350-2,000	Mostly cloudy
12	November 1960	78-84	70-76	300-2,000	Mostly cloudy

[1] Light intensity was measured with a light meter every two hours between 8 a.m. and 6 p.m. during June to September, and between 8 a.m. and 4 p.m. during October and November.

\- Information was not recorded.

Appendix Table 3

Chemical analysis of the soil used for growing source plants in all the experiments, and for test plants in experiments other than nitrogen level experiments

Soil pH	Phosphorus ppm[1]	Potassium m.e./100 g	Calcium m.e./100 g	Magnesium m.e./100 g	Total Nitrogen Per cent
6.8	31	0.54	13.8	5.10	0.09

[1] ppm = parts per million, m.e. = milli equivalents, g = grams

Appendix Table 4

Chemical analysis of the soil used for raising test plants in nitrogen level experiments

Nitrogen Level	Soil pH	Phosphorus ppm[2]	Potassium m.e./100 g	Calcium m.e./100 g	Magnesium m.e./100 g	Total Nitrogen Per cent
1	6.8	26	0.41	13.8	4.75	0.06
2	6.8	31	0.54	13.8	5.10	0.09
3	6.7	21	0.49	13.2	4.95	0.12
4	6.6	25	0.45	15.0	5.10	0.15
5	6.6	25	0.63	12.9	4.75	0.17

[2] ppm = parts per million, m.e. = milli equivalents, g = grams