Relation of Environment and Nutrition to Plant Susceptibility to Bean Yellow Mosaic Virus by Aphid Transmission

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K. G. SWENSON

ABSTRACT

The experiments reported here were concerned with the effect of different light and temperature environments, nutrition, and water supply on the susceptibility of pea and bean plants to infection with bean yellow mosaic virus by aphid transmission.

More pea plants were infected when kept at 30° C for two days after inoculation than when kept at 24 or 15° C for the same time. Plant age also affected susceptibility. Differences due to plant age were associated with the age of the inoculated leaves rather than the age of the whole plant. Leaves which were growing rapidly and expanding were less susceptible than fully expanded leaves, but their susceptibility was increased more by high post-inoculation temperature than was the susceptibility of fully expanded leaves.

In the first experiments, pea and bean plants were more susceptible when kept for two days before inoculation at low temperatures (15-18° C) than when kept at high temperatures (27-30° C) during the same time. In later experiments, this effect did not occur. It is suggested that a change in the virus culture was responsible for this difference.

In two experiments, the susceptibility of bean plants was affected by the amounts of nutrient elements in the soil; susceptibility increased with an increase in the general nutritional level. In numerous other experiments, the amount of nutrient elements available had no significant effect on susceptibility of pea or bean plants. Nor did large differences in the amount of water supplied to the plants affect susceptibility.

Various light treatments, including complete darkness for two days before and two days after inoculation, had no effect on plant susceptibility.

The data presented in this bulletin show that the result of evaluations of plant susceptibility to virus infection by mechanical inoculation do not apply to inoculations by aphids under the same conditions.

INTRODUCTION

Most of the numerous plant viruses transmitted by insects are carried from diseased to healthy plants in one of two ways: (1) internally, or (2) on the stylets. Internal transport of viruses is designated circulative or persistent transmission. Bean yellow mosaic virus belongs to the large group of viruses carried on the stylets of aphids.

The amount of virus acquired by vectors of circulative viruses increases with increasing time on diseased plants, so that low virus titer in the plant can be compensated for by longer feeding periods. The viruses are retained for long periods of time so that virus can be accumulated, stored, and, in some cases, increased by multiplication in the insect. Low probability of acquiring stylet-borne viruses, resulting from low virus titer in the diseased plant, cannot be offset by longer feeding on the diseased plants. Optimum acquisition periods are probes of 10 to 60 seconds duration. As duration of the acquisition probe increases, the probability of acquiring virus decreases. For example, alfalfa mosaic virus transmission by aphids dropped from 28 percent with acquisition probes of one minute to 2.5 percent with acquisition periods of 30 minutes (Swenson, 1952). Thus aphids cannot accumulate stylet-borne virus either by extended feeding or multiplication in the aphid.

Low plant susceptibility to circulative viruses may be offset by long periods of vector feeding because the viruses are retained for days or weeks. Stylet-borne viruses, on the other hand, are lost so rapidly that an aphid must place virus in a site susceptible to infection during the brief period that it is infective, if successful transmission is to occur. Few aphids are infective after 15 minutes and many lose infectivity within 5 minutes after leaving the diseased plant.

The transmission mechanism indicates the most appropriate line of experimentation. Stylet-borne viruses are much more directly dependent than are circulative viruses on virus titer in diseased plants and on the level of susceptibility of healthy plants (Swenson, 1968). Changes in plant susceptibility would be expected, therefore, to produce proportional changes in the frequency of transmission of stylet-borne viruses. Susceptibility of plants to virus infection by mechanical transmission, including some viruses transmissible by aphids, has been altered by various pre-inoculation light and temperature treatments, nutrition, and availability of water (Kassanis, 1957; Swenson, 1969).

Some attempts have been made to alter the susceptibility of bean and pea plants to infection with bean yellow mosaic virus (BYMV) by aphid transmission. Susceptibility of bean plants to BYMV was altered by changing the pre-inoculation temperature (Swenson and Sohi,

1961). The susceptibility of pea plants to infection with bean yellow mosaic virus by aphid transmission decreased with increasing pre-inoculation temperature between 15 and 33°C (Welton *et al.*, 1964). An opposite effect was obtained with different post-inoculation temperatures. More pea plants were infected when kept at 30°C for two days after inoculation than when kept for two days at 15°C (Welton *et al.*, 1964). Attempts to obtain an additive effect by combining optimum pre- and post-inoculation temperatures were unsuccessful in that transmission always depended on post-inoculation temperature only (Welton *et al.*, 1964).

The purpose of the experiments described in this bulletin was to learn the extent to which plant susceptibility to an aphid-borne virus, bean yellow mosaic virus, might be altered by environmental and nutritional conditions. Some light and nutritional treatments were combined with pre- or post-inoculation temperature treatments to test the possibility of interactions.

MATERIALS AND METHODS

All aphids used were *Myzus persicae* (Sulz.) cultured on *Brassica pekinensis* Rupr. Aphids were taken from cultures on *B. pekinensis* and kept in small flasks for several hours, or overnight, before use in experiments.

Bean yellow mosaic virus (BYMV) was maintained in *Vicia faba* L. by aphid transfer to successive plants. Infected *V. faba* was used as a source of virus for aphids in all experiments. Each aphid was allowed one naturally terminated probe of 10 to 45 seconds duration on the diseased plant and then transferred immediately to a healthy test plant. Probing time was measured from the time the tip of the rostrum touched the leaf surface until it was removed. Bradley (1964) commented that investigators frequently proceed as if aphids acquire stylet-borne viruses with equal frequency when probing on upper and lower surfaces of leaves, but that tests seldom are made to determine if this is true. In a replicated test, 26 of 50 aphids that made one probe on the upper surface of leaves of infected *V. faba* plants transmitted BYMV compared to transmission by 27 of 50 aphids that probed the lower surface of the same leaves.

Lincoln pea, *Pisum sativum* L., was usually used as a test plant. Aphids were allowed one probe of 10 to 60 seconds duration on each healthy plant. These test probes were measured in the same way as were the acquisition probes. Sometimes bean, *Phaseolus vulgaris* L. cv. Dwarf Horticultural, was used as a test plant and then the aphids were left on healthy plants for 30 to 60 minutes, which was about as long as any remained infective. All experiments in which beans were used as

test plants were done before the experiments in which peas were used as test plants. The advantages of the single-probe method over the conventional method of leaving aphids on test plants for their entire infective period were discussed by Swenson and Welton (1966). In all experiments, only one aphid was transferred to each test plant. Pea plants had two or three expanded leaves when inoculated. Bean plants were inoculated when the primary leaves had expanded but before the first trifoliate leaf appeared.

Test plants were grown in soil, Perlite, or washed sand. The soil was a mixture of three parts of silt loam to one part of peat moss. Commercial fertilizers were used for supplemental nitrogen, phosphorus, potassium, and lime, and they were thoroughly mixed with the soil in a mechanical mixer. Nitrogen was added in the form of ammonium nitrate, phosphorus as phosphoric acid (superphosphate), potassium as potassium monoxide (potash), and calcium as calcium carbonate. Perlite is an "exploded" mineral particle of volcanic origin. It contains an abundance of cationic minerals, such as potassium, calcium, and magnesium, but elements which occur as anions, such as nitrogen, phosphorus, and sulfur, are present in minute amounts or entirely lacking.

Plants were exposed to different environmental treatments in controlled environmental chambers. This was usually for two days immediately before and after inoculation. A two-day exposure to different light and temperature conditions was used because this period has been used in many studies of environmental effects on plant susceptibility to virus infection by mechanical inoculation (Kassanis, 1957). The daily light period was 16 hours in these chambers and light intensity about 1,100 foot-candles (fc), except when light was an experimental variable. Light was provided in the chambers by incandescent lamps and by Sylvania high-intensity Gro-Lux lamps which have an emission spectrum closer to that of the chlorophyll action spectrum than do other types of artificial light. The light period was from 6 a.m. to 10 p.m. Inoculation of plants in experiments began at about 8:30 a.m. and was usually completed by 11:30 a.m. Plants were grown in the greenhouse until they were placed in the controlled environment chambers. Natural light in the greenhouse was supplemented by Sylvania high-intensity Gro-Lux lamps so that the daily light period was always 16 hours. An attempt was made to maintain greenhouse temperature at 24°C, but the temperature was sometimes as high as 31°C for a few hours during the day in the summertime and as low as 17°C at night.

A factorial design was used in some experiments, and a simple randomized block design was used in others. Replicates were inoculated in a chronological sequence, and treatments were inoculated in a randomized order within replicates.

RESULTS

Temperature Experiments

Pre- and post-inoculation temperature. Pea plants were grown in Perlite with no supplemental nutrition until two days before inoculation. Plants received different combinations of nutritional treatments (Hoagland's solution or water only) and pre- and post-inoculation temperatures (15° or 30°C) for two days before and after inoculation, as indicated in Table 1. Beginning on the third day after inoculation, all test plants received Hoagland's solution daily until they were discarded. Factorial analysis showed that the difference in transmission between pre-inoculation temperatures was highly significant ($P<0.001$) and so was the difference in transmission between post-inoculation temperatures ($P<0.001$). The effect of post-inoculation treatment is obvious from Table 1, where almost invariably more plants were infected in treatments receiving 30°C post-inoculation temperature than in comparable treatments receiving 15°C post-inoculation temperature. Similarly, almost invariably more plants were infected in treatments receiving 15°C pre-inoculation temperature than in comparable treatments receiving 30°C pre-inoculation temperature. The only exception was treatment number nine in which more plants were infected than expected. There were no significant interactions and the effects of pre- and post-inoculation temperatures were additive. Addition of Hoagland's solution had little effect on the results.

In another experiment, plants were grown in soil and in washed sand and exposed to the same treatments as in the preceding experiment. The plants grown in soil were provided with an optimal nutritional level, whereas those grown in sand received nothing but tap water until two days after inoculation when irrigation with Hoagland's solution was begun. Peas will, however, grow reasonably well for two or three weeks in sand and water. Treatments and results are presented in Table 2. The only significant treatment effect was due to post-inoculation temperature ($P<0.001$). In a similar experiment, the effects of the same pre- and post-inoculation temperature combinations on plants grown in Perlite and soil were compared (Table 3). Once more, the only significant treatment difference was due to post-inoculation temperature ($P<0.001$).

Although Welton and others (1964) concluded that pre-inoculation temperature effect was not dependent on post-inoculation temperature, this point was checked once more. Pea plants grown in sand were exposed to pre-inoculation temperatures of 15 and 30°C and post-inoculation temperatures of 15, 24, and 30°C. Two BYMV isolates were used and were designated viruses 1 and 2. Results are pre-

Table 1. Effect of Pre- and Post-inoculation Nutrition and Pre- and Post-inoculation Temperature on Susceptibility of Pea Plants to Bean Yellow Mosaic Virus by Aphid Transmission

Treatment number	Nutrition		Temperature (°C)		Plants infected[1]
	Two days before inoculation	Two days after inoculation	Two days before inoculation	Two days after inoculation	
1	Hoagland's	Hoagland's	15	15	10
2	Hoagland's	Hoagland's	15	30	14
3	Hoagland's	Hoagland's	30	15	2
4	Hoagland's	Hoagland's	30	30	7
5	Hoagland's	Water only	15	15	10
6	Hoagland's	Water only	15	30	14
7	Hoagland's	Water only	30	15	4
8	Hoagland's	Water only	30	30	8
9	Water only	Hoagland's	15	15	10
10	Water only	Hoagland's	15	30	10
11	Water only	Hoagland's	30	15	2
12	Water only	Hoagland's	30	30	12
13	Water only	Water only	15	15	4
14	Water only	Water only	15	30	19
15	Water only	Water only	30	15	4
16	Water only	Water only	30	30	10

Summary of Main Factors

Main factor	Plants infected[2]	
Pre-inoculation nutrition		
Hoagland's sol.	69	NS
Water only	71	
Post-inoculation nutrition		
Hoagland's sol.	67	NS
Water only	73	
Pre-inoculation temperature		
15°C	91	$P<0.001$
30°C	49	
Post-inoculation temperature		
15°C	46	$P<0.001$
30°C	94	

Summary of Pre- and Post-inoculation Temperature Combinations

Pre-temperature	Post-temperature	Plants infected[3]
15°C	15°C	34
15°C	30°C	57
30°C	15°C	12
30°C	30°C	37

[1] Out of 36 inoculated.
[2] Out of 288 inoculated.
[3] Out of 144 inoculated.

Table 2. Effect of Different Pre- and Post-inoculation Temperature Treatments on Susceptibility of Pea Plants Grown in Soil and in Sand to Bean Yellow Mosaic Virus by Aphid Transmission

Treatment number	Growing medium	Temperature (°C) Two days before inoculation	Two days after inoculation	Plants infected[1]
1	Soil	15	15	6
2	Soil	15	30	12
3	Soil	30	15	2
4	Soil	30	30	11
5	Sand	15	15	4
6	Sand	15	30	10
7	Sand	30	15	4
8	Sand	30	30	5

Summary of Main Factors

Main factor	Plants infected[2]	Significance
Growing medium		NS
Soil	61	
Sand	46	
Pre-inoculation temperature		NS
15°C	60	
30°C	47	
Post-inoculation temperature		$P<0.001$
15°C	31	
30°C	76	

[1] Of 120 inoculated.
[2] Of 480 inoculated.

Table 3. Effect of Pre- and Post-inoculation Temperature on Susceptibility of Pea Plants Grown in Soil and Perlite to Bean Yellow Mosaic Virus by Aphid Transmission

Treatment number	Growth medium	Temperature (°C) Two days before inoculation	Two days after inoculation	Plants infected[1]
1	Soil	15	15	5
2	Soil	15	30	11
3	Soil	30	15	4
4	Soil	30	30	13
5	Perlite	15	15	1
6	Perlite	15	30	14
7	Perlite	30	15	3
8	Perlite	30	30	11

Summary of Main Factors

Main factor	Plants infected[2]	Significance
Growth medium		NS
Soil	33	
Perlite	29	
Pre-inoculation temperature		NS
15°C	31	
30°C	31	
Post-inoculation temperature		P<0.01
15°C	13	
30°C	49	

[1] Of 60 inoculated.
[2] Of 240 inoculated.

sented in Table 4. The two viruses were transmitted with almost equal frequencies and there were no significant interactions among viruses and temperatures. There were significant differences between pre- and post-inoculation temperature treatments, $P<0.05$ and $P<0.01$, respectively. A total of 120 plants were inoculated for each pre- and post-inoculation temperature combination, with the following results:

Temperature (°C)		
Pre-inoculation	Post-inoculation	Plants infected
15	15	19
15	24	25
15	30	36
30	15	14
30	24	18
30	30	23

Pre- and post-inoculation temperature effects were additive, indicating again that post-inoculation temperature did not determine the pre-inoculation temperature response. Combination of the pre-inoculation temperature, 15° C, and the post-inoculation temperature, 30° C, most favorable for transmission resulted in the greatest number of infected plants. Combination of the least favorable temperatures, 30° C before inoculation and 15°C after inoculation, resulted in the fewest infected plants.

In the foregoing experiments, environment of the plants was always the same for two days before and after inoculation. Therefore, any other environmental influences on pre-inoculation temperature response would have to have occurred when plants were in the greenhouse before or after this four-day period. Light intensity and quality

Table 4. Effect of Pre- and Post-inoculation Temperature on Susceptibility of Pea Plants Grown in Sand to Two Bean Yellow Mosaic Virus Isolates by Aphid Transmission

Treatment number	Virus	Temperature (°C) Two days before inoculation	Two days after inoculation	Plants infected[1]
1	1	15	15	9
2	1	15	24	13
3	1	15	30	19
4	2	15	15	10
5	2	15	24	12
6	2	15	30	17
7	1	30	15	5
8	1	30	24	11
9	1	30	30	12
10	2	30	15	9
11	2	30	24	7
12	2	30	30	11

Summary of Main Factors

Main factor	Plants infected	
Virus		NS
1	69[2]	
2	66	
Pre-inoculation temperature		$P<0.05$
15°C	80[2]	
30°C	55	
Post-inoculation temperature		$P<0.01$
15°C	33[3]	
24°C	43	
30°C	59	

[1] Out of 60 inoculated.
[2] Out of 360 inoculated.
[3] Out of 240 inoculated.

in the greenhouse changed during the year, but a 16-hour photoperiod was always provided by artificial lighting. Temperature in the greenhouse was a few degrees higher during mid-day in summer than in winter. Another difference was that plants might have varied in age, although this could not have been great because they were always used at approximately the same stage of growth.

Plant age. An experiment was designed to determine the possibility of pre-conditioning plants during their early growth period to the effects of pre-inoculation temperature during the two days immediately before inoculation. Two groups of peas were planted so that they would be 11 and 13 days old at inoculation time. At the time of plant-

Table 5. Effect of Pre- and Post-inoculation Temperature and Plant Age on Susceptibility of Pea Plants to Inoculation With Bean Yellow Mosaic Virus by Aphid Transmission

Treatment number	Initial temperature	Plant age[1]	Temperature (°C) Two days before inoculation	Two days after inoculation	Plants infected[3]
1	Constant (24°C)	13 days	15	15	6
2			15	30	9
3			30	15	3
4			30	30	11
5		11 days	15	15	3
6			15	30	13
7			30	15	6
8			30	30	11
9	Alternating[2]	13 days	15	15	10
10			15	30	7
11			30	15	5
12			30	30	8
13		11 days	15	15	6
14			15	30	13
15			30	15	1
16			30	30	12

Summary of Main Factors

Main factor	Plants infected[4]	Significance
Initial temperature		NS
Constant	62	
Alternating	62	
Plant age		NS
13 days	59	
11 days	65	
Pre-inoculation temperature		NS
15°C	67	
30°C	57	
Post-inoculation temperature		$P<0.001$
15°C	40	
30°C	84	

[1] From planting time until two days before inoculation.
[2] Day 24°C, night 15°C.
[3] Of 66 inoculated.
[4] Of 228 inoculated.

ing, half of each group of plants was placed in a controlled environment chamber at a constant 24°C, and the other half was placed in a chamber with a day temperature of 24°C and a night temperature of 15°C. Two days before inoculation, half of the plants from each chamber were transferred to a constant 15°C chamber and half to a constant 30°C chamber. After inoculation, the plants were further

divided into two groups kept at 15 and 30°C for two days. Thus, there were 16 different treatments (Table 5). All plants were kept at 24°C in controlled environment chambers on the third and fourth days after inoculation and then returned to the greenhouse. Some of the plants showed symptoms of virus infection three days later.

The results are presented in Table 5. Factorial analysis showed that of the main factors, temperature during the early growth period, plant age, pre-inoculation temperature, and post-inoculation temperature, only post-inoculation temperature had a significant effect on the number of plants infected ($P<0.001$). Of the interactions, only one was significant, the interaction between plant age and post-inoculation temperature ($P<0.05$). The difference between post-inoculation temperature effects was less for the plants that were 13 days old than for those that were 11 days old, as shown below (each figure is the number of plants infected out of 264 inoculated).

Plant age	11 days		13 days	
Temperature (°C)	15°	30°	15°	30°
Plants infected	16	49	24	35

The 13-day-old plants were, in general, somewhat larger than the plants used in previous temperature experiments.

The effects of different combinations of pre- and post-inoculation temperatures and plant age were compared in another experiment. Pre- and post-inoculation temperatures of 15 and 30°C were used and plant ages of 9, 13, and 17 days. The results are presented in Table 6. There was a significant post-inoculation temperature effect ($P<0.01$) but no significant difference due to pre-inoculation temperature and plant age as main factors. Interaction between post-inoculation temperature and plant age was again significant ($P<0.05$), as shown in the following tabulation (each figure is the number infected out of 40 inoculated):

	Plant age		
Post-inoculation temperature	17 days	13 days	9 days
15°C	4	8	2
30°C	11	10	14

There was no direct relationship between temperature effect and plant age. These results might be more consistent if age of the inoculated leaf, rather than age of the whole plant, determined susceptibility. Aphids were usually placed on the youngest leaf of test

Table 6. Effect of Pre- and Post-inoculation Temperature and Plant Age on Susceptibility of Pea Plants to Infection With Bean Yellow Mosaic Virus by Aphid Transmission

Treatment number	Temperature (°C) Two days before inoculation	Two days after inoculation	Plant age (Days)	Plants infected[1]
1	15	15	17	1
2	15	15	13	4
3	15	15	9	0
4	15	30	17	6
5	15	30	13	5
6	15	30	9	7
7	30	15	17	3
8	30	15	13	4
9	30	15	9	2
10	30	30	17	5
11	30	30	13	3
12	30	30	9	7

Summary of Main Factors

Main factor	Plants infected	Significance
Pre-inoculation temperature		
15°C	23[2]	NS
30°C	24	
Post-inoculation temperature		
15°C	14[2]	$P<0.01$
30°C	33	
Plant age		
17 days	15[3]	NS
13 days	16	
9 days	16	

[1] Of 20 inoculated.
[2] Of 120 inoculated.
[3] Of 80 inoculated.

plants. The youngest leaves of a group of plants of the same age would always be in about the same stage of growth, but the stage of growth of the youngest leaves might vary among plants of different ages.

In another experiment, infective aphids were allowed to probe on the most recent fully expanded leaf of healthy pea plants or on a leaf which was still unfolding and expanding. The plants were then kept at 15 or 30°C for two days. The following results were obtained (each figure is the number of plants infected out of 264 inoculated):

Post-inoculation temperature	Leaf age	
	Growing	Mature
15°C	11	25
30°C	29	30

The difference between post-inoculation temperatures was significant ($P<0.001$). Actively growing leaves were significantly less susceptible than mature leaves ($P<0.05$), and there was an interaction between temperature and leaf age ($P<0.05$). Infection of actively growing leaves was increased to a greater extent by the higher temperature than was infection of mature leaves.

Temperature during inoculation. The possibility existed that temperature at the time of inoculation might affect the response to pre-inoculation temperature treatments. Plants were frequently grown three to a pot. Each pot of plants was removed from the controlled environment chamber after the pre-inoculation treatment and inoculated. Inoculation of the three plants required 5 to 15 minutes, depending on aphid behavior. These inoculations were done in a room where temperature could not be closely controlled.

An experiment was designed to determine the effect of temperature during the inoculation period. Pea plants were grown in the greenhouse and placed in a controlled environment chamber at 15° C two days before inoculation. The plants were later divided among five temperatures for 10 minutes immediately preceding inoculation: 15°, 21°, 24°, 27°, or 30° C. This 10-minute period simulated the inoculation period in previous experiments when plants were exposed to uncontrolled temperatures, although this range of temperatures was considerably greater than was ordinarily encountered during inoculation.

It was expected that, if response to pre-inoculation temperature had depended on temperature during inoculation in previous experiments, the 10-minute exposures to different temperatures would result in different numbers of infected plants at some points in this range of temperatures. Only one plant was grown in each pot, and aphids were fed on diseased plants before the test plant was removed from the growth chamber for inoculation. Thus each test plant was removed from the controlled environment chamber only long enough for one aphid to make a single probe of 10 to 60 seconds duration. About 95 percent of the aphids probed immediately on the healthy plant so that the time required to remove a plant from the controlled environment chamber, inoculate it, and return it to the chamber varied from 30 to 90 seconds for these aphids. An additional 60 to 90 seconds was re-

quired for the remaining small proportion of the aphids. Plants in this experiment were 14 days old at the time of inoculation. The following results were obtained:

Pre-inoculation temperature (C°)	Plants infected
15°	9
21°	13
24°	9
27°	9
30°	9
Total	48

Each figure in the tabulation above represents the number of plants infected out of 96 inoculated for each temperature. The differences among the various temperatures were not significant.

Light Treatments

Pea plants were kept at either 15 or 30°C for two days immediately before and after inoculation, as in preceding experiments. During the two days before inoculation, half the plants at each pre-inoculation temperature were kept under 12-hour photoperiods and half under 16-hour photoperiods. Light intensity under 12-hour photoperiods was approximately 500 fc and that under 16-hour photoperiods was approximately 1,000 fc. All plants were kept under 16-hour photoperiods during post-inoculation temperature treatments. The eight light-temperature treatments and the results are presented in Table 7. Light treatments had no effect on the response to temperature. The only significant treatment effect was due to post-inoculation temperature ($P<0.01$).

Bean plants were kept at 24°C for two days before inoculation under 16-hour photoperiods with a light intensity of 1,100 fc, under 16-hour photoperiods with a light intensity of 550 fc, or in complete darkness. Half of each group was placed at 24°C and half at 30°C for two days after inoculation. The six light-temperature treatments and the results are given in Table 8. The only significant treatment difference was due to post-inoculation temperature ($P<0.05$).

The same light treatments were applied as post-inoculation treatments at 24°C in another experiment, with the following results:

16-hour photoperiod (1,100 fc) ..... 18 of 240 plants infected
16-hour photoperiod (550 fc) ..... 28 of 240 plants infected
Dark ..... 26 of 240 plants infected

These differences were not significant.

Table 7. Effect of Different Combinations of Light and Temperature Treatments on Number of Pea Plants Infected With Bean Yellow Mosaic Virus by Aphid Transmission

Light	Temperature (°C)		
Two days before inoculation	Two days before inoculation	Two days after inoculation	Plants infected[1]
12 hr.—500 f.c.	15	15	2
12 hr.—500 f.c.	15	30	6
12 hr.—500 f.c.	30	15	2
12 hr.—500 f.c.	30	30	8
16 hr.—1,000 f.c.	15	15	2
16 hr.—1,000 f.c.	15	30	3
16 hr.—1,000 f.c.	30	15	3
16 hr.—1,000 f.c.	30	30	9

Summary of Main Factors

Main factor	Plants infected[2]	Significance
Light		NS
12 hr.—500 f.c.	18	
16 hr.—1,000 f.c.	17	
Pre-inoculation temperature		NS
15°C	13	
30°C	22	
Post-inoculation temperature		$P<0.01$
15°C	9	
30°C	26	

[1] Of 30 inoculated.
[2] Of 120 inoculated.

Table 8. Effect of Different Light Post-inoculation Temperature Treatments on the Number of Bean Plants Infected With Bean Yellow Mosaic Virus by Aphid Transmission

Light[1]	Temperature (°C)[1]	Plants infected[2]
16 hr.—1,100 f.c.	24	6
16 hr.—1,100 f.c.	30	15
16 hr.— 550 f.c.	24	7
16 hr.— 550 f.c.	30	11
Dark	24	7
Dark	30	6

[1] For two days before inoculation.
[2] Out of 90 inoculated.

Pea plants were grown in the greenhouse in sand and in soil and transferred to controlled environment chambers at 15°C two days before inoculation. Half of the plants in each growth medium were kept under a 16-hour photoperiod at about 1,100 fc and half under a 16-hour photoperiod at about 550 fc. The plants were placed in the greenhouse after inoculation. There were no significant differences among the four treatments in the number of plants infected. The following results were obtained:

Growth medium	Light intensity	Plants infected
Soil	1,100 fc	6 of 60
Soil	550 fc	9 of 60
Sand	1,100 fc	10 of 60
Sand	550 fc	9 of 60

Pre- and post-inoculation light treatments were combined in another experiment. Pea plants were grown in the greenhouse and transferred to controlled environment chambers two days before inoculation. During these two days, they were kept in either continuous light or in continuous dark. Half of the plants in each group were transferred to continuous light for two days immediately after inoculation, and the other half were transferred to continuous dark for this period. There were no significant differences among these light treatments in the number of plants infected. The following results were obtained:

Pre-inoculation	Post-inoculation	Plants infected
Light	Light	12 of 60
Light	Dark	11 of 60
Dark	Light	7 of 60
Dark	Dark	10 of 60

Bean Nutrition

General. Swenson and Sohi (1961) stated that the susceptibility of bean plants to infection with BYMV by aphid transmission was increased by raising the general fertility level of the soil in which the plants were grown, but no data were presented. In two experiments made in the greenhouse in October and November, fertilizers were added to soil to give a low and a high nutritional level. Actually, the amount of major elements present in both the low and the high treatment was above that required for good plant growth, although these particular treatments resulted in somewhat acid soils. Soil analyses indicated the following amounts of available major elements:

	P	*K*	*Ca*	*N*	*pH*
Low (Expt. 1)	32.5 ppm	0.81 me	10.2 me	0.110%	4.95
Low (Expt. 2)	28.5 ppm	0.75 me	9.7 me	0.108%	4.98
High	48.9 ppm	1.18 me	10.2 me	0.188%	4.70

Analysis for the high nutrient level was the same for both experiments since soil from the same lot was used for both experiments. Inoculation of bean plants used in all plant nutrition experiments was by the conventional method of leaving each aphid on one test plant during its entire infective period. In the first experiment, 30 of 114 bean plants grown at the low nutrient level were infected, compared to 45 of 114 plants infected at the high nutrient level ($P>0.05$). In the second experiment, 31 of 72 plants were infected at the low nutrient level and 47 of 72 plants were infected at the high nutrient level ($P<0.001$). No differences in susceptibility of bean plants were detected in two subsequent experiments (in January and May) using the same nutritional levels. Nutrient utilization may, however, have depended upon temperature and light conditions, as well as amounts of nutrients available in the soil, so that these experiments are not necessarily contradictory.

Phosphorus. The effect of four levels of phosphorus on plant susceptibility was tested in two experiments. The amount of phosphorus in the soil varied from 11.4 to 12.5 ppm to 206 to 246 ppm, thus including a range from deficiency to a great excess of phosphorus. The amounts of other elements were somewhat low but quite constant: potassium varied from 0.23 to 0.26 me among the treatments in the two experiments, calcium from 9.2 to 10.0 me, and nitrogen from 0.083 to 0.112 percent. One experiment was made in September, the other in December. Fifty plants were grown at each of the four different phosphorus levels in each experiment and inoculated with BYMV by aphid transmission. There were no significant differences in the numbers of plants infected. In another experiment, plants were grown in two lots of soil containing the following amounts of nutrients:

	P	*K*	*Ca*	*Mg*	*N*	*pH*
1	25 ppm	0.44 me	13.7 me	5.08 me	0.090%	6.8
2	70 ppm	0.32 me	11.4 me	5.10 me	0.088%	6.6

These two soil mixtures differed relatively little except in the amounts of phosphorus. They differed markedly from the soil mixtures in the first two experiments on phosphorus effects in that the amounts of potassium and calcium were greater and the pH was higher. Sixty

plants were grown in each mixture and inoculated in April. Twenty-three plants were infected in each group. Thus the amount of phosphorus in the soil had no effect on plant susceptibility even though plants were grown under a wide range of conditions insofar as light, soil pH, and the amount of other major elements were concerned.

Nitrogen. Bean plants were grown in soil containing 0.06 percent nitrogen and 0.130 percent nitrogen. Amounts of other major nutrient elements were essentially the same in the two treatments: pH 6.8, in each case; phosphorus 27.4 and 20.7 ppm, respectively; potassium, 0.44 and 0.46 me, respectively; and calcium, 13.8 and 14.1 me, respectively. Twenty-one of 80 plants in each treatment were infected with BYMV by aphid transmission. These plants were grown and inoculated in October. In another experiment, nitrogen was added to soil to give mixtures containing 0.090 and 0.134 percent nitrogen. Amounts of other major elements present were essentially the same as in the previous experiment. One hundred ninety-two plants were grown in each soil mixture and inoculated with BYMV by aphid transmission in May and June. There was no significant difference in the number of plants infected.

Pea Nutrition

A similar series of experiments concerning the relationships of the general nutritional level and the amounts of nitrogen and phosphorus, in particular, to susceptibility of pea plants to infection with BYMV by aphid transmission was made by Welton (1963). In no case did he find that the amounts of major elements available in the soil had any effect on plant susceptibility. In addition, the experiments described earlier in this bulletin wherein pea plants were grown in soil, sand, and Perlite, with and without Hoagland's solution, gave no indication that plant nutrition had any significant effect on susceptibility to BYMV by aphid transmission.

Water Supply

Bean plants were grown at two levels of soil moisture, field capacity and twice field capacity. Twenty-nine of 90 plants grown at field capacity were infected, compared to 33 of 90 plants grown at twice field capacity. In another experiment (Welton 1963), one group of pea plants was grown with an ample water supply and another group with soil moisture so low that the plants were stunted and the leaves bluish in color. The second group developed normal foliage when an adequate water supply was provided after inoculation. Forty-one of 75 plants in this group were infected compared to 43 of 75 plants in the group which received an ample water supply.

DISCUSSION

Perhaps the principal value of these data is to help place in proper perspective some of the notions concerning plant susceptibility to virus infection. Most of the information has been obtained from experiments wherein plants were inoculated mechanically, and results do not agree at all with the relatively few data obtained from experiments on aphid transmission (Swenson, 1969). In general, high temperature and low light intensity before inoculation, high nutritional level, and ample water enhance plant susceptibility to virus infection by mechanical inoculation. Susceptibility to BYMV of leaves of *Chenopodium amaranticolor* decreased with increasing age on the same plant (Swenson *et al.*, 1964). In the experiments described in this bulletin, post-inoculation temperature was the only factor which consistently affected plant susceptibility. Light, nutrition, and water supply had little or no effect. No simple relationship was found between leaf age and plant susceptibility. These preliminary data relating to plant nutrition and water supply indicate that agricultural practices such as fertilization and irrigation are likely to have little direct effect on plant susceptibility to BYMV infection. They might, however, have an indirect effect by enabling plants to support larger aphid populations or by keeping them in a physiological condition susceptible to virus infection for a longer time.

The single-probe method of inoculating plants by aphids was used in most experiments described herein because it is a more sensitive method of estimating plant susceptibility than the conventional method of leaving aphids on a test plant during their entire infective period. Because most previous work has been done by the conventional method and because transmission frequency was often low in the experiments described herein, a conversion from single-probe to conventional data is given in Table 9.

The failure to show the specific conditions in which a response to pre-inoculation temperature would be obtained probably reflects a change in the virus culture used rather than failure to identify the necessary conditions. The virus culture used herein was the one used by Welton and others (1964). All other materials, methods, and facilities were the same. It is unlikely that all the experiments in which a response to pre-inoculation temperature occurred (Swenson and Sohi, 1961; Welton *et al.*, 1964; and some herein) could have included some difference in conditions or method that did not occur in the varied experiments where a pre-inoculation temperature effect was not obtained. In the laboratory, BYMV strains have lost their aphid transmissibility (Swenson, 1957; Swenson, *et al.*, 1964) and ability to infect beans (Kamm, 1967). Thus, a change in temperature response is not im-

Table 9. Comparison of Aphid Transmission of Bean Yellow Mosaic Virus to Pea Plants by Single-probe (S-P) and Conventional (C) Methods (from Swenson and Welton, 1966)

Transmission by S-P	Estimated transmission by C
%	%
5	16.5
10	29.4
15	39.9
20	48.8
25	55.6
30	61.7
35	66.9
40	71.5
45	75.5
50	79.0

probable. The fact that a pre-inoculation temperature response was obtained in some experiments conducted after those experiments in which it did not occur may indicate a transitional stage in which both types of virions were present in the stock culture. Virus source plants for experiments were always prepared by aphid transfers from the virus stock culture. These source plants were discarded after use in experiments and played no part in the maintenance of the stock culture.

REFERENCES CITED

Bradley, R. H. E. 1964. Aphid transmission of stylet-borne viruses. *In* M. K. Corbett and H. D. Sisler (eds.), *Plant Virology,* pp. 148-174. Gainesville: Univ. of Florida.

Bradley, R. H. E. 1966. Which of an aphid's stylets carry transmissible virus? Virology, *29:*396-401.

Kamm, J. A. 1967. Transmission variation and loss of aphid transmissibility of bean yellow mosaic virus. Ph.D. dissertation, Oregon State University, 87 pp.

Kassanis, B. 1957. Effects of changing temperature on plant virus diseases. Advan. Virus. Res., *4:*221-241.

Swenson, K. G. 1952. Aphid transmission of a strain of alfalfa mosaic virus. Phytopathology, *42:*261-262.

Swenson, K. G. 1957. Transmission of bean yellow mosaic virus by aphids. Jour. Econ. Entomol., *50:*727-731.

Swenson, K. G. 1969. Plant susceptibility to insect-transmited viruses. *In* K. Maramorosch (ed.), *Viruses, Vectors, and Vegetation.* New York: Interscience (in press).

Swenson, K. G., and S. S. Sohi. 1961. Factors determining the rate of bean yellow mosaic virus transmission by the aphid *Myzus persicae* (Abstract). Phytopathology, *51:*67.

Swenson, K. G., S. S. Sohi, and R. E. Welton. 1964. Loss of transmissibility by aphids of bean yellow mosaic virus. Ann. Entomol. Soc. Amer., *57:*378-382.

Swenson, K. G., and R. E. Welton. 1966. Evaluation of plant susceptibility to infection with bean yellow mosaic virus by aphid transmission. Phytopathology, *56:*269-271.

Welton, R. E. 1963. Susceptibility of peas to aphid inoculation with bean yellow mosaic virus. Ph.D. dissertation, Oregon State University, 130 pp.

Welton, R. E., K. G. Swenson, and S. S. Sohi. 1964. Effect of temperature on plant susceptibility to infection with bean yellow mosaic virus by aphid transmission. Virology, *23:*504-510.