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Title: THE INHERITANCE AND NATURE OF A SYMPTOMLESS INFECTION BY A WILLAMETTE STRAIN OF PEA STREAK VIRUS IN PISUM SATIVUM L.

Abstract approved: James R. Baggett

Pea streak virus (Marmor trifolii Holmes) is widespread and occasionally destructive in Eastern Oregon and Washington, and in many other pea growing areas. Since control by the use of insecticides to kill the insect vector is only partially successful, resistant varieties of peas are needed.

In a study to confirm the mode of inheritance of resistance to a strain of pea streak virus (PSV), resultant data suggested that resistance is governed by a single recessive gene. Resistant plants were infected and supported a substantial virus concentration, but were symptomless. The virus concentration in resistant plants, as determined by local-lesion assay in Chenopodium amaranticolor, approximated that of plants showing severe symptoms.

The effects of various environmental factors on symptom expression by several types of peas were studied to facilitate studies

and breeding. The symptom expression was affected by seasonal change, soil fertility, and plant growth conditions. Plant height was reduced by PSV infection in susceptible varieties but not in the symptomless or resistant type of plants. Symptom expression was more pronounced when plants were grown in soils which did not favor vigorous growth. When growth was reduced by chemical retardants or excess fertilizer, symptoms were more pronounced.

# The Inheritance and Nature of a Symptomless Infection by a Willamette Strain of Pea Streak Virus in *Pisum sativum* L.

by

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# THE INHERITANCE AND NATURE OF A SYMPTOMLESS INFECTION BY A WILLAMETTE STRAIN OF PEA STREAK VIRUS IN PISUM SATIVUM L.

## INTRODUCTION

Pea streak (Marmor trifolii Holmes), first noted by Linford in the United States in 1928, occurs in many pea production areas of the world. According to Hampton and Ford (18) pea streak, was the most conspicuous disease of peas in Eastern Washington and Eastern Oregon. It also frequently occurs in Southern Idaho (44). It has sometimes been the cause of considerable yield reduction. In 1957, for instance, it was reported to be the most serious virus disease of peas ever noted in the Southern Idaho area. According to Schroeder et al. (33), "pea streaks" occur naturally in New York state, where, in 1958, outbreaks of severe "streak" occurred in a number of commercial pea fields.

Resistance to pea streak virus (PSV) is being sought after by breeders. Such resistance would insure against the destructive potential of this important disease. Immunity has not been found, though excellent field resistance is observed in pea varieties and lines which are susceptible in greenhouse tests. Some partial resistance has also been found in greenhouse tests in the pea breeding program being conducted at Oregon State University.

A form of PSV resistance was found in 1965 in which plants were susceptible to infection but developed no symptoms. This characteristic for infection latency was found to be conditioned by a single recessive gene. This was determined by crossing two susceptible varieties, 'Geneva 41' and 'Midfreezer', to each of several resistant OSU breeding lines, and testing the $F_1$, $F_2$, and backcross generations. In all crosses with 'Geneva 41' as susceptible parent, the $F_1$ plants and their reciprocals were completely susceptible to OSU 331 strain of PSV. $F_2$ populations from these crosses in most cases satisfactorily fit 3 susceptible to 1 resistant (3:1) ratios and backcross data confirmed the $F_2$ results. However, these same populations failed at certain times to fit 3:1 ratios, having far too many symtomless plants (either "escapes" or plants with latent infection). Also, populations from crosses with 'Midfreezer' as susceptible parent, consistently failed to fit the 3 susceptible to 1 resistant expected ratios.

In the course of this genetic study incomplete symptom expression among susceptible types was a frequent problem. Meaningful genetic ratios were therefore precluded particularly in certain crosses. It was apparent that a better understanding of conditions affecting symptom expression, and the relationship between symptom occurrence and virus titer, was needed.

Because of the importance of the disease and observed

deviations in the genetic studies of previous work, the following objectives were undertaken in this study:

1. Elucidate previously obtained $F_2$ ratios by means of additional $F_2$ populations and $F_3$ family tests selected from single $F_2$ plants.
2. Characterize the symptom expression of a group of pea cultivars as to:
   a) Occurrence and severity of symptoms.
   b) Presence or absence of virus particles in inoculated plants.
   c) Relation of symptom type to virus concentration in the plant.
3. Relate symptom expression to environmental factors and attempt to influence symptom expression by changes in environment or growth of the pea plant.

Figure 1. Resistant Parent B114-36 and Susceptible Parent 'Geneva 41' Inoculated with PSV-331.

# REVIEW OF LITERATURE

Several aspects of pea virus disease work are related to studies of inheritance and symptom expression of pea streak virus. The following review therefore includes literature concerning the causal viruses and symptomology of the pea streak disease, resistance to pea virus diseases in general, and factors affecting symptom expression of virus diseases.

## Viruses Induce Streak Diseases in Pisum sativum L.

Linford, in 1928, first reported a streak disease of pea in the United States (22). Similar diseases have since been found in several other parts of the world, but no description of a causal agent was given until 1938 when Zaumeyer (42) discussed the relationship of pea streak to several strains of alfalfa mosaic virus. Zaumeyer named this virus pea streak virus I. The following year another pea streak virus, Pisum virus 3, was described by Chamberlain (8) in New Zealand. Cucumber mosaic virus was reported on peas by Zaumeyer (43). Hagedorn and Walker in 1949 (16) designated the causal virus of a Wisconsin pea diseases was Wisconsin pea streak virus. Schroeder et al. (33) demonstrated that a streak of peas in New York state was incited by combinations of specific viruses that occur in nature, such as red clover vein mosaic virus with either strains of

bean yellow mosaic virus 2 or Pisum virus 2. Zaumeyer et al. isolated a red clover vein mosaic virus strain (46) and a strain of red clover necrosis virus (45) both of which produced streak symptoms in peas. Kim and Hagedorn (21) compared the host ranges, physical properties and particle characteristics of streak-inducing viruses from New York, Wisconsin, Minnesota and Idaho. Results indicated that the four isolates represented three separate and distinct streak-inducing viruses. Hampton and Ford (18) noted that the pea streak virus in Washington and Oregon was related to the Wisconsin and Idaho isolates reported by Kim and Hagedorn (21). Osborn (25) first described red clover vein mosaic virus in 1937. This virus is identical to that described by Hagedorn and Walker (17) and Hagedorn and Hanson (15) as causing "pea stunt." Wetter, Quantz, and Brandes (38) studied the relationship of pea streak virus and red clover vein mosaic virus and found no differential hosts that would allow their separation. There were, however, differences in symptomatology, particulary on peas and bellbeans. These viruses could be differentiated by electron microscopic measurements of their particles. The normal lengths of pea streak virus particles were 619 mμ and those of red clover vein mosaic virus 645 mμ. According to Zaumeyer et al. (46) serological studies and a comparison of particle size of these virus show that they are related.

The pea streak viruses described in the U.S.A. are closely

similar in the following highly significant points (23):

1. Almost identical host ranges and symptomology.
2. Inability to infect Phaseolus vulgaris.
3. Ability to cause symptoms in diverse varieties of peas.
4. Very similar morphology indicated by electron micrographs of those that have been studied.

## Symptomology

The most obvious symptom of the pea streaks is necrosis of the stem and petioles (34, p. 324). Zaumeyer (44) reported that under field conditions the most conspicuous symptoms caused by the Idaho strain of pea streak virus are purple to brown necrotic streaking of the stems and petioles and a necrotic spotting and malformation of the pods. Stem and petiole streaking are usually noted first near the upper portion of the plant, but stem streaking later may extend from the top to the bottom. The pods that are formed before the plant becomes seriously infected take on a dark purplish-gray or brown color, and frequently do not reach maturity. The initial symptoms are a slight purple streaking of the stems, and often, a clearing of the lower portion of the stipules. The latter recurve and the leaflets curl downward. The veins of the leaves are often cleared. The stem internodes of the upper part of the plant are shortened, and the leaves, which are smaller than normal, are rosetted and slightly

chlorotic but not mottled. The apical growing points usually curl to one side, and the stems and petioles become very brittle. The veinlets of the leaves and stipules may become necrotic, and often necrosis is observed in the interveinal tissues. Infected leaves finally become flaccid, the tip of the plants may wilt, and often the whole plant dies. Some of these symptoms are not found on all infected plants, and many variations are noted, particularly if only some of these symptoms appear.

The symptoms are not so striking in the greenhouse as in the field. Although the first evidence of the disease is a reddish-brown necrotic streaking of the stems and petioles, the most characteristic symptom is wilting of the plants. Discolorations are often noted on the leaves and stipules above the inoculated ones. These leaves wilt and die, and progressive wilting and death of the upper leaves and stipules follow.

Kim and Hagedorn (21) demonstrated that the symptoms caused by the Idaho strain of Wisconsin pea streak virus resemble the original virus described by Hagedorn and Walker (16).

Schroeder et al. (33) reported that with pea streaks incited by combinations of viruses in New York, the most striking symptoms in the field occurred on the pods, and ranged from a brown, roughened and/or pitted condition on filled or partially filled pods to completely flat, purple-brown pods. Streaks of similar color on the

stems and peduncles preceded pod formation and remained as prominent symptoms. Vascular discoloration often accompanied the stem streaking. Erratic symptoms that appeared were a slight mottle, a faint indication of vein clearing, axillary bud proliferation and a yellowing of the terminal foliage, with or without veinal necrosis or necrotic flecking. In the greenhouse, symptoms were usually a faint chlorosis of the leaves, immediately above those inoculated, followed closely by a grayish-tan streaking and slight epinasty of the terminal growth. Sometimes a mild rosetting appeared at the terminal growth. Leaves later became progressively chlorotic with slight veinal necrosis, and ultimately wilted and died.

## Resistance to Pea Viruses

Relatively little has been reported about resistance to pea streak diseases. McWhorter (23) stated that no variety of Pisum sativum L. resistant to pea streak viruses has yet been found. Ford and Baggett (11) in reporting the reactions of Plant Introduction lines of Pisum sativum to several pea viruses, found there were differences in degree of susceptibility. Some Plant Introduction lines were resistant to alfalfa mosaic virus, very few were resistant to pea streak virus (strain p-42), and none were resistant to clover yellow mosaic virus. Considerable information is available concerning inheritance of resistance to other pea viruses. Yen and Fry (41)

showed that resistance to pea mosaic virus was conditioned by the mo mo genotype. Johnson and Hagedorn (19) later indicated that resistance to bean yellow mosaic virus (pea isolate 1) was similarly inherited. Yen and Fry (41) and Johnson and Hagedorn (19) observed that heterozygotes (Mo mo) exhibited a delayed response to inoculation, but the demarcation between Mo mo and Mo Mo was not clear-cut enough to separate them. Cousin (9) in 1965, reported that resistance to virus of common pea mosaic behaved in the $F_2$ and $F_3$ generations as a single recessive character. Schroeder and Barton (28) showed that resistance to pea enation mosaic virus was conditioned by a single dominant gene. This resistance is not in the nature of immunity but rather in the ability of the plant to grow and reproduce normally even though it may be systemically infected with pea enation mosaic virus.

## Environmental Factors Affecting Virus Infection and Symptom Expression

Seasonal changes. Bhargava (7) reported an extreme example of seasonal behavior in Phaseolus vulgaris, vars. 'Prince' and 'Bountiful', when inoculated with cucumber mosaic virus; during the summer the beans are apparently immune, whereas during winter they produce black local lesions in numbers adequate for quantitative assays. Smith and Bald (2, p. 41-42) reported that it is common in

winter for tobacco seedlings to become naturally infected with tobacco necrosis viruses, but rare in summer.

Light intensity. Bawden and Roberts (5) noted that reduced light intensity in summer increased susceptibility of plants to virus. The virus content of plants was higher under reduced light intensity.

Temperature. Kassanis (20) found, with a number of viruses, that keeping plants at 36° C for some time before inoculation increased their susceptibility. Yarwood (40) has investigated heat-induced susceptibility with a number of viruses in beans. Schroeder et al. (31) in 1966 reported that symptom development in heterozygous (Mo mo) plants of Pisum sativum inoculated with certain strains of bean yellow mosaic virus can be manipulated by changing the temperature of the environment. Changes in temperature can affect the severity and type of symptom (2, p. 43).

Host nutrition. Spencer (35) pointed out that the amount of nitrogen supplied to beans and tobacco affected susceptibility to tobacco mosaic virus. Susceptibility was highest when growth was somewhat retarded by excess nitrogen. Moderately high potassium decreased susceptibility without affecting plant growth (36). Bawden and Kassanis (3) found that susceptibility of N. glutinosa to tobacco mosaic virus was decreased by a deficiency or an excess of nitrogen or phosphorus.

Soil conditions. The growth of plants is so profoundly affected

by soil conditions that these almost certainly affect the symptoms of many virus diseases, but these variables have been studied less than light intensity and temperature (2, p. 47).

# MATERIAL AND METHODS

The experiments were conducted in the greenhouse during the fall, winter and spring of 1967 to 1969, and in the field during the summer of 1968.

## General Method of Plant Culture in the Greenhouse

Pea plants were primarily grown in number ten cans in a mixture of field soil (Chehalis Silt Loam) and sand with 8-24-8 commercial fertilizer at a level teaspoon per can. However, experimentation with soil mixes is described later. Seven or eight pea seeds were planted in each can. They were treated with Spergon to reduce seed rot and damping off and covered with a measured amount of sterilized sand. Some host plants for assay and maintenance were grown in other size cans. The Chenopodium plants were grown from seed, then transplanted in loam-peat moss mixture at about two weeks after seeding.

Although the greenhouse room was equipped with heating and cooling equipment, there was still considerable variation in temperature. Night temperature was usually 65° F while day temperature was set at 70° F in most cases. It was not uncommon, however, for the temperature to be 80° F or higher during late spring days. Light intensity also varied greatly in the greenhouse. Because the

Chenopodium species used are short day plants, supplementary light was provided during the winter months to delay flowering. All plants were watered at one or two day intervals depending upon the need. The greenhouse room was fumigated about once a week to keep them insect free.

## Inoculation Methods

Regular inoculations of pea and bellbean. All test pea plants and virus maintenance pea plants were inoculated about 9 or 10 days after planting, when the first and second true leaves were fully expanded. The virus maintenance bellbean plants were inoculated at about two weeks after seeding.

The virus was transmitted mechanically in all experiments, using a modification of the technique described by Rawlins and Tompkins (26). The source plant tissue was ground thoroughly in a mortar and pestle. The juice so expressed was then diluted 1:3 with 0.01 M phosphate buffer ($K_2HPO_3$+$KHPO_3$), and applied to the test plant gently and firmly with a forefinger. Carborundum, used to enhance the success of inoculation, was lightly and evenly dusted on the leaves and plants to be inoculated. After inoculation, the inoculated leaves were rinsed with tap water to remove the excess carborundum and inoculum. Readings were taken two to four weeks after inoculation, depending upon time of symptom appearance.

Inoculation method for local-lesion assay. For finding a suitable local-lesion host for the strain of PSV used, host range tests were made in February, 1968, in the greenhouse. The results obtained are summarized in Table 1.

When assayed on susceptible pea varieties, samples from non-inoculated leaves of the two Chenopodium species and in Phaseolus varieties were found to contain no virus. Vicia faba, Vigna sinensis, Trifolium pratense, and T. hybridum were found to be systemically infected. Both species of Chenopodium developed destinct local lesions, and were thus acceptable for assay purposes.

For assay of virus presence or titer, the Chenopodium plants were large enough for use after three to four weeks from transplanting. However, older plants were sometimes used if the new growth was vigorous. The inoculum in this case was prepared by diluting crude juice to 40:1 with the phosphate buffer. It was applied to the carborundum dusted leaves, with a cotton-tipped swab using uniform pressure and number of strokes. In order to reduce errors due to the effect of leaf position and plant to plant variation, an inoculation scheme was designed, as shown in Table 2. Each of the four random samples from each variety was inoculated separately to four different Chenopodium leaves with different leaf positions. Each variety from the field or greenhouse was therefore tested on 16 different Chenopodium leaves. The average number of lesions per leaf

Table 1. Summary of Host Range Information for OSU 331 Strain of PSV.

| Host | Greenhouse Symptoms | # Inf. / # Inoculation |
|---|---|---|
| *Phaseolus vulgaris* | | |
| Bountiful | No symptom | 0/6 |
| Red Mexican | " | 0/5 |
| Pinto U.I. 111 | " | 0/6 |
| *Chenopodium quinoa* | Chlorotic local-lesion | 3/3 |
| *Chenopodium amaranticolor* | Small chlorotic local-lesion | 5/5 |
| *Vigna sinensis* | Dark green spots local-lesion like | 5/5 |
| *Pisum sativum* | | |
| Wilt Res. Perfection | Stem necrotic, tip distorted | 4/4 |
| *Vicia faba* | Brown necrotic spots, curly leaves, systemic infection | 6/6 |
| *Trifolium pratense* | Vein-clearing, systemic infection | 1/1 |
| *Trifolium hybridum* | Yellowish brown small spots | 1/1 |

Table 2. Inoculation Scheme for Local-lesion Assay of Virus Concentration in Six Pea Varieties.

| Leaf Position Number | Plant Number | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 1 | $A_1$ | $B_2$ | $C_2$ | $D_3$ | $E_4$ | $A_2$ | $B_3$ | $C_4$ | $E_1$ | $F_2$ | $A_3$ | $B_4$ | $D_1$ | $E_2$ | $F_3$ | $A_4$ | $C_1$ | $D_2$ | $E_3$ | CK |
| 2 | $F_4$ | $A_1$ | CK | $C_3$ | $D_4$ | $F_1$ | $A_2$ | $B_3$ | $C_4$ | $E_1$ | $F_2$ | $A_3$ | $B_4$ | $D_1$ | $E_2$ | $F_3$ | $B_1$ | $C_2$ | $D_2$ | $E_3$ |
| 3 | $E_4$ | $F_4$ | $A_1$ | $B_2$ | $C_3$ | $D_4$ | $F_1$ | $A_2$ | $B_3$ | $C_4$ | $E_1$ | $F_2$ | $A_3$ | $C_1$ | $D_1$ | CK | $F_3$ | $B_1$ | $C_2$ | $D_3$ |
| 4 | $D_3$ | $E_4$ | $E_2$ | $A_1$ | $B_2$ | $C_3$ | $D_4$ | $F_1$ | $A_3$ | $B_4$ | $C_4$ | $E_1$ | $F_2$ | $A_4$ | $C_1$ | $D_2$ | $E_3$ | $F_4$ | $B_1$ | $C_2$ |
| 5 | CK | $D_3$ | $E_4$ | $B_2$ | $A_2$ | $B_3$ | $C_3$ | $D_4$ | $F_1$ | $A_3$ | $B_4$ | $D_1$ | $E_2$ | $F_3$ | $A_4$ | $C_1$ | $D_2$ | $E_3$ | $F_4$ | $B_1$ |

* Each letter represents one inoculum from one source variety.

was calculated, using all 16 readings, and used to represent the virus concentration of a given variety sample.

## Maintenance of Virus Inoculum

The susceptible pea variety 'G 41' was used as the inoculum source plant for all $F_2$ and $F_3$ tests. The inoculum was maintained either fresh in living plants or dried. Dried inoculum for storage was prepared by enclosing infected pea leaves in wide-mouth jars, containing anhydrous calcium chloride, which were sealed tightly and placed in refrigeration at $40^{\circ}$ F.

For studies of factors affecting symptom expression, the virus was maintained in systemically-infected bellbean Vicia faba. This host was used to possibly increase the virulence of virus.

## Source and Description of the Virus Used

The OSU 331 strain of pea streak virus (PSV-331) was used throughout this study. It was originally isolated and identified as red clover vein mosaic virus by Dr. R. E. Ford, previous U.S.D.A. plant virologist at Oregon State University. The original isolate was taken from peas in the Donald-Aurora area of the Willamette Valley in Oregon. Subsequently, Dr. R. O. Hampton through serological methods has determined this isolate to be pea streak virus. The original virus supply used in this study was from dry infected pea plant

material.

## Description of Infection Types

Completely symptomless. The infection never causes visible symptoms. In plant virology this type of permanently nonapparent infection is called a latent infection. The varieties in this case have no apparent sensitivity and are thus termed resistant in these studies.

Usually symptomless. Plans of this type are usually free from symptoms. Occasional mild symptoms may appear, probably due to environmental factors. In plant virology this phenomenon is commonly referred to as symptom masking.

Klendusic. Plants characterized by this reaction are difficult to infect but usually produce normal symptoms when infected.

Severe symptom type. Severe symptoms are usually produced by plants of this type, and the plants are easily infected.

## Pea Varieties

The principal pea varieties and breeding lines used during this study are described below:

Geneva 41 (G 41). A commercial pea variety from New York State Agricultural Experiment Station, which is enation mosaic resistant. It was in the severe symptom type.

Midfreezer (MF). A commercial freezing variety, which was

classed as klendusic.

Dark Gray Sugar (DGS) and Wiwo (WW). Both are commercial varieties and were in the class described as usually symptomless.

OSU 42 and OSU 176-2. Enation mosaic resistant breeding lines, selected at Oregon State University. These were of the symptomless type.

## Genetic Study

Two separate tests were made in the genetic study. $F_2$ populations and $F_3$ families selected from single $F_2$ plants were planted in the summer of 1967, on the Vegetable Research Farm, Oregon State University. In this section, plants of the symptom producing type were classified as susceptible. Those not producing symptoms, even though infected were considered resistant. $F_2$ populations were tested against the segregation ratio of 3 susceptible to 1 resistant. $F_3$ population data was obtained by testing separate $F_3$ families and then making a composite of the individual plant results. The same number of plants were used for each family except in a minority of cases where germination was poor. These data were tested against a 5:3 ratio which should result from testing a massed $F_3$ population. Separate $F_3$ family data were tested against the theoretical ratio of 3 susceptible to 1 resistant. In this case all families having any plants with symptoms were included together in the susceptible class, and

those without any plants with symptoms were classified as resistant families. In most cases, plants in the genetic studies were inoculated a second time if they failed to show symptoms after the first inoculation. The parentages of segregating populations are listed in Table 3.

Table 3. Parentage of Segregating Populations.

| Designation of Cross | Parentage |
|---|---|
| B 289 | B 111-27 (R) x G 41 (S) |
| B 292 | B 114-36 (R) x G 41 (S) |
| B 310 | OSU 42 (R) x Midfreezer (S) |
| B 310R | Midfreezer (S) x OSU 42 (R) |
| B 312 | OSU 42 (R) x G 41 (S) |
| B 312R | G 41 (S) x OSU 42 (R) |
| B 313 | OSU 176-2 (R) x G 41 (S) |
| B 313R | G 41 (S) x OSU 176-2 (R) |

R: resistant parent S: susceptible parent

Soil and Chemical Experiments

For studies of the effects of different soil conditions on symptom expression, the following soil types and treatments were used. Farm soil or greenhouse soil, were used with or without sterilization and fertilization, and also with and without added sawdust. Other

media used were pure sterilized sand, sawdust, peat-moss, and vermiculite.

In experiments on growth retardation, a series of dilutions of the herbicide trifluralin were mixed thoroughly in sterilized farm soil prior to planting. The growth retardants Alar (succinic acid 2, 2-dimethyl hydrazide) and Amo-1618 (2-inoprophyl-4-dimethylamino-5-methylphenyl 1-piperidinecarboxylate methyl chloride) were used at 2000 ppm concentration as a spray on a series of plants immediately after inoculation.

Field Observation of Symptom Expression

A field planting was made in the summer of 1968 for the observation of symptoms in the same six varieties studied in the greenhouse. In this experiment screened field cages were placed over the row at the time of emergence to keep the peas free from insects which could carry contaminating viruses. An all-purpose insecticide was sprayed inside the cages each time they were opened or once or twice a week to keep them insect free. The peas were irrigated with overhead sprinklers four for five times during the growing season. Inoculation was made at ten days after planting using the same general methods used in the greenhouse. Inoculum was from greenhouse-grown virus infected 'G 41' plants. Readings on symptoms were taken two to four weeks after inoculation. Assays on the susceptible variety 'G 41' for virus

presence and local-lesion assays on Chenopodium quinoa leaves for virus concentration were made in greenhouse at the end of the test. It was hoped that the cage environment would approximate that of the field plots immediately adjacent to the cages. However, plant responses suggested that the two situations were markedly different.

# RESULTS

## Genetic Study

The reactions to PSV-331 of $F_2$ populations and $F_3$ progenies selected from single $F_2$ plants are given in Tables 4, 5, and 6.

It was assumed that the seven or eight plants per family used here was sufficient to identify the families homozygous for the recessive resistant trait. Approximately 70% of the $F_3$ and 95% of the $F_2$ plants were apparently non-infected prior to the second inoculation. The symptom expressions ranged continuously from severe to very mild. The $F_2$ plants had very healthy growth and the plants showing symptoms showed very mild mottling and somewhat curly terminal leaves at the late stage of growth. Because of the continuous variability in the time of appearance and the severity of symptoms, plants were classified only as having symptoms or as showing no symptoms.

All $F_2$ populations had many more symptomless plants than the expected 25%. We did not reproduce 3:1 ratios even in the crosses (OSU 42 x G 41, OSU 176-2 x G 41) which, in previous studies at this laboratory, had closely fit this ratio. However, in other tests made in these previous studies, some of these populations behaved as was found in the present work. Because there was obviously no fit to a

Table 4. Inheritance of Resistance to PSV-331 in $F_2$ Populations

| $F_2$ Populations * | Total | Number of Plants | | | |
|---|---|---|---|---|---|
| | | Expected | | Observed | |
| | | Sus. | Res. | Sus. | Res. |
| B 310 | 301 | 226 | 75 | 12 | 289 |
| B 310R | 304 | 228 | 76 | 21 | 283 |
| B 312 | 136 | 102 | 34 | 38 | 98 |
| B 312R | 104 | 78 | 26 | 23 | 81 |
| B 313 | 298 | 223 | 75 | 66 | 232 |
| B 313R | 352 | 264 | 88 | 90 | 272 |

* The parentage of each cross is shown on Table 3.

Table 5. Inheritance of Resistance to PSV-331 in $F_3$ Populations.

| $F_3$ Populations | Total | Number of Plants | | | | $X^2$ 5:3 | .05 Level | .01 Level |
|---|---|---|---|---|---|---|---|---|
| | | Expected | | Observed | | | | |
| | | Sus. | Res. | Sus. | Res. | | | |
| B 289 | 390 | 244 | 146 | 263 | 127 | 3.951 | | 6.635 |
| B 292 | 384 | 240 | 144 | 78 | 306 | --- | | |
| B 293 | 397 | 249 | 148 | 273 | 124 | 6.204 | | 6.635 |
| B 310 | 333 | 208 | 125 | 157 | 176 | --- | | |
| B 310R | 330 | 206 | 124 | 190 | 140 | 3.306 | 3.841 | |
| B 312R | 349 | 219 | 130 | 146 | 203 | --- | | |
| B 313 | 407 | 254 | 153 | 194 | 213 | --- | | |

* Composites of individual plant results from $F_3$ family tests.

Table 6. Inheritance of Resistance to PSV-331 in $F_3$ Progenies.

| $F_3$ Progenies | Total | Number of Plants | | | | $X^2$ 3:1 | .05 Level | .01 Level |
|---|---|---|---|---|---|---|---|---|
| | | Expected | | Observed | | | | |
| | | Sus. | Res. | Sus. | Res. | | | |
| B 289 | 50 | 37.5 | 12.5 | 33 | 17 | 2.160 | 3.841 | |
| B 292 | 50 | 37.5 | 12.5 | 34 | 16 | 1.307 | " | |
| B 293 | 52 | 39 | 13 | 42 | 10 | 0.923 | " | |
| B 310 | 50 | 37.5 | 12.5 | 36 | 14 | 0.240 | " | |
| B 310R | 52 | 39 | 13 | 37 | 15 | 0.410 | " | |
| B 312R | 49 | 37 | 12 | 34 | 15 | 0.993 | " | |
| B 313 | 52 | 39 | 13 | 32 | 20 | 5.025 | | 6.635 |

3:1 ratio, the $F_2$ data were not analyzed by the Chi-square test.

Some of the mass $F_3$ populations fit a 5:3 ratio better than the $F_2$ populations fit the expected 3:1 ratio. However, the fit in this case was also generally unsatisfactory. Tests of $F_3$ progenies selected from single $F_2$ plants, where any symptom expression resulted in classification of a family as susceptible (either homozygote or segregating) gave good fits to a ratio of 3 susceptible to 1 resistant. The actual genotype of individual plants was in most cases not known. Thus, plants without symptoms could have been symptomless or non-infected.

In population B 310R however, assays for presence of PSV on susceptible 'G 41' pea plants were made from most families with symptomless plants, and from some families in which all plants developed symptoms. As shown in Table 7, assay of 13 of the 15 resistant families (no plants with symptoms in any family) showed virus present in eight, with the remaining five families apparently non-infected. There were 20 families which had both symptomless plants and plants with symptoms. From 17 of these, assays were made of the symptomless plants only, and all of these assays were positive for virus infection. Of the 17 families in which all plants showed symptoms, 12 were assayed and all of these were virus infected. These data indicated that the resistant $F_3$ families could have been escapes, true symptomless types, or immune. However,

Table 7. Detailed Results from the Inoculation of a Single Population of $F_3$ Families B 310R(G41 x OSU 176-2).

| Family Number | Final Symptom Classification | | Virus Present* | Family Number | Final Symptom Classification | | Virus Present* |
|---|---|---|---|---|---|---|---|
| | Sus. | Res. | | | Sus. | Res. | |
| 1. | 7 | 0 | --- | 27. | 5 | 1 | Yes |
| 2. | 5 | 2 | Yes | 28. | 6 | 1 | --- |
| 3. | 0 | 5 | No | 29. | 3 | 3 | Yes |
| 4. | 5 | 0 | Yes | 30. | 0 | 5 | --- |
| 5. | 3 | 5 | Yes | 31. | 0 | 6 | Yes |
| 6. | 0 | 6 | No | 32. | 6 | 0 | --- |
| 7. | 0 | 4 | Yes | 33. | 0 | 6 | Yes |
| 8. | 7 | 0 | --- | 34. | 3 | 4 | Yes |
| 9. | 3 | 4 | --- | 35. | 0 | 7 | Yes |
| 10. | 2 | 2 | Yes | 36. | 0 | 5 | Yes |
| 11. | 3 | 3 | Yes | 37. | 5 | 3 | Yes |
| 12. | 1 | 5 | Yes | 38. | 5 | 3 | Yes |
| 13. | 0 | 6 | --- | 39. | 0 | 7 | No |
| 14. | 7 | 0 | Yes | 40. | 7 | 0 | Yes |
| 15. | 7 | 0 | Yes | 41. | 6 | 0 | Yes |
| 16. | 4 | 2 | --- | 42. | 0 | 3 | No |
| 17. | 7 | 0 | Yes | 43. | 3 | 4 | Yes |
| 18. | 4 | 3 | Yes | 44. | 4 | 3 | Yes |
| 19. | 0 | 7 | No | 45. | 6 | 1 | Yes |
| 20. | 0 | 3 | Yes | 46. | 6 | 0 | --- |
| 21. | 7 | 0 | Yes | 47. | 6 | 0 | Yes |
| 22. | 6 | 0 | Yes | 48. | 0 | 7 | Yes |
| 23. | 6 | 0 | Yes | 49. | 7 | 0 | Yes |
| 24. | 2 | 4 | Yes | 50. | 4 | 2 | Yes |
| 25. | 8 | 1 | Yes | 51. | 7 | 0 | Yes |
| 26. | 0 | 7 | Yes | 52. | 7 | 0 | --- |

* As determined by assay on G 41.

throughout this work extensive assays on peas and Chenopodium amaranticolor plants strongly suggest that immunity probably does not exist in the varieties of peas studied. It may be that symptomless types also have a greater tendency to escape.

Genotypic ratios obtained by classifying families as homozygous susceptible, segregating, and homozygous resistant did not fit the theoretical 1:2:1 ratios in most cases. One of the $F_3$ populations B 310R, previously discussed on connection with assay results, produced a ratio of 17:20:15 which very roughly fits the 1:2:1 ratio with a probability of 2.92 (the critical value of $\chi^2$ for $\alpha = 0.10$ and 2 d.f. is 4.605). Limiting factors in testing most populations were small sample sizes and the inherant problem of obtaining full expression of the symptom producing genotype.

## Symptom Comparisons of Pea Varieties

Because of the problem in the inheritance studies of the number of symptomless infected plants exceeding the expected, emphasis was given to the various aspects of symptom expression in the varietal material involved. To further type the comparative symptom expression in the pea varieties, they were observed under various conditions in the greenhouse and field.

In the field, after mechanical inoculation and in insect-proof cages, all varieties grew luxuriously and produced no sumptoms

except that, late in the growing season 'G 41' and 'Midfreezer' gradually developed very mild terminal symptoms. Plants outside of the cages may have been infected with enation mosaic or pea streak. However 'G 41', OSU 42 and OSU 176-2 are resistant to enation mosaic virus.

The observations, mostly made under greenhouse conditions, of symptom development of the six pea varieties involved in this work are described below. Variety 'G 41' and 'Midfreezer' usually were infected by PSV-331 and produced the most severe symptoms, 'Wiwo' and 'Dark Gray Sugar' are in the usually symptomless type, while OSU 42 and 176-2 were completely symptomless type.

G 41. In the greenhouse, ten days after inoculation, the initial symptoms were wilting and the death of inoculated leaflets. A systemic streak developed from the basal part of the stem upward to the terminal. Meanwhile, the upper leaflets became malformed and wilted, and finally dropped off (Figure 2). Infected plants usually died eventually, but sometimes infected plants remained alive for a long time, with considerable stunting, faint vein-clearing, and rigid curly leaves (Figure 3). In some cases a distinct relative recovery was observed where the new growth of 'G 41' plants became less severely affected. This recovery usually was followed by a return to severe symptom production. Symptoms in the field were much lighter inside the cages than outside or in the greenhouse. The inoculated plants

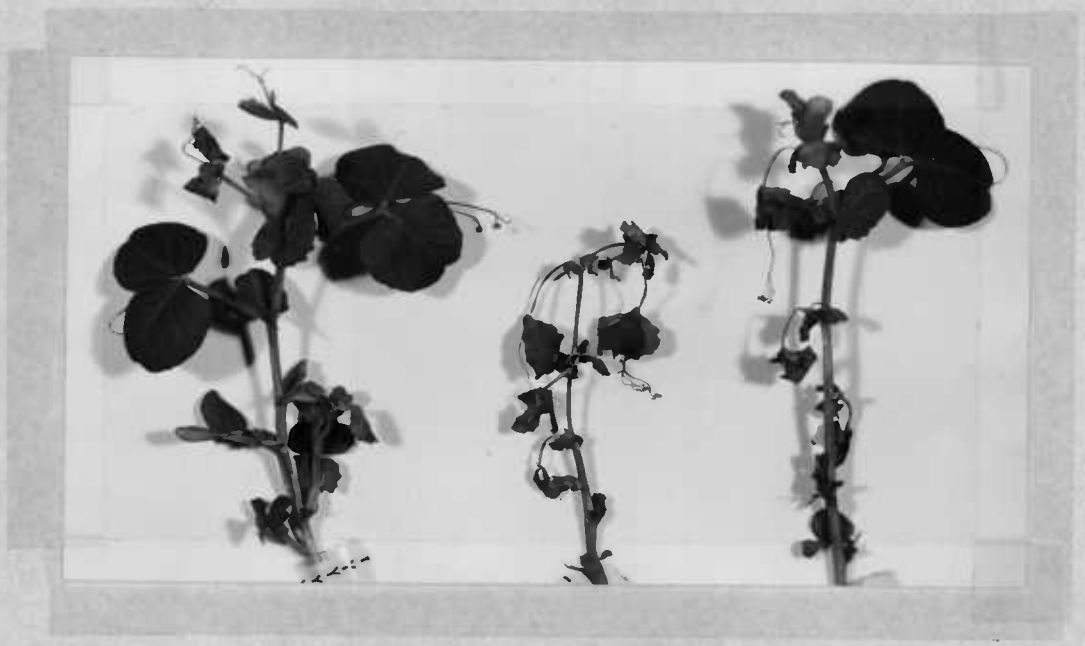


Figure 2. Degrees of Symptom Development of PSV-331 in Young Plants of Susceptible 'G 41'.

Figure 3. Mild Symptoms on Susceptible 'G 41' Inoculated with PSV-331.

inside the cages were as vigorous as the check plants, but the top foliage showed slight distortion and vein-clearing late in the season.

Midfreezer. The symptoms in the greenhouse appeared very much like those of 'G 41' plants except that this variety showed more pronounced foliage symptoms and less wilting and stunting. Sometimes the symptoms later reappeared and new growth became stunted again. In the field, foliage symptoms such as vein-clearing, yellowing, and curling-back of leaflets were produced.

Wiwo and Dark Gray Sugar. These two varieties usually showed no symptoms in the greenhouse and field tests, but occasionally produced light vein-clearing.

OSU 42 and OSU 176-2. These two varieties never showed symptoms throughout this study in either greenhouse and field conditions.

In the greenhouse test with 'G 41' and 'Midfreezer', cutting off the old growth resulted in more severe symptoms on the new branches grown from the basal part of the plant. When symptomless 'Wiwo' and 'Dark Gray Sugar' plants were cut back, slight foliage symptoms resulted. However, OSU 42 and 176-2 plants always remained symptomless after such treatment.

## Tests for Presence of the Virus

Table 8 shows the results of assay of the inoculated plants of

Table 8. Presence of Virus in Pea Varieties as Shown by Assay Inoculation on Susceptible 'G 41'.

| Variety | Greenhouse Source | | Field Source * | |
|---|---|---|---|---|
| | Virus Present | Severity on G 41 Test Plant | Virus Present | Severity on G 41 Test Plant |
| G 41 | Yes | Severe | Yes | Severe |
| Midfreezer | Yes | Severe | Yes | Severe |
| Wiwo | Yes | Mild | Yes | Mild |
| Dark Gray Sugar | Yes | Mild | Yes | Mild |
| OSU 42 | Yes | Very mild | Yes | Very mild |
| OSU 176-2 | Yes | Very mild | Yes | No sumptom |

* Two replications were sampled and assayed separately but were combined here because the results were almost identical. The field plants outside the cages were also sampled, but were not included because they appeared to be contaminated by other viruses.

the six varieties on 'G 41'. The results indicated that there was a consistent relation between the symptom type of the source plant and the symptoms produced on the 'G 41' test plants. It appears that symptomless (OSU 42 and OSU 176-2) and mild symptom types ('Wiwo' and 'Dark Gray Sugar') in some way reduced the virulence of the virus. The concentration of the virus as shown by the local-lesion assay, described below, did not follow this pattern.

Local-lesion Assay for Virus Concentration

Because Chenopodium quinoa was used as a local-lesion assay host to test the virus concentration of the varieties grown in the field, while Chenopodium amaranticolor was used for the assay host of the varieties grown in the greenhouse, these two tests cannot be directly compared. However, the comparison of varieties is shown in Figures 4 and 5 for the greenhouse and field tests respectively. Average numbers of lesions produced per leaf are assumed to be an indication of relative concentration of the virus in the variety sample tested and was presumed to be reliable because of the inoculation scheme (described in Table 1). Figures 6, 7, and 8 show the appearance of typical local-lesions on Chenopodium quinoa for the assay of each variety in the field test.

In Figures 4 and 5, there was no clear or consistent relation between virus concentration and symptom level. Mild symptom types

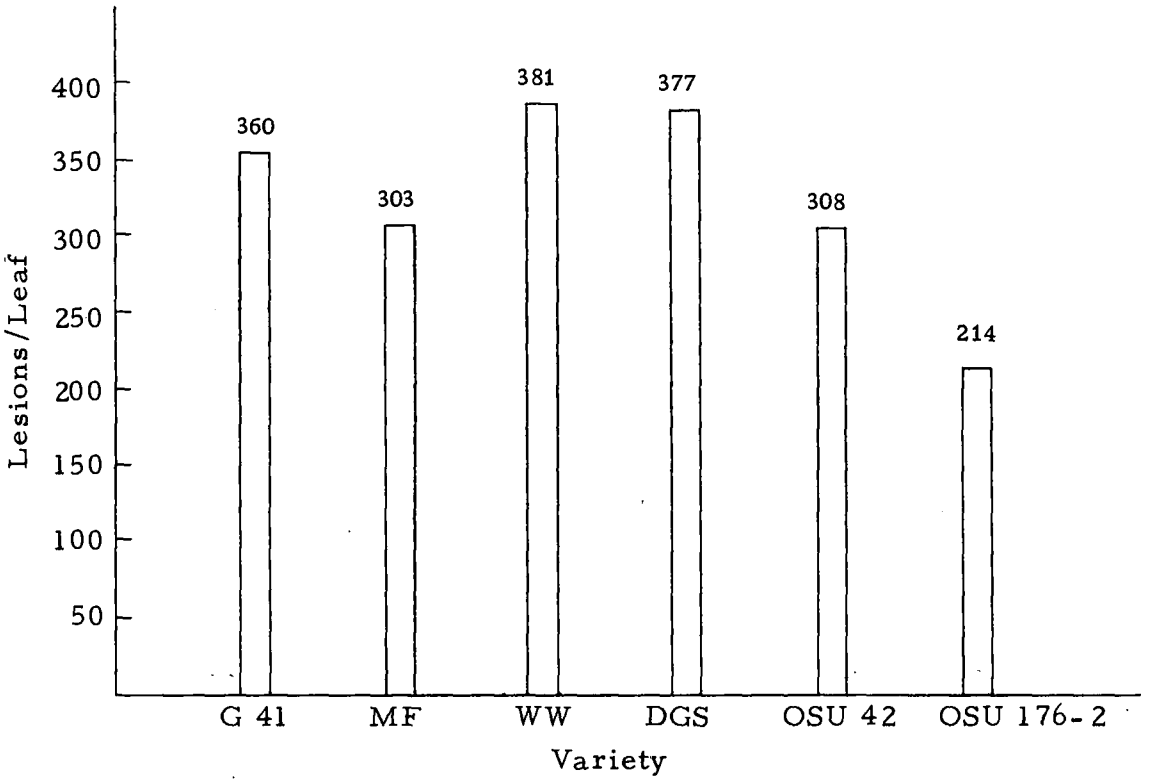



Figure 4. Comparison of the Concentration of PSV-331 in Six Pea Varieties Inoculated in the Greenhouse, as Determined by Assay on Chenopodium amaranticolor.

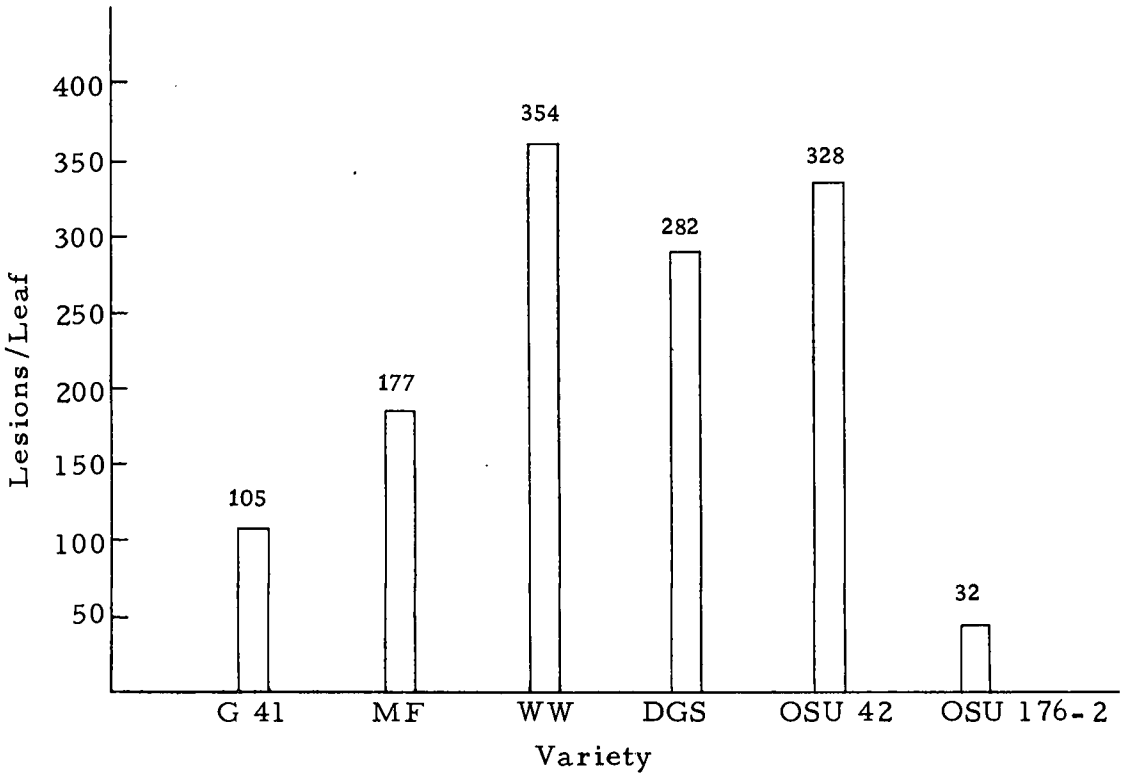
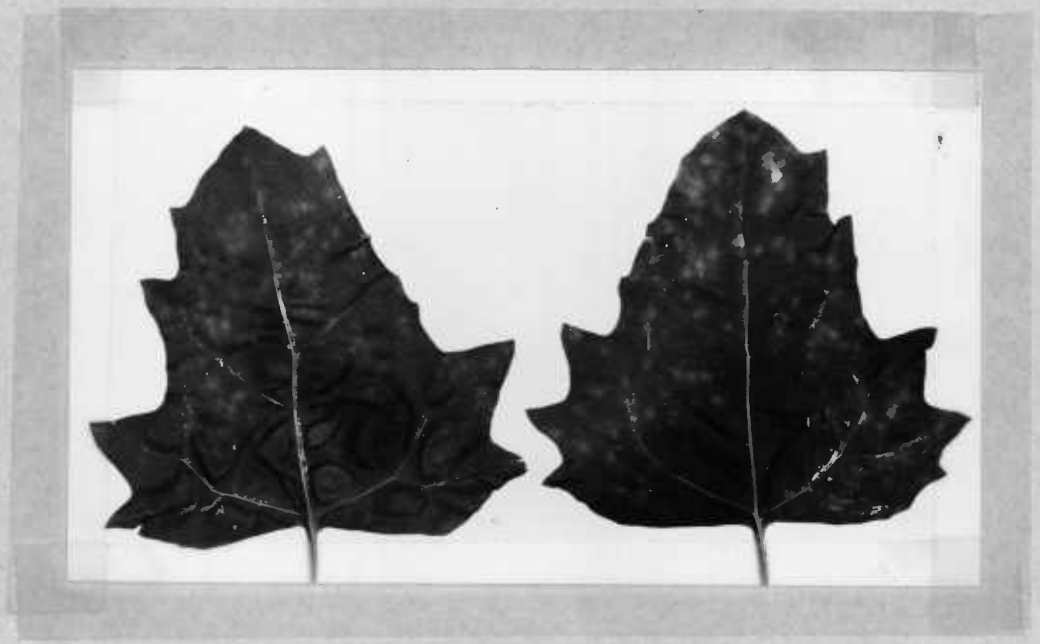
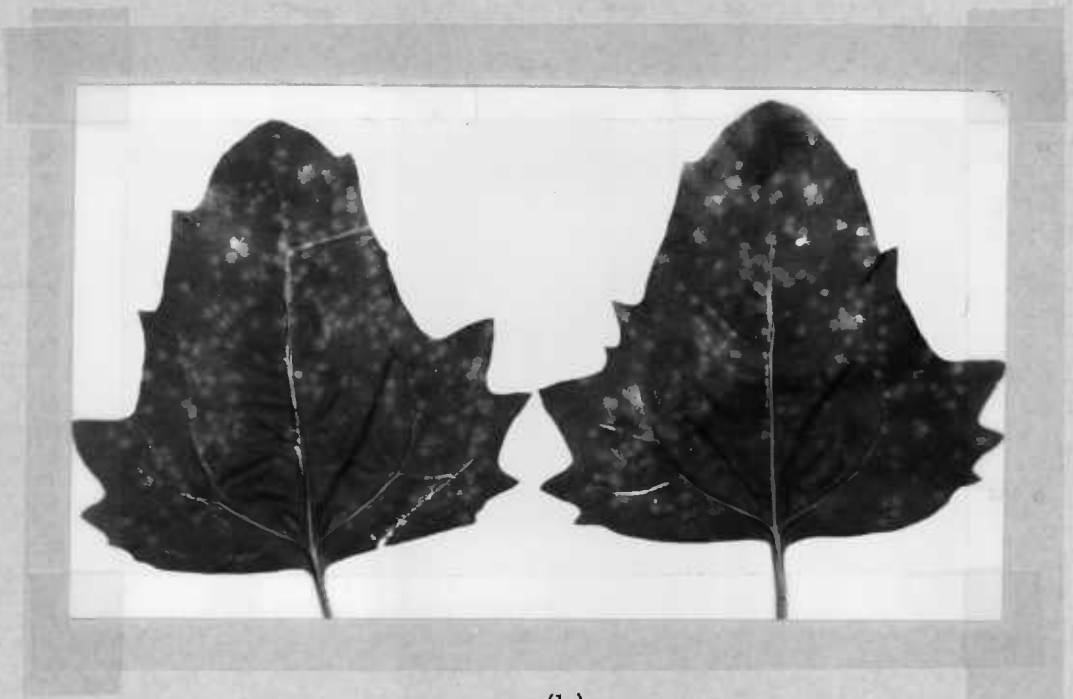



Figure 5. Comparison of the Concentration of PSV-331 in Six Pea Varieties Inoculated in the Field, as Determined by Assay on Chenopodium quinoa.

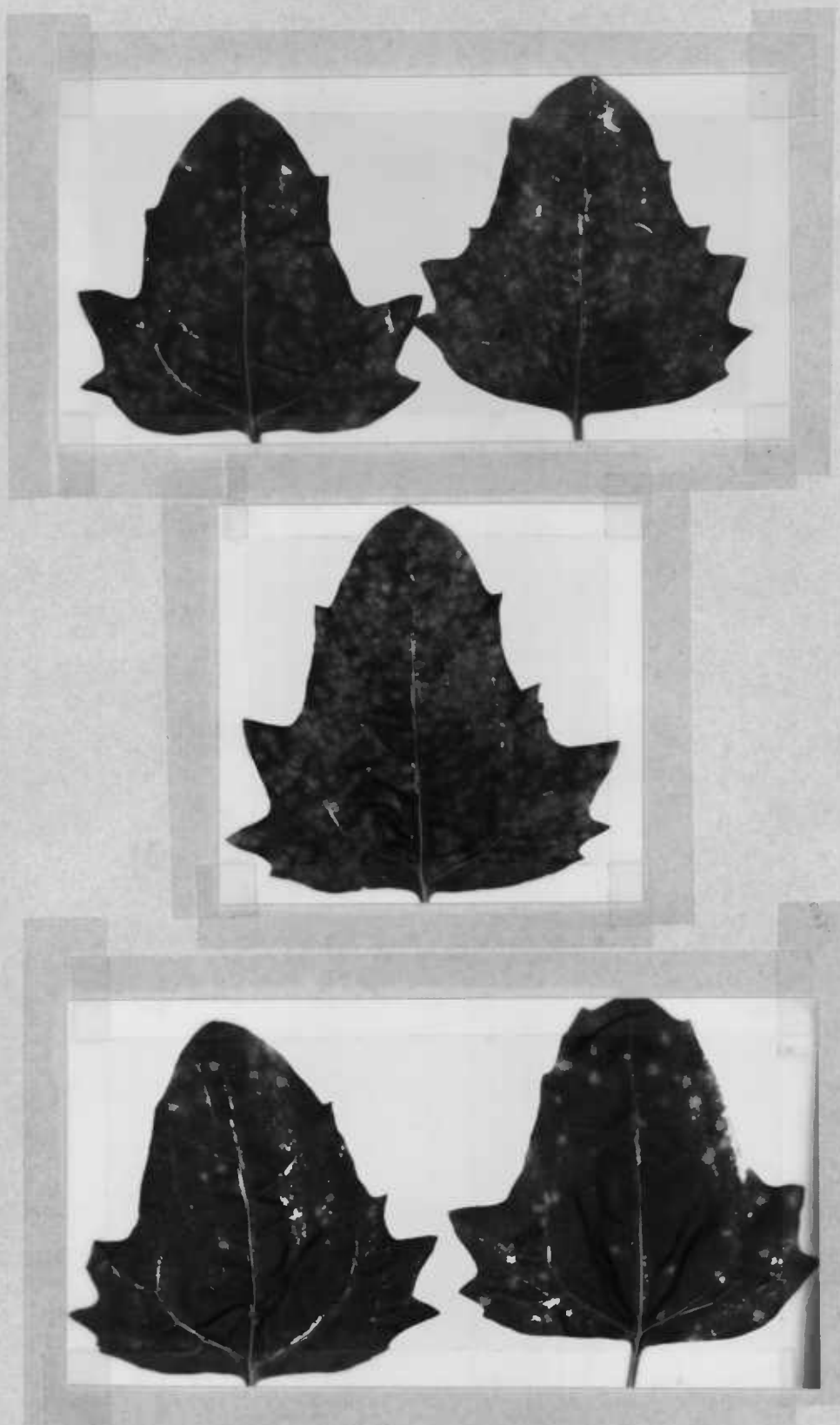


(a)



(b)

Figure 6. Local-lesions Produced on Chenopodium quinoa by an Assay Sample from 'G 41' (a) and 'MF' (b).

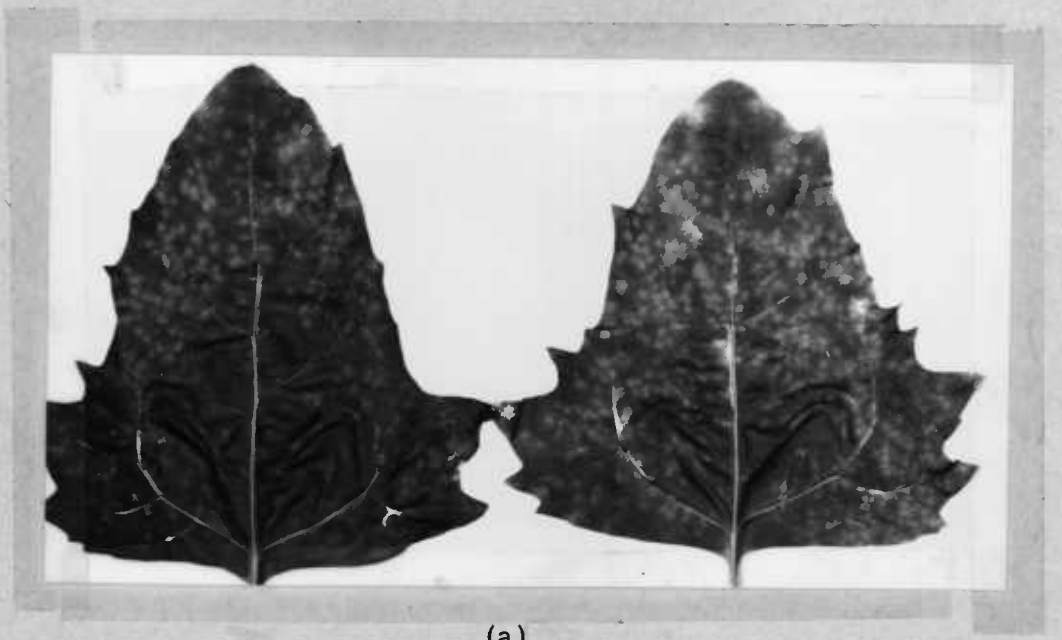


(a)

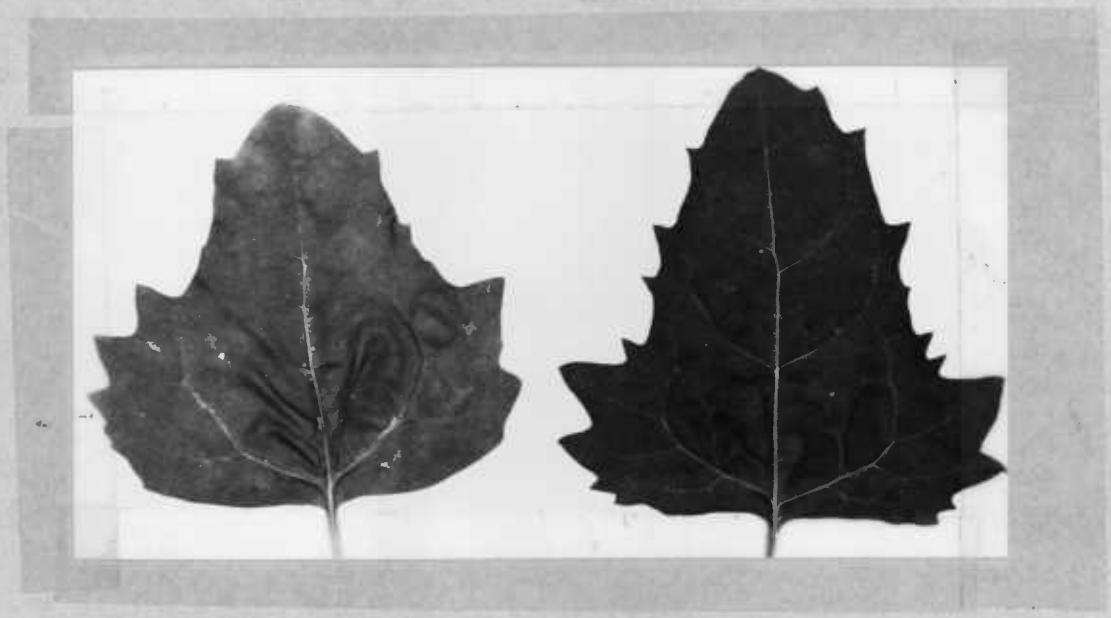
(b)

(c)

Figure 7. Local-lesions Produced on Chenopodium quinoa, by an Assay Sample from 'DGS' (a), 'WW' (b), and OSU 176-2 (c).



(a)



(b)

Figure 8. Local-lesions Produced on Chenopodium quinoa by an Assay Sample from OSU 42 (a) and Water Inoculated Control (b, left), Non-inoculated Control (b, right).

'Wiwo' and 'Dark Gray Sugar' were high in both tests, OSU 42 was moderate to high, but OSU 176-2 was very low in the field test. In the case of OSU 42 and OSU 176-2, since symptoms were not visible, the presence of non-infected plants in the composite sample may have affected the virus concentration of the sample as determined by assay.

Seasonal Effects on Symptom Expression

This series of tests was carried out during late November of 1967 through early May of 1968. During this period the weather conditions can be approximately classified into three different phases. The early period (midwinter) was characterized by cool overcast weather with relatively little bright sunshine. During the middle period (early spring), cool cloudy weather was prevalent although sunny days were more frequent than in the early period. In the late period (late spring) of this experiment, sunny days were more common and light intensity was higher than in the two earlier periods. There were marked relationships between weather conditions and the time of symptom expression. The results, summarized in Table 9, show that the symptoms produced on 'G 41' and 'Midfreezer' were greatly affected by seasonal change. The variety 'G 41' produced severe symptoms during the winter time, then gradually showed late and mild symptoms when sunny days were more frequent and light

Table 9. Effect of Seasonal Change on Symptom Expression[a] in Pea Varieties.

| Inoculation Date | Numbers of Plants | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G 41 | | | Midfreezer | | | OSU 42 | | | OSU 176-2 | | |
| | S | M | N | S | M | N | S | M | N | S | M | N |
| 11/25 | 28 | 0 | 0 | 13 | 0 | 16 | 0 | 0 | 27 | 0 | 0 | 32 |
| 12/15 | 30 | 0 | 0 | 28 | 0 | 2 | 0 | 0 | 30 | 0 | 0 | 33 |
| 1/6 | 30 | 1 | 0 | 14 | 17 | 3 | 0 | 0 | 28 | 0 | 0 | 33 |
| 1/25 | 29 | 0 | 0 | 30[c] | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 29 |
| 2/10 | 19 | 8 | 0 | 31[c] | 0 | 0 | 0 | 0 | 29 | 0 | 0 | 31 |
| 2/28 | 27 | 3[b] | 0 | 18[c] | 0 | 10 | 0 | 0 | 30 | 0 | 0 | 30 |
| 3/15 | 16 | 12[b] | 0 | 19[b] | 0 | 8 | 0 | 0 | 28 | 0 | 0 | 29 |
| 4/5 | 24[b] | 9[b] | 3 | 35[b] | 0 | 0 | 0 | 0 | 39 | 0 | 0 | 40 |
| 4/22 | 17[b] | 7[b] | 8 | 0 | 20[b] | 18 | 0 | 0 | 36 | 0 | 0 | 40 |
| 5/10 | 0 | 34[b] | 0 | 0 | 31[b] | 1 | 0 | 0 | 34 | 0 | 0 | 35 |

[a] S - severe symptom M - mild symptom N - no symptom

[b] Symptoms showed only at the late period of growth.

[c] Symptoms disappear after first infection then reappear at the late period.

intensity was higher during the middle to late spring. On 'Mid-freezer', symptoms produced during midwinter were as severe as those of 'G 41', but recovery was common in early spring and late mild symptoms were produced during the late spring. On OSU 42 and OSU 176-2, symptomless infections occurred throughout the whole study period.

## Soil and Chemical Experiments

Three experiments were conducted in the greenhouse, in the spring of 1969, to determine the effect of different soil conditions on symptom expression, using primarily the susceptible variety 'G 41'. Descriptions and results of these tests are given below and in Tables 10 and 11, and Figures 9 to 13.

Experiment 1. In this experiment, made in February, 1969, the effects of sterilization and various rates of fertilizer in several soils were compared. Primarily involved were a field loam, designated farm soil, which was generally high in natural fertility and a greenhouse mix of peat and silt which was low in general fertility. Sterilization apparently modified each type of soil by increasing fertility; and complete fertilizer raised the fertility and in some cases produced a toxic soluble salt level. It was not possible to determine or recognize other effects of sterilization which might have influenced results. Of the plants grown in sterilized farm soil, those with three

and four teaspoons of 8-24-8 fertilizer per can (equivalent to 12, 000 and 16, 000 lbs/acre respectively) produced the most severe symptom (Figure 9, Table 10). Three weeks after inoculation the plants started dying; after four weeks, none survived except the non-inoculated check plants left in the same can. The cause of the death was considered to be not only the virus effect but apparently the high salt content in the soil. Although the check plant survived, it was very much retarded. The symptoms were milder when the soil contained reduced amounts of fertilizer. Symptomless infection occurred when the plants were grown in sterilized farm soil without any fertilizer. The growth rate of control plants in the unfertilized farm soil was almost identical with the infected ones. Unsterilized farm soil produced more severe symptoms than sterilized farm soil. There were much stronger symptoms in plants grown in greenhouse soil compared to those in farm soil (Figure 10). Plants grown in unsterilized unfertilized greenhouse soil which is a mixture of light silt and peat produced typical severe greenhouse symptoms of PSV-331. In this case, infected 'G 41' plants were severely stunted with distorted growth, foliage vein-clearing, yellowing, and rigid curled-back leaves (Figure 11). Farm soil never produced stunting symptoms except in the excess fertilizer treatments. These results are included in Table 10, where they can be compared with treatments from Experiment 2. Adaptation of these results, after further study, might

Table 10. The Effects on the Severity of Symptoms of Soil, Fertilizer and Chemical Treatment on 'G 41'.

| Treatment | | | Severity of Symptoms[a] |
|---|---|---|---|
| Experiment 1 (February 1969) | | | |
| Sterilized Farm Soil | + | 4 tsp. of 8-24-8[b] | 5.0 |
| Sterilized Farm Soil | + | 3 tsp. of 8-24-8 | 4.5 |
| Sterilized Farm Soil | + | 2 tsp. of 8-24-8 | 2.6 |
| Sterilized Farm Soil | + | 1 tsp. of 8-24-8 | 1.6 |
| Sterilized Farm Soil | + | 1/2 tsp. of 8-24-8 | 1.5 |
| Sterilized Farm Soil | + | 0 tsp. of 8-24-8 | 1.0 |
| Sterilized Farm Soil | + | 1/3 sawdust | 2.7 |
| Unsterilized Farm Soil | | no fertilizer | 2.8 |
| Unsterilized Farm Soil | + | 1/3 sawdust | 3.4 |
| Unsterilized Greenhouse Soil | | no fertilizer | 4.3 |
| Experiment 2 (March 1969) | | | |
| Sterilized Farm Soil | + | 2000 ppm of growth retardant: Amo | 3.2 |
| | | Alar | 3.3 |
| Sterilized Farm Soil | + | Herbicide-trifluralin[c] | |
| | | 10 X | 1.3 |
| Sterilized Farm Soil | + | 5 X | 1.8 |
| Sterilized Farm Soil | + | 1 X | 1.6 |
| Sterilized Farm Soil | + | 0.5 X | 1.3 |
| Sterilized Farm Soil | + | 0.1 X | 1.7 |
| Sterilized Farm Soil | + | 0 X | 1.0 |
| Sterilized Farm Soil | Non-inoculated control | | 1.0 |
| Peat-moss | | | 1.4 |
| Sterilized Sand | | | 1.2 |
| Vermiculite | | | 1.8 |
| Sawdust | | | 1.5 |

[a] Severity index was the average of individual scores of about 25 plants, where: 1--symptomless, no visible symptoms, almost identical with the non-inoculated check plants. 2--plants showed terminal symptoms only, yellowing, vein-clearing, and curled-back leaves. 3--plants slight stunted with foliage symptoms. 4--plant severe stunted with foliage symptoms, but remain survived. 5--infected plants died early.

[b] One teaspoon 8-24-8 fertilizer is equivalent to 4000 lbs/acre.

[c] The base rate of 1X is equivalent to 0.75 lbs/acre.

Figure 9. Symptoms of 'G 41' Where Plants Grown in Sterilized Farm Soil with Three Teaspoons of 8-24-8 Fertilizer.

Figure 10. Severity of 'G 41' Where Grown in Unsterilized Greenhouse Soil (left) and Unsterilized Farm Soil (right).

Figure 11. Severe Symptoms of OSU Strain of Pea Streak Virus on 'G 41' Grown in Unsterilized Greenhouse Soil.

Figure 12. Comparison of Symptom Expression of 'G 41' Grown in Four Different Soil Treatments. Left to right: Unsterilized Farm Soil; Unsterilized Greenhouse Soil; Sterilized Farm Soil; Sterilized Farm Soil plus Three Teaspoons of Fertilizer.

produce a means of improving greenhouse tests for resistance.

Experiment 2. This experiment involved treatments with two different growth retardants, Alar and Amo. Each was used at 2000 ppm concentration. In a second group of treatments a series of dilutions of the herbicide trifluralin were mixed in the soil before planting. Effects of these treatments are shown in Table 10, for comparison with several soil and fertilizer treatments.

There was no significant difference on symptom expression between two kinds of growth retardants, but both produced a marked effect on the severity of symptoms. These plants were more stunted with severe foliage symptoms. The results of the herbicide treatments showed no marked effects on symptom expression. Plants grown in peat-moss, sterilized sand, vermiculite, and sawdust were very poor in growth and produced mild symptoms only. The poor growth may have been caused by difficiencies of soil nutrients or to unfavorably high acidity in the case of the peat-moss treatment.

Samples from symptomless plants of each treatment were assayed on Chenopodium amaranticolor for the presence of virus and results were positive as expected, while the assay for the check plants were negative.

Experiment 3. In this experiment the relationship between the severity of symptoms and plant height under certain soil treatments were observed. These treatments and these effects on symptom

severity are given in Table 11. There was no strong effect on plants in either farm or greenhouse soil from hydrated lime used in an attempt to correct a possible minor element deficiency or acidity problem which had been present where plants were grown in greenhouse soil. There were differences in height and an interesting relationship between height of virus infected and non-inoculated plants was apparent in this experiment (Figure 13). The height differences between infected and non-inoculated in the susceptible 'G 41' plants were more pronounced compared to that obtained inoculated OSU 42 plants compared with non-inoculated OSU 42 plants. The small differences between the OSU 42 plants were probably due to the effect of rub inoculation.

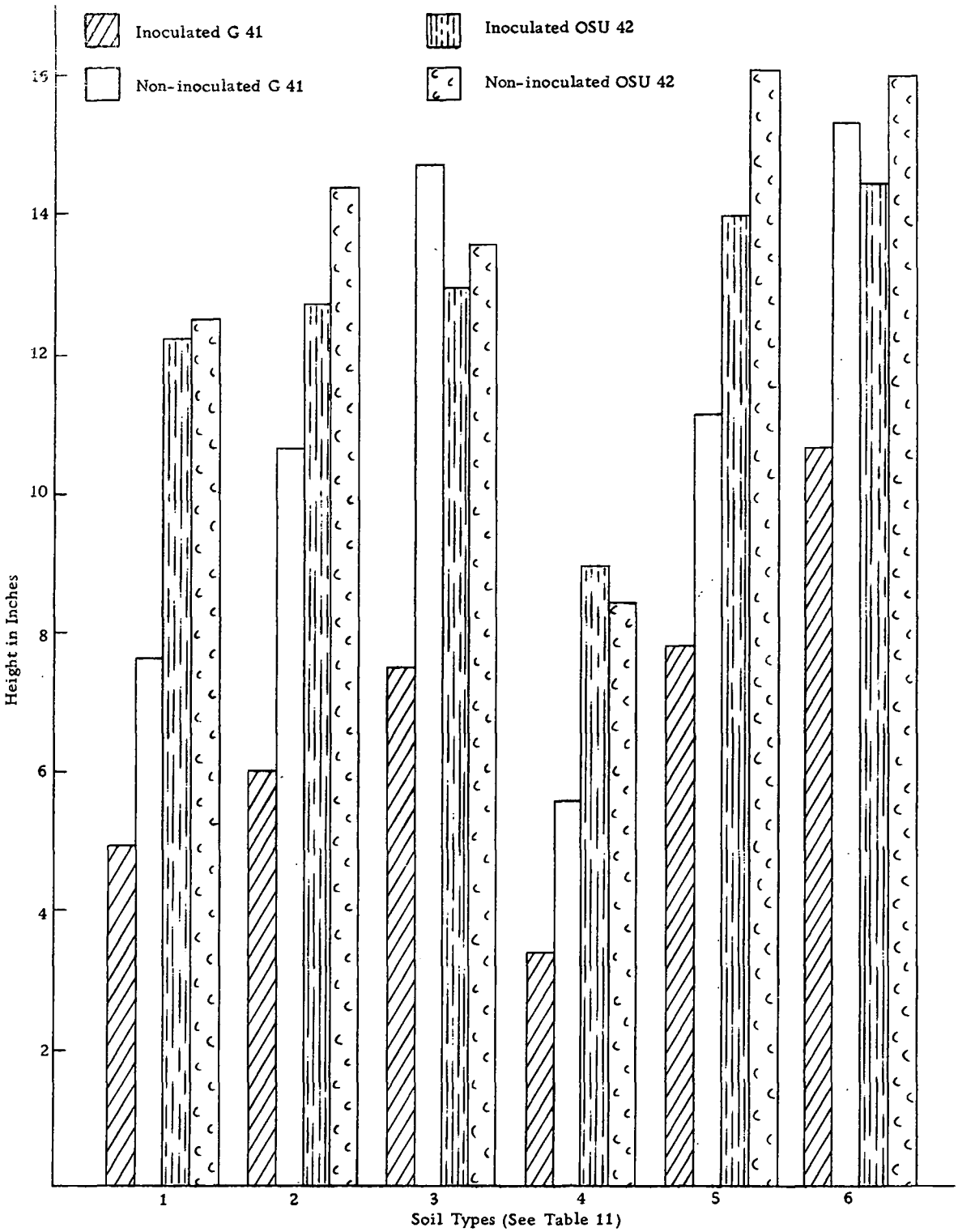



Figure 13. Comparisons of Height Affect between Inoculated and Non-inoculated under Various Soil Fertilizer Treatments (Four Weeks after Inoculation).

Table 11. The Effects on the Severity of Symptoms of Various Soil Fertilizer Treatments (Experiment 3).

| Soil Type | Treatment | Severity[a] G 41 | Severity[a] OSU 42 |
|---|---|---|---|
| Farm soil | ----- | 3.2 | no symptom |
| Farm soil | 1 tsp. 8-24-8 fertilizer | 3.0 | no symptom |
| Farm soil | 1 tsp. fertilizer + 1 tsp. hydrated lime[b] | 2.4 | no symptom |
| Greenhouse soil | ----- | 3.8 | no symptom |
| Greenhouse soil | 1 tsp. 8-24-8 fertilizer | 2.6 | no symptom |
| Greenhouse soil | 1 tsp. fertilizer + 1 tsp. hydrated lime | 2.0 | no symptom |

[a] Severity index calculated as the same method as Table 10.

[b] One teaspoon of hydrated lime is equivalent to a rate of 3390 lbs/acre.

# DISCUSSION

A previous study done in this laboratory indicated that resistance (infection in which no symptoms were expressed) to PSV-331 was governed by a single recessive gene. In that study, and in the present work, expected 3:1 ($F_2$) and 5:3 ($F_3$) ratios of "symptom-producing":"symptomless-infected" progenies were not always obtained. Observed ratios were often smaller than 3:1 or 5:3 ratios, respectively. Such departure from expected ratios suggests an influence of one or more of the following factors: (i) unsuccessful inoculation of some portion of the plants, causing them to be mistaken for symptomless-infected plants, (ii) supression of virus symptoms under certain environmental conditions, resulting in an unexpected proportion of symptomless-infected plants, or (iii) a reduction in virulence of PSV-331, thus reducing its inoculum potential or resulting a milder symptom response in inoculated pea plants. Yen and Fry (41), reporting on the inheritance of immunity to pea mosaic virus found that immunity was controlled by a single recessive gene, but that there was a delayed symptom expression in the heterozygote. Their susceptible heterozygote was noted to be only partially tolerant to infection, as typified by a delay in symptom expression. The possibility that the heterozygous condition caused a delayed or reduced response was not considered during the course of our study.

Although incubation periods were not recorded, observations made on the $F_2$ and $F_3$ populations indicated that this same phenomenon might have been involved in the present experiments. The use of different symptom types, such as, severe symptom type, mild symptom type, or symptomless infection type in parental material might play an important role on the symptom expression in the progenies. The progenies which had 'Midfreezer' as susceptible parent failed to fit a 3:1 expected ratio, while progenies from crosses with 'G 41' as a susceptible parent more often gave a close fit to 3:1 segregation ratio in $F_2$ and $F_3$ generations.

Perhaps modifying genes are present in certain of the parents used. Modification of the expression of genetically controlled susceptibility of a pea virus has been reported by Schroeder et al. who found that the symptom development in heterozygous plants of Pisum sativum inoculated with certain strains of bean yellow mosaic virus can be manipulated by changing the temperature of the environment. However, temperature effects were not conclusively demonstrated here.

Symptom expression caused by PSV-331 on susceptible pea varieties varied considerably from time to time. The results of a series of tests revealed that the symptom expression may be affected by seasonal changes in temperature and/or light intensity. The symptoms were more severe in the winter when short days and low

light intensity were more pronounced. Intermediate symptom types occurred during the early spring, and symptoms were late occurring, and very mild or absent in late spring. Sander (27), working with RCVMV, indicated that apparent infectivity was highest in midwinter and approached zero in late spring. Since RCVMV is rather unstable, it is possible that the virus content was affected by seasonal changes in greenhouse temperature and/or light intensity. He also suggested that there may be seasonal variations in the amount of inhibitors present in the plants. Another possibility is that the susceptibility of the test plants may be affected appreciably by seasonal change in temperature and/or light intensity. Though in our study we were using PSV, this virus is considered to have a distinct serological relationship to RCVMV (38) and thus similar effects may have been involved.

The results of local-lesion assay revealed that the severe symptom types 'G 41' and 'Midfreezer' did not necessarily produce the most lesions on the assay host. Contrarily, the trace symptom types 'Wiwo' and 'Dark Gray Sugar' produced the most lesions, and the symptomless type varieties OSU 42 and 176-2 varied in their concentration of virus. The concentration of virus particles in OSU 42 was as high or even higher than 'G 41' and 'Midfreezer', but OSU 176-2 produced the lowest number of lesions on assay plants. These results appear to support the idea that mild symptom type

plants which can tolerate the infection by virus, can also permit and tolerate greater virus multiplication. Bawden (2, p. 69) stated that some hosts that are symptomless carriers can accommodate virus multiplication without being noticeably affected. The relative virulence of viruses, or of virus strains, may not be determined by the amounts to which they accumulate in infected plants. Bawden also comments (2, p. 46),

> The differences in severity of symptoms occasioned by changing the environment seem, often than not, to be correlated with the amount of virus in the plants. There is, though, no such general correlation between severity of host reaction and virus concentration; similarly, because one strain of a virus is more virulent than another towards a given host does not necessarily mean that it occurs in greater amounts; and a host that reacts severely to a virus strain does not necessarily contain more virus than one that reacts moderately.

In the present study, little correlation was observed between symptom expression and different temperatures. The temperatures of $65^{\circ}$ F, $70^{\circ}$F, $75^{\circ}$F were used, but we failed to observe differences, perhaps because of the nonconstant temperature maintenance and the small differences in interval between the temperatures used.

Different growing conditions and soil nutrients seem to greatly influence the severity of symptoms, though these effects were somewhat unpredictable. Very mild symptoms were produced under either very poor soil condition such as with sand, peat moss, vermiculite, sawdust or very rich soils such as sterilized farm soil with optimum

fertilizer. The best symptoms found among all soil, fertilizer, and chemical treatments, was with unsterilized, unfertilized greenhouse soil (Figure 11). This soil produced normal but rather non-vigorous control plants, and consistently good symptoms in the inoculated plants. In the growth retardant experiment, leaves were normal appearing in the uninoculated controls yet the plant was much shortened. Symptoms were early appearing and severe in the inoculated plants. This suggests that rate of growth affects symptom development, in this case in a negative way. In other known cases, well-nourished, vigorous plants are most susceptible to infection or symptom production. Perhaps the most general statement that can be made is that better conditions for growth of a plant result in earlier and more characteristic symptoms though no doubt there are many exceptions. However, Spencer (35) showed that susceptibility to tobacco mosaic virus was highest when growth was somewhat retarded by excess nitrogen. Spencer (36) also found that the systemic movement of the virus was accelerated with high nitrogen. There was an early appearance of systemic symptoms with either a deficiency or an excess of nitrogen when tobacco was infected with the yellow strain of tobacco mosaic virus. The results from these experiments with the PSV-331 indicated that a toxic excess of fertilizer in the soil made the plant somewhat retarded and produced severe symptoms. Rapid vigorous growth promoted by high fertility

and high light conditions were always detrimental to symptom production in this study, in contrast to other reported cases. In some known cases, rapidly growing plants will permit virus free plant parts, because virus multiplication has lagged behind. However, this relationship may not apply here because we have shown nearly equal virus concentration in some cases where the plants were symptomless. The plants seemed usually more adversely affected by infection when nutrition or other factors were unfavorable than when they were supplied with abundant nutrients and favorable environment.

Because of difficulties in obtaining uniform infections and symptoms, we were not able to get valid yield data to demonstrate the yield reduction affected by virus infection. However, the experiment with plant height effect suggested that there was little or no difference in growth between symptomless inoculated and non-inoculated plants, but there was greatly reduced height in the case of inoculated and non-inoculated plants of a severe symptom type variety (Figure 13). Ford and Baggett (12) reporting on relative severity of legume virus (included pea streak virus) in peas, measured by plant growth reduction, indicated that plant height was directly correlated with relative severity of infection of pea plants.

Why field symptoms were so mild is still not known for certain, but there are some possibilities such as the shading effect of the

plastic screen cages, which produced very luxuriant growth, or that the inoculum may have been attenuated before or during this test. Passage through bellbean (Vicia faba) late in this study seemed to restore some virulence as indicated by symptom severity on 'G 41' pea plants.

It is at least clear that symptoms reflect reactions between specific factors of the host, the virus strain, and the environment. In this study we were able only to recognize a few of these interactions.

# SUMMARY AND CONCLUSION

Genetic studies were inconclusive but partially confirmed previous findings that the resistant (symptomless) type is controlled by a single recessive gene. We were not able to obtain good $F_2$ data, but $F_3$ family tests provided usable information. Only progenies with 'G 41' as susceptible parent statistically fit the expected single gene ratios.

Host range studies demonstrated that Chenopodium quinoa and Chenopodium amaranticolor were good local-lesion hosts for assay purposes.

The symptom expression induced by PSV-331 was characterized by seasonal change. Cloudy cool weather, as in midwinter, usually gave severe symptoms. Sunny weather and high light intensity gave mild or symptomless type of infection.

Assay studies in six pea varieties, using 'G 41' peas as assay host, showed that the infectivity of virus from severe symptom types was higher than from mild symptom and symptomless types. However, the virus concentration in sap from symptomless plants or those with mild symptoms as determined by assay on Chenopodium quinoa or C. amaranticolor was equal to that from plants with severe symptoms.

Symptom expression was also related to plant growth conditions. Rapidly growing and vigorous plants produce mild and late terminal symptoms only, or even remained symptomless.

Cutting off the infected plants just above the base produced more severe symptoms on the new branches arising from the basal part of the plant.

Soil conditions affected symptom expression. Soils low in nitrogen or in general fertility produced the most typical greenhouse symptom while the soil of high fertility resulted in almost symptomless plants. Intermediate soil produced mild symptom.

Growth retardant experiments demonstrated that plants grown in good soil conditions, but somewhat retarded by chemicals, produced more severe symptom than unretarded plants. Excessive rates of complete fertilizer, which produced high salt damage in the plants, produced more severe symptoms than normal rates.

No significant effect on symptoms was found in plants treated with the herbicide trifluralin.

There was a significant difference in height between inoculated and non-inoculated plants in the susceptible variety 'G 41' but no significant difference in height was found between inoculated and non-inoculated plants in the symptomless variety OSU 42.

Whether a given plant can be a host for a given strain of a virus, and whether infection can lead to symptoms or not, is

determined by the genetic constitution of both the plant and virus strain. Any enviornmental change may affect host physiology in a way that may influence virus multiplication or the response of the host to infection.

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