THE INHERITANCE OF RESISTANCE TO BEAN YELLOW MOSAIC VIRUS IN *PHASEOLUS VULGARIS* L.

by

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THE INHERITANCE OF RESISTANCE TO BEAN YELLOW MOSAIC VIRUS IN *PHASEOLUS VULGARIS* L.

INTRODUCTION

The common bean, *Phaseolus vulgaris* L., is grown commercially in large acreages in the United States, either for dry-shell or green-pod consumption. About 300,000 acres of snap or green beans alone, with a value of approximately $77,000,000, were grown in the United States in 1955. Green beans are also among the leading vegetables grown in home gardens throughout the nation.

In Oregon, the leading state in the production of processed green beans, approximately 10,000 acres, with a gross value to the grower of $10,000,000, were grown in 1955 for canning and freezing. The most important variety in this area is the Blue Lake, a pole type which requires trellis culture. The growing of green beans in Oregon is therefore a high-cost, concentrated industry, in which plant disease problems may be economically critical.

Among the diseases affecting beans in Oregon and other areas, one of the most widespread and consistent in occurrence is that caused by the bean yellow mosaic virus. Naturally transmitted by aphids to beans, from such perennial hosts as various clovers and gladioli, bean yellow mosaic virus is present in varying degrees in virtually every field of beans grown in Western Oregon. Infected plants of ordinary susceptible varieties, such as the Blue Lake, are usually severely dwarfed and distorted. The pods are often badly misshapen and reduced in size, and may be of little commercial value.

Although the actual damage, on a percentage basis, is ordinarily slight throughout the area, more serious losses are not uncommon when insect vectors and perennial host plants are abundant. When bean plantings are made adjacent to fields of gladioli, severe infections of nearly 100% of the plants may occur. In 1950, widespread infections of bean yellow mosaic, and of bean common mosaic, occurred in the Willamette Valley of Oregon in the F.M. 65 Blue Lake variety, causing heavy general losses. Similar losses have been reported in other areas. In 1947 in the Cache Valley of Utah, an outbreak of bean yellow mosaic caused 165 of the 240 acres of Blue Lake pole beans planted for processing to be abandoned (2, p.384). Such outbreaks are usually related to high aphid populations in combination with the presence of virus inoculum in wild or crop hosts. Widespread infection could occur at any future time when these conditions become favorable.

Although the occurrence of bean yellow mosaic can possibly be reduced, in some cases, by adequate aphid control programs, and the elimination of perennial host plants, the use of resistant bean varieties would be an ideal general control measure. A field bean variety, Great Northern U.I. 31, has been found to possess strong resistance to pod-distorting strains of BYMV [1] isolated from severe field infections of beans in the Willamette Valley of Oregon, and to natural infection by viruses transmitted by aphids from gladioli plantings. This variety is known, however, to be susceptible to other

1 BYMV will be used hereafter for bean yellow mosaic virus.

BYMV strains including some which occur in Idaho and Washington. Because of the great apparent variability in BYMV strains which may be found in the various bean growing areas of the nation, resistance may be of only temporary value unless plant materials possessing strong general resistance are available for breeding purposes.

Several varieties of the runner bean, Phaseolus coccineus L., have also been tested, and are resistant or immune to many, and possibly all strains of BYMV, including strains isolated from beans in Oregon and Central Washington, and strains occurring in Western Oregon gladioli plantings.

The purpose of this study has been to determine the mode of inheritance of resistance to BYMV in crosses between the available resistant varieties, and susceptible commercial bean varieties. Such information should greatly facilitate the planning and execution of programs for the breeding of commercial bean varieties resistant to bean yellow mosaic.

REVIEW OF LITERATURE

The Bean Yellow Mosaic Virus Group

Bean yellow mosaic was first described as a distinct virus disease by Pierce (11, pp.87-115), in 1934. Symptoms in a susceptible variety were described as a yellowing and downward turning of the first trifoliate leaves, followed by a distinct green and yellow mottle in higher leaves, and a severe dwarfing of the plant. In certain extremely susceptible varieties such as Mexican Tree, the first trifoliate leaves and epicotyl were killed, while in Brittle Wax and Giant Stringless, leaves were curled, and pods mottled and distorted. Corbett Refugee, Robust, and Great Northern U.I. 1, varieties resistant to bean common mosaic, were mildly affected, developing distinct mottles with little distortion or dwarfing in later growth stages. No seed transmission of this virus was found in extensive tests. On the basis of symptomology, host range, and the lack of seed transmission, this virus disease was considered to be distinct from the common mosaic disease of beans, previously described by Reddick (12, pp.530-534), Fajardo (3, pp.469-494), and others; and the causal virus was designated bean virus 2.

In more recent usage, this virus is known as bean yellow mosaic virus (BYMV). This nomenclature will be used during the remainder of the thesis.

BYMV was shown, by Grogan and Walker (5, pp.489-493) in 1948, to be related to bean common mosaic virus on the basis of cross-protection tests. In Stringless Green Refugee and Sensation Refugee 1066, bean common mosaic virus was found to effectively immunize plants against infections by BYMV, and conversely, BYMV gave partial or complete protection against the other virus. The chief differences between the two viruses are in host range, and the fact that bean common mosaic virus is seed-transmissible, while BYMV is not.

The virus described by Pierce is usually considered to be the typical or classical strain of BYMV. Several other distinct strains have been reported since 1934. In 1948, Grogan and Walker (4, pp.301-314) described a virus strain, isolated in Wisconsin, which produced severe pod distortion in many bean varieties, and which was concluded to be a strain of BYMV on the basis of symptoms in certain hosts and its failure to be transmitted by seed. Reactions in various bean varieties were divided into three groups: (1) no symptoms, (2) stunting, leaf deformation and mottle, without necrosis, (3) top necrosis with partial recovery, or a general necrosis which killed the plant, and (4) a modified necrosis causing yellowing and drop of tip leaves, but no death of the terminal. Pods borne on infected plants of any type were severely warted and misshapen. This strain differed from a typical BYMV strain, studied at the same time, in that it did not infect several leguminous hosts, including _Vicia faba_ L., _Melilotus officinalis_ (L.)Lam., _Melilotus alba_ Desr., and _Glycine max_ (L.)Piper. Inactivation properties were reported to conform to those described by Pierce (11, pp.100-104) for bean common mosaic virus and typical BYMV.

The occurrence of a severe form of BYMV in Western Oregon was described by McWhorter and Boyle (10, pp.1-4) in 1946. This virus, termed "X-disease," produced severe leaf, stem, and tip necrosis, which sometimes resulted in death of the plant. Typical mottles were also produced, at times, following the necrotic symptoms. Along with ordinary non-necrotic strains, it was considered to be the chief cause of economic loss in home and field plantings of beans and peas in Oregon at that time.

Zaumeyer and Fisher (14, pp.45-49), in 1953, described a necrotic-lesion producing strain of BYMV, isolated from a malformed bean pod in a field in Eastern Washington. All 44 bean varieties tested were classified as susceptible and were of three classes: (1) susceptible to necrotic lesions and systemic mottle, (2) susceptible to necrotic local lesions only, and (3) susceptible to systemic mottle only. Local lesions produced were distinct circular spots, or spreading veinal necrosis, and were produced on trifoliate leaves of a few varieties only. The host range differed from the type strain in that Phaseolus lunatus L., two species of Vigna, Nicotiana tobacum L., and Nicotiana rustica L. were infected, while Pisum sativum L., Trifolium incarnation L., Trifolium pratense L., and Trifolium repens L. were not susceptible.

Thomas and Zaumeyer (13, pp. 11-15), in 1953, described a virus from Blue Lake beans at Twin Falls, Idaho, which produced yellow-necrotic lesions on three species of tobacco. In beans, strong epinasty, chlorosis, and veinal necrosis were produced on the inoculated leaves, followed by severe systemic mottle in some varieties, and top necrosis and death in others. It was considered to be a severe-yellow-mosaic strain of BYMV, because of a similarity of properties, host range, and symptoms on bean varieties. No cross-protection was obtained from bean common mosaic virus, however.

Resistance to Bean Yellow Mosaic Virus

Relatively few common bean varieties have been reported resistant to the various forms of BYMV. In the original study of this virus by Pierce (11, pp.98-100) in 1934, all varieties tested were susceptible to systemic infection. The varieties resistant to bean common mosaic, Corbett Refugee, Robust, and Great Northern U.I. 1, developed systemic infection which was mild in comparison with that of other varieties.

Three varieties, Great Northern U.I. 59, G.N. U.I. 123, and G.N. U.I. 81, were reported resistant to the pod-distorting strain by Grogan and Walker (4, p.303) in 1948. All varieties tested were susceptible to infection by the typical BYMV strain used in their experiments.

Great Northern U.I. 15 and G.N. U.I. 59, however, were reported by Afanasiev and Morris (1, pp.101-104), in 1952, to be susceptible to viruses which occur in Montana bean fields, and which are considered to be typical BYMV strains. Some resistance to these strains was observed in Montana Great Northerns 1 and 43-15. In repeated greenhouse tests, these varieties showed only a low percentage of mild infections.

Hungerford and Hillyer (7, p.621), in 1954, studied the reaction of various bean varieties to virus strains isolated in Idaho and Washington and considered similar to the type strain of Pierce. Montana Great Northerns 1 and 43-15 were found to be as severely infected as Great Northerns U.I. 123 and 59. Also susceptible were G.N. U.I. 16, 31, and 56.

Great Northerns U.I. 31 and 16, which have been found in the present study to be resistant to systemic infection by a severe Western Oregon strain of BYMV, and Great Northern U.I. 123, were reported by Zaumeyer and Fisher (14,p.45) to form only necrotic local lesions when inoculated with the necrotic-lesion strain. These same three varieties, G.N. U.I. 16, 31, and 123, were reported by Thomas and Zaumeyer (13,p.11) to be the only bean varieties not infected with the severe strain of BYMV which produced local lesions on tobacco.

Great Northerns U.I. 16 and 31, according to Hungerford (6, p.7), originated as selections from crosses of G.N. U.I. 59 and curly-top resistant Red Mexican U.I. 34. G.N. U.I. 59 has been previously mentioned as being resistant to the pod-distorting strain by Grogan and Walker (4, p. 303).

Resistance to BYMV in the species <u>Phaseolus</u> <u>coccineus</u> L. has been reported by several workers. Pierce (11, pp.106-107) inoculated <u>P. coccineus</u> L. plants with the typical strain in 1934, and indicated there was no infection. Zaumeyer and Fisher (14, p. 46), in 1953, found no infection in plants inoculated with the necrotic-lesion strain described at that time. Thomas and Zaumeyer (13, p.13), also in 1953, reported the Streamline variety of <u>P.</u> <u>coccineus</u> L. to be resistant to the severe form of BYMV which produced local lesions on tobacco.

Gladioli as a Source of Field Infection

During the course of this study, gladioli were used as a source of natural infection for the field testing of parental materials and certain progeny lines of beans. The production of an infection of BYMV in beans by virus from the gladiolus was first described by McWhorter and co-workers (9, pp.177-178) in 1947. The frequent observation of severe infection of beans planted adjacent to commercial gladioli fields in Western Oregon, led to the confirmation of this plant as a host of BYMV. Viruses, including both the necrotic and typical forms of BYMV, were isolated from gladioli in successful inoculations of *Vicia faba* L.

MATERIALS AND METHODS

Definition of Resistance

A resistant bean plant, variety, or strain, is defined as one in which no systemic symptoms are produced when rub-inoculation is employed. Localized necrosis may occur on the inoculated leaves only.

Bean Varieties

The principal bean varieties and breeding lines used during these studies are described below. When not otherwise stated, the supply of seed used in the experiments was produced on the farm of the Vegetable Crops section, Department of Horticulture, Oregon State College.

Phaseolus vulgaris L.

O.S.C. 21 Blue Lake (hereafter designated O.S.C. 21). This line originated from a single plant selection made from the F.M. 1 Blue Lake variety (Ferry Morse Seed Company) in 1951. Originally selected for early vigor of growth, O.S.C. 21 was retained because it displayed some promise as a quality Blue Lake type, but was never exploited beyond a preliminary testing stage. Typical of the current Blue Lake varieties, O.S.C. 21 is a true pole bean line, with indeterminate growth, long internodes, and twining habit. The pods are dark green, round, fleshy, fiberless, small-seeded, and fairly straight, but with a tendency for moderate segmentation in the late processing stages. The mature seeds and the flowers are white. It is resistant to ordinary strains of bean common mosaic, but susceptible to infection by BYMV, developing a severe systemic mottle, accompanied by leaf and stem distortion, general stunting, and pod distortion when infected with the strains used in this study.

O.S.C. 22 Blue Lake (O.S.C. 22). The origin and general characteristics are the same as for O.S.C. 21. Because of a greater pod quality, O.S.C. 22 has received production and processing tests on a much larger scale than those given O.S.C. 21.

Great Northern U.I. 31 (G.N. 31). This variety was developed by the University of Idaho for resistance to the curly-top disease, and the types of bean common mosaic prevalent in Southern Idaho. It was selected from a cross of Great Northern U.I. 59 with Red Mexican U.I. 34 (6, p.7). It possesses the typical Great Northern characteristics: white, flat seeds; flat, stringy, fibrous, non-fleshy pods of a light green color; and semi-pole type growth with a heavy pod set and bushy growth at the base of the plant. G.N. 31 is resistant to curly-top, bean common mosaic, certain types of bean yellow mosaic, and halo bacterial blight.

The original seed stock was supplied by the University of Idaho (Department of Plant Pathology). When plants grown from this original seed supply, or from that produced the first year at this station, were inoculated with either strain of BYMV used in this study, an occasional distinctly susceptible plant was observed. Seed was saved from several of these plants, and the resulting progeny plants were all severely infected when inoculated, indicating that such susceptible plants were distinct genotypes present in the G.N. 31 stock as mixtures. In the spring of 1954, a number of uniformly resistant lines were established by rigorously testing a number of plants, saving seed from each plant individually, and testing a number of progeny plants in each line. No susceptibles were found in any of the lines, which were increased and used for hybridization and as resistant check plants during the remainder of the study.

Montana Great Northern 43-15 (Mont. 43-15). This Great Northern variety, developed by the Montana Agricultural Experiment Station, is very similar in general characteristics to G.N. 31. It has been reported by the Montana workers to be highly resistant to the strains of BYMV prevalent in Montana bean growing areas (1, pp.101-104), but is highly susceptible to the virus strains used in this study. The original seed for testing was received from Dr. Afanasiev of Montana State College.

Dwarf Horticultural. This standard dwarf variety of the horticultural type has open foliage, large pods which are flat and heavily mottled, and large red-mottled seeds. Although mosaic-resistant commercial strains may be available, the standard Dwarf Horticultural strain used as a virus stock plant in these studies, is highly susceptible to bean common mosaic, and bean yellow mosaic. It is also very susceptible to root rots and other fungus diseases. Supplies of seed were obtained from the Ferry Morse Seed Company.

Phaseolus coccineus L.

Six lines and varieties of P. coccineus L., the runner bean, were tested and employed in hybridization, although only the first listed below was used in crosses which were tested extensively. Both varietal names, when available, and Oregon State College Accession numbers are given below. In subsequent sections of the text, only the accession numbers, and in some cases the species name, will be used. Origins, other than the immediate source of seed, are not known.

In general, the Phaseolus coccineus L. strains are very vigorous, profusely flowering, and bear large, flat, very fibrous, stringy pods containing large seeds. All are true pole types.

E. M. Meader, of the University of New Hampshire, supplied the original seed of all strains except the last one listed, which was obtained from the Associated Seed Growers, Inc., Salinas, California.

The following is a list of the varieties or strains of P. coccineus L. studied.

Accession 2014 - Barteldes Lima - white seeded, white flowered.
" 2019 - Black Runner - black seeded, red-orange flowered.
" 2012 - Butterbean - white seeded, white flowered.
" 2018 - white seeded, white flowered.
" 2016 - Cornwall Giant - red-purple mottled seeds, orange flowers.
-- -- Scarlet Runner - red-purple mottled seeds, orange flowers.

Virus Strains

Two distinct strains of Bean Yellow Mosaic Virus were used in this study to determine the behaviour of parental varieties and the inheritance of resistance. The descriptions which follow were derived in part from information obtained during the experimental period.

The designations Y and W have been assigned to these two strains. They are similar in that each produces epinasty of the primary leaves, severe systemic mottle, and pod distortion in susceptible bean varieties; but differ in host range, as shown in Table 1, page 16. The pod-distorting characteristic (Figure 3 and 4, p.19) indicates a certain similarity between these strains and the pod-distorting strain of BYMV reported by Grogan and Walker (4, pp.301-314). No special studies have been made, however, to classify the Y and W-strains and to determine their relationships with strains reported in the literature.

The origin and characteristics of each strain are as follows:

Y-strain. The Y strain of BYMV was isolated from a severely infected planting of F.M. 65 Blue Lake beans in 1950 in the Willamette Valley of Oregon. Studies of seed transmissibility and cross-protection relationships with bean common mosaic virus (Figure 2, p. 18) were made soon after its isolation and before the beginning of this study. All tests for seed transmission have been negative. A strain of bean common mosaic virus gave complete cross-protection against the Y-strain in F.M. 65 Blue Lake. No further cross-protection tests have been made.

The thermal inactivation temperature in a ten-minute water bath is about 60 C, as observed when plant juice was heated to reduce the possibility of contamination by other viruses. Some infectivity remained after treatment at 60 C in each of three separate purifications carried out during the course of the study. The highest temperature at which this strain would remain infective was not determined.

A summary of the available host range information for the Y-strain may be found in Table 1, page 16.

W-strain. The W-strain was provided by Dr. W. J. Virgin of the California Packing Company, Sunnyvale, California, who considered it to be a severe strain, valuable for use in breeding beans for resistance to BYMV. Little study has been made of its characteristics and classification. There has been some indication that a contaminating strain or distinct virus has been maintained with the W-strain, or that possible mutational changes have taken place since it was acquired. In the early stages of this study, the W-strain was decided upon, in favor of the Y-strain, because of greater severity and infectivity, with the result that several populations of F_2 plants and 225 F_3 families were tested exclusively with this strain. Its use was discontinued in the spring of 1955 in all tests of progenies with G.N. 31 as the resistant parent, because at that time systemic infection was observed in this variety. No systemic infection had previously been observed in G.N. 31 inoculated with the W-strain. Because of its distinct symptoms, and high infectivity which resulted in a smaller percentage of escapes, some further use was made of this strain in tests of progenies of O.S.C. 22 X 2014 (*P. coccineus* L.). Accession 2014 has never been infected by either virus strain in these studies.

The W-strain was inactivated, in the early stages of the work, by heating for ten minutes at 60 C, but since its observed change in behaviour in the G.N. 31 variety, a test was made in which some survival was recorded at this temperature.

Seed transmission has not been observed, although no special study has been made of this characteristic, or of cross-protection relationships with bean common mosaic virus.

Host Range Information for the Y and W-strains. Host range or host preferance information available for the Y and W-strains is presented in Table 1. These data were obtained largely from tests conducted in conjunction with the inheritance study. In some cases the numbers of plants involved are low, and results are possibly not reliable when negative. The data for tests of common bean varieties represent one particular test only, although hundreds of plants of Dwarf Horticultural, O.S.C. 21, G.N. 31, and others, were inoculated during the course of the study as stock or control plants for progeny tests. The test recorded for G.N. 31, in which systemic infection was observed, was made in the summer of 1955. Prior to the fall of 1954, no systemic infection was observed in this variety when it was inoculated with the W-strain. The results for inoculations of *P. coccineus* L. lines represent all the plants tested, except for 2014, which was inoculated as a resistant control in several progeny tests.

Table 1. Summary of Host Range Information Available for the Y and W Strains of BYMV.

Species Inoculated	Number infected/Number inoculated	
	Y-strain	W-strain
Phaseolus vulgaris L., variety:		
Topcrop	5/5	3/3
Pencil Pod Wax	5/5	5/5
Landreth's Stringless	5/5	6/6
Kentucky Wonder	4/4	5/5
Tendergreen	6/6	5/5

Table 1. - continued.

Species Inoculated	Number infected/Number inoculated	
	Y-strain	W-strain
Phaseolus vulgaris L., variety:		
Contender	3/3	3/3
Blue Ribbon Bush	5/5	5/5
Hyscore	5/5	5/5
Purple Pod	4/4	5/5
Red Kidney	5/5	5/5
Puregold	0/18	5/5
Great Northern U.I. 59	3/12	-
G.N. 123	8/12	-
G.N. 81	5/12	-
Red Mexican U.I. 3	8/12	-
Red Mexican U.I. 34	11/12	-
Pinto U.I. 78	11/12	-
Pinto U.I. 111	11/12	-
Pinto U.I. 72	12/12	-
Idaho Refugee	9/19	-
O.S.C. 21 Blue Lake	5/5	11/11
G.N. 31	0/10	5/5
Bountiful	15/15	11/11
Dwarf Horticultural	11/11	11/11
Phaseolus coccineus L., var.:		
Barteldes Lima (Accession 2014)	0/46	0/18
Black Runner (Acc. 2019)	-	0/21
Butterbean (Acc. 2012)	-	0/17
White Flowered Acc. 2018	-	0/15
Scarlet Runner	0/17	0/23
Cornwall Giant (Acc. 2016)	-	0/12
Vicia faba L.	9/20	3/9
Glycine max (L.)Piper	0/12	8/12
Vicia villosa Roth.	0/12	12/12
Trifolium pratense L.	0/40	0/34
Trifolium incarnation L.	4/19	10/23
Trifolium repens L.	0/31	0/18
Trifolium hybridum L.	3/24	7/13
Melilotus alba Desr.	0/40	7/26
Melilotus officinalis (L.)Lam.	2/26	11/26
Pisum sativum L., variety:		
W. R. Perfection	0/12	0/12
Little Marvel	0/11	0/11
Surprise	0/12	0/12
Wando	0/12	0/12
Tall Telephone	0/12	10/12
Freezonian	4/12	6/12

Figure 1. Typical symptoms of the Y (left) and W (right) strains of BYMV in leaves of the Bountiful bean variety.

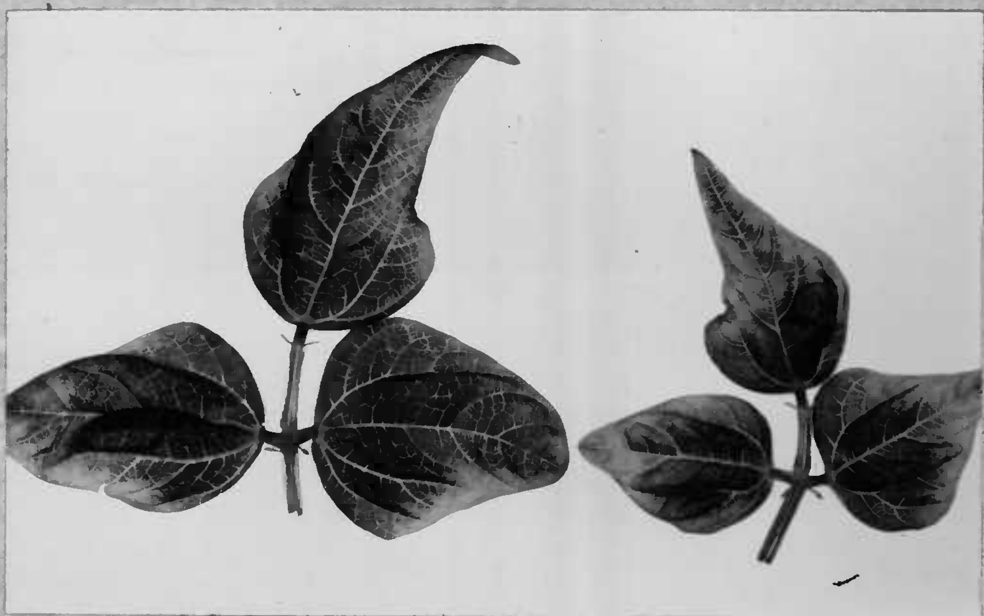

Figure 2. Young leaves of the Dwarf Horticultural bean variety infected with the bean common mosaic virus.

Figure 3. Pod distortion in the Topcrop bean variety infected with the Y strain of BYMV; check on the top.

Figure 4. Distorted pods of Dwarf Horticultural infected with the Y strain of BYMV; check on the right.

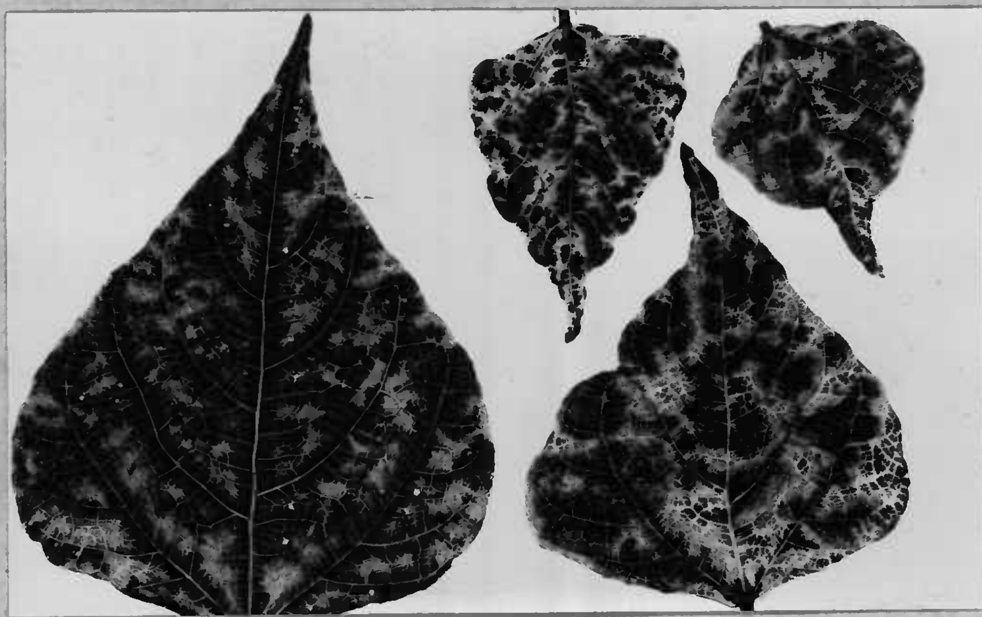

Figure 5. Symptoms produced in the Dwarf Horticultural bean variety inoculated with the Y strain of BYMV.

Figure 6. Symptoms of the Y strain of BYMV in Vicia faba L.

General Methods of Plant Culture

Plants were grown in three general ways, depending upon the season of the year, the needs for the particular phase of work, and the facilities available. These three general situations are described below.

Bench Greenhouse. During most seasons, plants were grown in one-gallon cans, on permanent greenhouse benches. The soil used consisted of about two-thirds field soil, primarily Chehalis loam, and one-third mason sand. For all principal tests, the soil was unsterilized, and about four grams of complete commercial fertilizer was mixed into the upper half before planting. To insure uniform germination, the top of the soil was smoothly packed and the seeds were covered with measured quantities of mason sand.

Ground-bed Greenhouse. During the spring and early summer, a portion of the work was done in greenhouses where plants were grown in ground beds of fine sandy loam soil. Seeds were planted about three inches from a band application of ammonium phosphate (11-48-0) commercial fertilizer, in rows two to three feet apart. Plants were usually grown on string trellises.

Field. Field plantings were made in May or early June in Chehalis loam. Prior to planting, a band application of 400 pounds of 11-48-0, or 600 pounds of 10-16-8 commercial fertilizer was made. Seeds were planted individually by hand, or with a belt-type hand planter that was designed to plant any size or type of seed without danger of seed mixtures. Plants were spaced from three to six inches apart, or one to two feet when it was necessary to harvest seed from each plant individually. Except for F_3 families grown for testing, the plants were grown on wire-supported string trellises, or on wooden stakes. Sprinkler irrigation was applied every ten days, or when needed.

General. Insects were controlled as much as possible, by dusting or fumigation, in all plantings.

A general practice adopted during this study was that of scarifying seeds in order to insure prompt and uniform germination, and thereby facilitate more uniform inoculation. This practice was needed because of the tendency for seeds, when thoroughly dried, to develop hard seedcoats that were impermeable to water. Scarification was accomplished by rubbing the seeds on sandpaper to produce a small abrasion in the seed coat.

Light and Temperature. No control of light conditions was possible except for the whitewashing of greenhouses during the summer to reduce the intensity of sunlight. It is recognised that the usual light intensities during the cloudy winter weather in Western Oregon are not adequate for optimum plant growth. Although such environmental conditions as light and temperature may affect the expression of plant disease symptoms, it was not possible to clarify such effects in the present study.

Temperature control was possible to an extent in greenhouse culture. The bench greenhouse was equipped with hot-water coils, and manually operated ventilators. During winter months, the day temperatures were maintained at 65 to 70 F when possible, and night temperatures fluctuated from 50 to 60 F. During the late spring and early fall, temperatures fluctuated considerably with variable weather conditions.

The ground-bed greenhouses were equipped with thermostatically controlled, hot water, blower-type heaters, and manually operated ventilators. Night temperatures were maintained at 50 to 60 F. Day temperatures ranged from 70 to 75 F during cloudy weather, but fluctuated from about 60 to 85 F during variable or sunny periods when it was necessary to maintain open ventilators.

Hybridization

Hybridization between bean varieties was accomplished by means of hand emasculation and pollination. Flowers of the plant used as the seed or pistillate parent were emasculated and prepared for pollination one day before normal opening, by pulling aside the outer petals, splitting the keel, and removing the immature stamens with sharply pointed forceps. From open flowers of the male or staminate parent plant, the stigma, which usually carries a generous supply of free pollen at this stage, was removed and rubbed upon the exposed stigma of the female flower.

Immediately after pollination, the flowers were enclosed in a hollow wrapping of moist tissue paper to prevent drying of the exposed stigmatic surface, and reduce the possibility of contamination by foreign pollen. The tissue paper wrappers were removed in about five days to prevent the deformation of the pods.

The crosses made during this study are listed below, in chronological order. Spring crosses were made on plants grown in greenhouse ground beds, while summer crosses were made in the field.

Spring-1953. The initial crosses were made between O.S.C. 21 and G.N. 31. Parent plants were not tested for disease reaction. The reciprocal crosses made were the following:

O.S.C. 21 X G.N. 31

G.N. 31 X O.S.C. 21

Hereafter, the notation O.S.C. 21 X G.N. 31, will refer to the reciprocal crosses, unless otherwise specified.

Summer-1953. An interspecific cross was made as follows:

O.S.C. 22 (*Phaseolus vulgaris* L.) X 2014 (*P. coccineus* L.)

In addition, the F_1 generation of O.S.C. 21 X G.N. 31, from crosses made in the spring of 1953, was reciprocally backcrossed to each parent, as listed below:

F_1 X O.S.C. 21

O.S.C. 21 X F_1

F_1 X G.N. 31

G.N. 31 X F_1

Spring-1954. Greenhouse crosses were again made between O.S.C. 21 and G.N. 31 because those made in the spring of 1953 involved untested G.N. 31 plants. It was considered necessary to repeat these crosses under more controlled circumstances, because of the presence, in the G.N. 31 stock, of a low percentage of distinctly susceptible individuals, as mentioned previously in the description of this variety (p. 11). The thirty-one G.N. 31 plants used for crossing were grown under favorable conditions in the ground-bed greenhouse and tested rigorously for susceptibility to both the W and Y virus strains. Inoculation with the W-strain on the primary leaves, and later with the Y-strain on the first or second trifoliate leaf, failed to produce symptoms, except for vein necrosis of the inoculated leaves, although the 25 O.S.C. 21, and 14 Dwarf Horticultural plants were severely infected at the time of the inoculation with the Y-strain. When two to three months of age, each G.N. 31 plant was sampled from the upper leaves, and test inoculations were made on Dwarf Horticultural. All tests were negative. One G.N. 31 plant developed a mild systemic mottle when 4½ months of age, however. A sample from this plant produced systemic infection in Dwarf Horticultural, and in turn, early mild systemic mottles in a group of young G.N. 31 plants. This infection was considered to be caused by a naturally transmitted virus strain, different from the Y and W-strains, but the crosses involving the original infected G.N. 31 plant were not included in F_3 tests.

As a further test of the G.N. 31 parent plants, seeds were saved from each, and from 15 to 40 plants of each progeny were grown in the field in 1954 and inoculated with the W-strain. No distinct infection resulted, although several plants in the entire planting developed mild symptoms. Inocula from these plants were found to infect young G.N. 31 plants in the greenhouse, and were considered to contain field transmitted viruses other than the Y and W strains. Several uniformly resistant lines of G.N. 31 were obtained from these tests and used during the remainder of the study for check plants or for hybridization.

Summer-1954. Reciprocal backcrosses were made between G.N. 31, and the F_1 generation of the crosses made in the spring of 1954 between this variety and O.S.C. 21:

F_1 X G.N. 31

G.N. 31 X F_1

The G.N. 31 plants used in these backcrosses were from the original seedstocks, and were inoculated twice, once on the primary leaves, and once on the first trifoliate leaves, to eliminate possible susceptible individuals.

G.N. 31 was also hybridized with Montana Great Northern 43-15. Only G.N. 31 X Mont. 43-15 was made; the reciprocal cross was not obtained.

O.S.C. 21 was hybridized with each of the P. coccineus L. varieties listed on page 13, in order to obtain information on the susceptibility of the F_1 generation.

Seed Production and Harvest

Seed for the F_2 and F_3 generation tests was produced in the field, except for a portion of the F_3 seed, which was produced in the ground-bed greenhouse.

In the case of the 1953 reciprocal crosses between G.N. 31 and O.S.C. 21, the F_2 seeds were mass harvested, with the reciprocals separate. F_2 seeds from the crosses O.S.C. 22 X 2014 were also mass harvested.

F_2 seeds from the 1954 reciprocal crosses between O.S.C. 21 and

G.N. 31, and the cross of G.N. 31 X Mont. 43-15, were harvested separately from individual F_1 plants, and maintained through the testing program as F_2 families.

The production of seed for the F_3 generation was accomplished by planting F_2 seeds at wide spacings, and harvesting seeds separately from each individual plant. In the case of the 1954 crosses between O.S.C. 21 and G.N. 31, and G.N. 31 X Mont. 43-15, F_3 families were also grouped and identified according to the F_2 family from which they originated. This identity was maintained during the testing program.

General Inoculation Methods

Maintenance of virus strains and the source of inoculum. Dwarf Horticultural bean plants were used as the source of inoculum for all tests of resistance. The virus strains were maintained, by repeated transfers, in this variety from the beginning of the study until October, 1954. At that time, as a precaution against serious modifications of the virus behaviour through mutation or virus mixtures, a quantity of each strain was preserved by means of the dehydration technique described by McKinney (8, pp.615-620). This method consisted of placing 15 grams of young infected leaves, wrapped in cheesecloth, in wide-mouth pint mason jars, containing an equal weight of anhydrous calcium chloride. The jars were sealed tightly and placed in a refrigerator for one to two weeks, after which the dried leaves were transferred to four-ounce screw top jars containing approximately

1.5 grams of anhydrous magnesium perchlorate. These jars were stored indefinitely in a refrigerator.

A second supply was preserved in April, 1955, from plants inoculated with material stored the previous October. The procedure used following this preservation consisted of the inoculation of Dwarf Horticultural plants, and subsequently making two or three sub-transfers into groups of young plants before again using preserved leaves as a source of inoculum. Inoculum maintained in this manner was used for the major portion of the testing program with the Y-strain. The W-strain was used during this period only for certain tests involving *P. coccineus* L. crosses.

Age and condition of test plants. Plants were normally inoculated in the early primary leaf stage. It was found through observation that greater inoculation success was attained if plants were inoculated during the first or second day after the unfolding of the primary leaves. Less leaf injury and a higher percentage of infected plants resulted at this time than at later stages when the primary leaves were more fully expanded. During the early stages of the study, this relationship was not realized, with the result that the age of inoculation was somewhat variable, and in some cases the success of the test was possibly reduced. From the fall of 1954 until the termination of the work in the spring of 1956, the period which included the major inoculations, the age of the plant was given considerable attention.

In certain cases, a second inoculation was made on the first or second trifoliate leaf, while in other tests plants were reinoculated on young lateral leaves - produced as a result of a cutting-back of the plant.

Preparation of inocula. The inoculum used for testing was prepared from young infected leaves thoroughly ground by means of a mortar and pestle. During the grinding of the leaves, sufficient tap water was added to the preparation to attain the desired dilution. This dilution was reached, in the earlier stages of the study, by measuring the plant juice along with a known quantity of water added to facilitate grinding, and in the final year of work, by weighing the leaves before the grinding process. With the exception of certain preliminary tests, measured dilutions of four to five parts of water to one part of plant juice were employed.

Inoculation technique. Before inoculation, each leaf was lightly dusted with 400-mesh silicon carbide powder. The infective plant juice was rubbed gently but firmly over the leaf surfaces with one or several fingers. Cotton or cheesecloth pads were used for the early inoculations, but their use was discontinued. A comparative test in February, 1954, involving 200 O.S.C. 21 plants, indicated that the finger method gave equal or superior results, with less leaf injury, greater convenience, and faster inoculation.

In all greenhouse tests, the plants were washed with a sprinkling hose soon after inoculation, but this practice was not consistently followed for the field inoculations.

In most cases, inoculations were made during the morning - usually before 10:00 A.M.

Resistance Tests

A brief description of the resistance tests made for each cross is given on the following pages. Tests of parental varieties are not included in the descriptions. Preliminary tests, with varying numbers of plants, were made for each parent line or variety, and in addition, a number of plants of the parents were included in each test as susceptible and resistant controls.

O.S.C. 21 X G.N. 31

F_1 generation. (1) A very small preliminary test was made in the ground-bed greenhouses in April, 1953, soon after the first F_1 seeds were available. In this test, nine F_1, two O.S.C. 21, and three G.N. 31 plants were inoculated with the W strain of BYMV. (2) A second test, involving 80 plants was made in the summer of 1953 in the field. Plants of each reciprocal cross were grown in four plots of ten plants each. Five plants in each plot were inoculated with the W-strain, and five with the Y-strain. When 43 days of age, all plants without symptoms were reinoculated on the youngest leaves. Final infection counts were made at the end of the season. (3) The F_1 generation of the crosses made in the spring of 1954 was tested in the field in the summer of 1954. A total of 33 plants were inoculated with the W-strain. Reinoculations were made at three weeks of age of the F_1 and O.S.C. 21 plants not showing symptoms, and of all G.N. 31 plants. Infections were recorded in July and August.

F_2 generation. Because a continuous and wide variation of symptoms and a high escape rate made the classification of individual segregates difficult and uncertain, only a limited amount of F_2 testing was done. Preliminary F_2 tests, made in the field in 1953, and in the bench greenhouse in November, 1953, will not be mentioned further, because of the very high percentages of escapes which were obtained. The following additional F_2 tests were made in the greenhouse during the study, and contributed supporting data to the F_3 tests which followed. These tests were designed to identify plants homozygous for resistance, by means of a test of their F_3 progenies. The need for this approach was apparent early in the study.

(1) In January, 1954, the following plants were tested:

199 - F_2 of the cross O.S.C. 21 X G.N. 31 (reciprocal not included).
104 - O.S.C. 21, inoculated check plants.
106 - G.N. 31, inoculated check plants.

In addition, approximately equal numbers of each were grown as uninoculated checks, because of the possible occurrence of abnormal growth under winter conditions. Inoculations, with a 10:1 dilution of the W-strain, were made on three occasions, at five-day intervals, because of uneven germination. Because five day intervals allowed some plants to reach five days of age before inoculation, it is now recognized that many of these plants were probably too old when inoculated and that the success of the initial inoculations may have been reduced to some extent.

Plants were removed and counted when they were visibly infected. At six weeks of age, all remaining F_2 and O.S.C. 21 plants were cut back to encourage lateral growth, and at eight weeks, reinoculations with the W-strain were made on the young leaves. Seeds were harvested from all plants which did not become infected throughout the test, and progenies were grown and tested in the field in the summer of 1954 with a 5:1 dilution of the W-strain. F_4 plants from the most resistant progenies were also tested later in the greenhouse.

(2) In March, 1954, the following materials were inoculated with the W-strain:

O.S.C. 21 X G.N. 31 F_2	- 476	plants
G.N. 31 X O.S.C. 21 F_2	- 442	"
O.S.C. 21	- 219	"
G.N. 31	- 59	"

A few plants of each variety and progeny were also grown as uninoculated checks. The general procedure in this test was the same as that for the previous test. However, sunlight conditions were much better during most of this period, and symptom expression was greatly improved. Plants remaining four weeks after inoculation were cut back and reinoculations made the following week. Additional visibly infected plants were eliminated, and several questionable plants were proved infected on the basis of test inoculations on Dwarf Horticultural. From 10 to 40 progeny plants from each uninfected F_2 plant were tested in the field in 1954 with the W-strain.

<u>F_3 generation.</u> (1) In the summer of 1954, 225 random F_3 families of the 1953 crosses were tested in the field with the W-strain. The number of plants tested in each family depended upon the amount of seed available, and ranged from 15 to 60. About two weeks after the first inoculation, all distinctly infected plants were eliminated and

the remaining plants were reinoculated on young leaflets. Final counts were made at the end of the growing season. The following winter, using small seed remnants in the bench greenhouse, retests were made of all families that were uninfected or contained only several infected plants in the field. This test also employed the W-strain. Several families which were very mildly infected or uninfected in the greenhouse retests, were inoculated as F_4 lines the following spring (1955) in the ground-bed greenhouse, using both the Y and W virus strains.

(2) 409 F_3 families of the original crosses between O.S.C. 21 and G.N. 31 were tested in the spring of 1955 with the Y-strain. Five plants each of 270 families were planted in the ground-bed greenhouse on March 28. A second randomized planting of the same 270 families was made on April 4, but was used only when it was necessary to confirm the results of the first planting. Detailed symptom descriptions were made for these families, for use in a discussion of the effects of genetic segregation on the symptom expression of BYMV. The remainder of the 409 families tested during this season were planted in the bench greenhouse in three groups, the last of which was planted on June 1. Five or six plants of each family were tested. For all of the families tested during this season, plants were retained and observed until pods were present in most cases. Family classifications were made on the basis of the severity and type of symptoms present. No reinoculations were made, but families giving questionable results were replanted and retested in the late spring of 1955.

(3) Six plants each of 595 F_3 families from the 1954 crosses between O.S.C. 21 and G.N. 31 were tested in the bench greenhouse in the fall of 1955 and the early spring of 1956. The inoculum and general procedures were the same as for the spring-1955 tests described above. Plants were inoculated with a 5:1 dilution of the Y-strain, and families were discarded when susceptibility was apparent. Uninfected or questionable families were discarded after approximately six-weeks observation, and retests were made in a planting made on February 17, 1956.

<u>F_1 backcross generation</u>. (1) First generation backcross plants of the 1953 crosses, which included backcrosses to the susceptible parent, were inoculated with the W-strain in the ground-bed greenhouse in the spring of 1954. Plants were not reinoculated. Resistant check plants, because of a space limitation, consisted of 40 G.N. 31 plants which were planted earlier for crossing purposes and inoculated with the W-strain, followed by the Y-strain. Readings were taken and plants with systemic mottle were eliminated at intervals, until the test was terminated when the remaining plants were about eleven weeks old.

(2) Backcross plants of the 1954 crosses between O.S.C. 21 and G.N. 31, consisting in this case only of backcrosses to the resistant parent, were tested in the summer of 1955 in the bench greenhouse. In this case plantings were made in soil-filled benches, as well as in gallon cans. All plants were inoculated with the Y-strain. Approximately four weeks after the first inoculation, uninfected plants were cut back and subsequently reinoculated. Although this test was made

during the summer, light conditions were not ideal because the greenhouse was whitewashed to reduce heat, and because it was necessary to crowd the plants considerably.

G.N. 31 X Mont. 43-15

A 5:1 dilution of the Y-strain was used for all inoculations made in tests of these progenies. All plants were grown in gallon cans in the bench greenhouse.

F_1 generation. Only 15 F_1 plants of this cross were available for testing. Six of these were tested in July, 1955, in conjunction with the F_2 test of the same cross. The remaining nine plants were tested in March, 1956, with the final F_3 family retests.

F_2 generation. 299 F_2 plants were inoculated in August, 1955. Susceptible plants were eliminated as observed, and uninfected survivors were retained for five weeks before being discarded.

F_3 generation. Tests of 366 F_3 families were made in the fall and winter of 1955, and the spring of 1956, using the same procedures as were employed for the F_3 families of the 1954 crosses between O.S.C. 21 and G.N. 31. Replantings were made in March, 1956, of families which were uninfected in the first test.

Phaseolus vulgaris L. X *P. coccineus* L.

F_1 generation. F_1 tests for these crosses were very limited. Six plants of O.S.C. 22 X 2014 F_1 were inoculated in the field in 1954 with the W-strain. In the summer of 1955, in the greenhouse, small numbers of plants were tested for each of several crosses of O.S.C. 21 X *P. coccineus* L. lines. These crosses are listed in the results section for the interspecific crosses.

F_2 generation. Three separate tests were made of F_2 plants from the cross O.S.C. 22 X 2014. Because of differences in the methods used, each is described separately below.

(1) On December 6, 1954, in the bench greenhouse, 156 plants were inoculated with the W-strain. Test inoculations were made on Dwarf Horticultural for a number of plants with possible infection, but those with no indication of symptoms were counted as uninfected, and the test was discontinued on February 10, 1955, six weeks after the inoculation.

(2) On February 20, 1955, 288 plants were inoculated in the greenhouse ground beds with the Y-strain. Three weeks later, a second inoculation was made on the youngest leaves of all plants which did not show distinct symptoms. Final records of visible symptoms were taken eight weeks later, on May 6, after which tests were made on Dwarf Horticultural for all plants with questionable or negative readings.

(3) The third planting consisted of 188 plants inoculated with the W-strain, and 97 with the Y-strain, on June 1, 1955, in the bench greenhouse. This test was made to obtain additional inheritance information, and to compare the severity of the two virus strains on this progeny. All plants not apparently infected in three weeks were reinoculated with the respective virus strain. The test was terminated in ten weeks, after all symptomless or questionable plants were tested by an inoculation on Dwarf Horticultural.

Grafting Experiments

Small experiments were conducted in the summer of 1955 to determine whether G.N. 31 and 2014 (P. coccineus L.) would become systemically infected when approach-grafted to infected plants of a susceptible variety.

Ten young G.N. 31 plants were approach-grafted to previously infected Dwarf Horticultural plants, and six were grafted to O.S.C. 21 or susceptible F_3 progeny plants. Four 2014 plants were grafted to infected O.S.C. 21. Grafts were made by splitting off half of the stem of each of the plants, which were growing in the same container, then binding the cut surfaces together with masking tape. The plants were retained until they reached an advanced stage of maturity.

Natural Infection with Gladioli as the Source of Inoculum

In the summer of 1955, a planting was made adjacent to a small plot of gladioli on the farm of the Department of Botany and Plant Pathology. The purpose of this test was to observe the behaviour of all parents used in this study, various progeny materials, and miscellaneous commercial varieties, when exposed to natural infection by insect transmitted viruses carried in gladioli. Because the gladioli were not planted sufficiently early, it was necessary to delay the planting of the test plants until July 11, which is much later than the normal planting date for commercial bean crops in Oregon.

The general arrangement of this planting is shown photographically in Figure 7, page 38. The north edge of the bean planting was located 13 feet south of the first of three rows of mixed gladioli, and separated from them by a row of cucumbers, and a row of untrellised F.M. 1 Blue Lake beans which were not a part of this experiment, but which were heavily infected early in the test period, and probably served as an additional source of inoculum. Row spacing for the test plots, and the adjacent materials, was four feet. All indeterminate bean varieties or lines were allowed to grow on four-foot stakes.

One plot of each variety or line was planted at random, except for O.S.C. 21, Mont. 43-15, G.N. 31, and Dwarf Horticultural, each of which was included in each of the four rows. Two plots of G.N. 31 X Mont. 43-15 F_2 were included.

Figure 7. Beans planted near gladioli in a study of natural field infection, with cucumbers and unstaked Blue Lake beans between the bean plots on the right, and the gladioli on the left.

Analysis of Data

Experimental ratios were compared statistically with theoretical genetic ratios by means of the chi square test for goodness of fit.

Calculations were made using the formula:

$$\text{chi-square} = \sum \frac{D^2}{E}$$

where D equals the difference between observed and expected (theoretical) values, and E equals the expected value. Because all ratios included only two classes, the Yates correction factor of -0.5 was applied, unless the difference between the observed and expected values was less than 0.5. The corrected formula, when the Yates correction factor was applicable, was as follows:

$$\text{chi-square} = \sum \frac{(D - 0.5)^2}{E}$$

RESULTS OF RESISTANCE TESTS

O.S.C. 21 X G.N. 31

Parent Varieties

Data for inoculations of O.S.C. 21 and G.N. 31 may be found in all tables of results for resistance tests in which these varieties were used as susceptible and resistant controls, respectively.

The proportion of O.S.C. 21 plants infected was variable, but generally high. Symptoms were relatively severe in all cases, but varied somewhat according to the season; the terminals of plants inoculated in winter were often killed at an early stage and the plant was generally more dwarfed than those tested in spring or summer. Typical leaf symptoms are shown in Plate 12, and plant symptoms are shown in Plates 1 and 3.

G.N. 31 was not systemically infected by the Y virus strain during this study, although hundreds of plants were inoculated. As discussed in the description of this variety, on page 11, the original stock contained a small percentage of distinctly susceptible plants that were found to breed true for susceptibility and were assumed to be mixtures. Uniform lines of G.N. 31 were used after they were established in 1954, and no susceptible plants were observed in plantings inoculated with the Y-strain after that time. Inoculations of this variety with the Y-strain in the summer, spring, or fall, normally produced discrete, reddish-brown, necrotic local lesions on the inoculated leaf. These discrete lesions were usually followed by

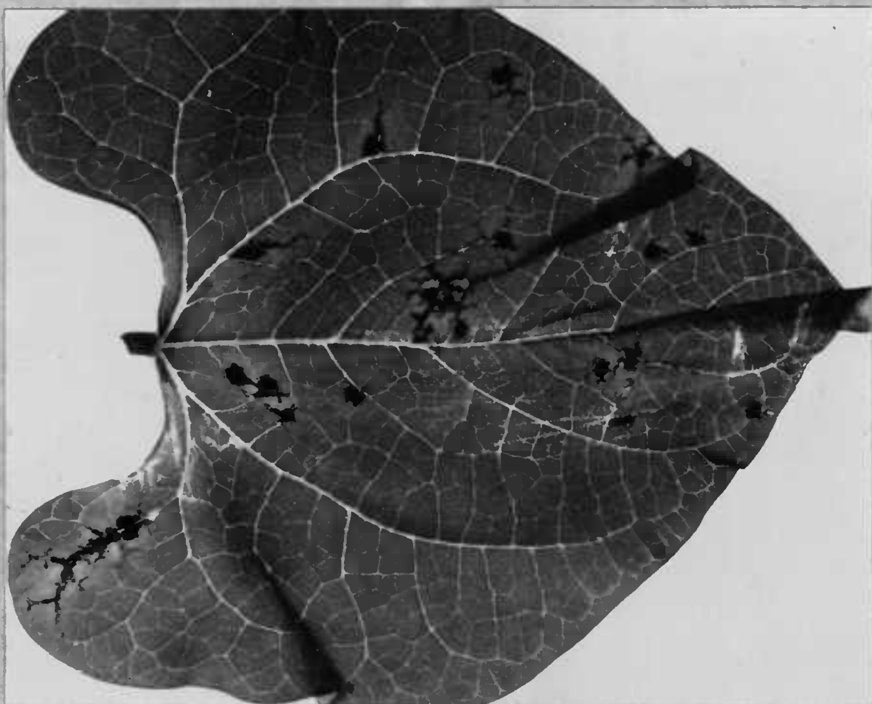

Figure 8. A primary leaf of G.N. 31 inoculated with the Y strain of BYMV, showing discrete necrotic lesions and the beginning of vein necrosis.

spreading necrosis of the veins (Figure 8). Plants inoculated under winter conditions usually did not develop clearly visible lesions.

During the first two years of the study, inoculations of G.N. 31 with the W-strain produced only inoculated leaf symptoms similar to those described above for the Y-inoculated plants. Subsequent to the fall of 1954, the W-strain caused systemic vein necrosis and mild mottle, in addition to the usualy inoculated leaf symptoms, in approximately one-half of the inoculated plants (Figure 9, p. 42). Use of the W-strain was discontinued at that time for all crosses in which G.N. 31 served as the resistant parent.

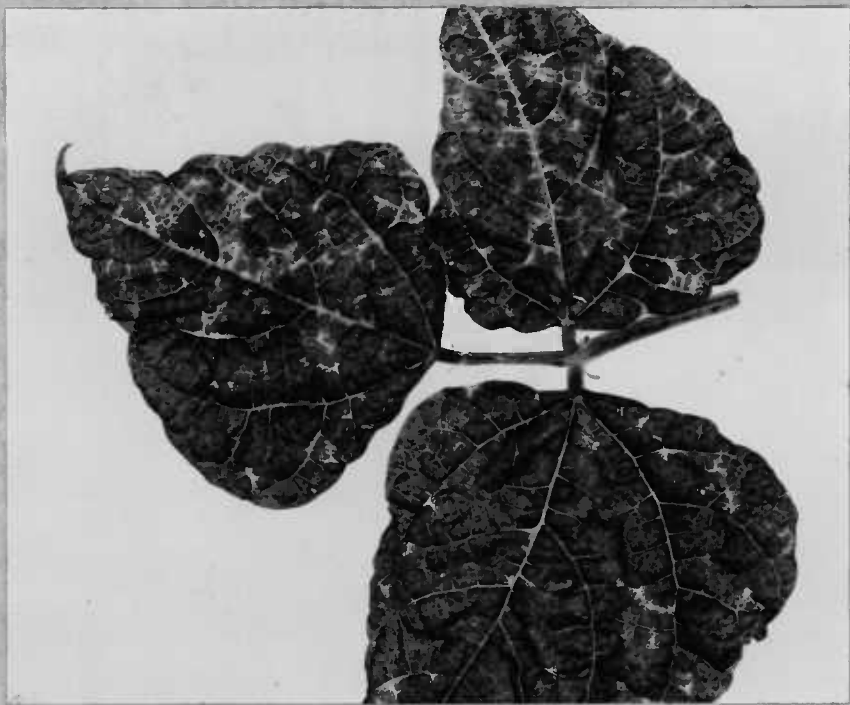

Figure 9. Systemic infection of G.N. 31 inoculated with the W strain of BYMV in January, 1955.

F1 Generation

The results of all F_1 generation tests for O.S.C. 21 X G.N. 31 are presented in Table 2, page 43. Dominance of susceptibility in this generation is clearly shown by these tabulated data.

The number of F_1 plants free from infection was only slightly larger than the number of uninfected O.S.C. 21 plants in each case. Symptoms resembled those of O.S.C. 21 in type and severity, and in the time of their appearance.

Table 2. Behaviour of the F_1 Generation of O.S.C. 21 X G.N. 31 Reciprocal Crosses when Inoculated with the Y and W Strains of BYMV

Test	F_1 Progeny or Check Variety	Plants Tested	Plants Infected	Plants Uninfected
1. Greenhouse 1953 W-strain	G.N. 31 X O.S.C. 21	6	5	1
	O.S.C. 21 X G.N. 31	3	3	0
	O.S.C. 21	2	2	0
	G.N. 31	2	0	2
2. Field 1953 W-strain	G.N. 31 X O.S.C. 21	20	18	2
	O.S.C. 21 X G.N. 31	20	17	3
	O.S.C. 21	19	18	1
	G.N. 31	20	0	20
Y-strain	G.N. 31 X O.S.C. 21	20	19	1
	O.S.C. 21 X G.N. 31	20	20	0
	O.S.C. 21	19	18	1
	G.N. 31	20	0	20
3. Field 1954 W-strain	G.N. 31 X O.S.C. 21	15	11	4
	O.S.C. 21 X G.N. 31	18	17	1
	O.S.C. 21	28	27	1
	G.N. 31	77	0	77
Totals	G.N. 31 X O.S.C. 21	61	53	8
	O.S.C. 21 X G.N. 31	61	57	4
	O.S.C. 21	68	65	3
	G.N. 31	119	0	119

F_2 Generation

Results of the F_2 progeny test of January, 1954, are shown in Table 3, page 44. Because of the continuous variability in the time of appearance and the severity of symptoms, plants were classified only as infected or non-infected. The chi-square tests for the F_2 ratios were applied only in the case of final results after the field progeny tests.

Table 3. Results of January-1954 Inoculations of O.S.C. 21 X G.N. 31 F_2 with the W Strain of BYMV

Progeny or Check	Plants Tested	Greenhouse Results		Final Results		X^2	P *
		Infected	Free	Infected	Free	63:1	
O.S.C. 21 X G.N. 31 F^2	199	179	20	196	3	.003	.98 - .95
O.S.C. 21	104	97	7	-	-		
G.N. 31	105	0	105	-	-		

* Range of probability of the deviation from the theoretical ratio

Approximately 28% of the F_2 plants and 23% of the O.S.C. 21 check plants were apparently uninfected prior to the second inoculation. At the termination of the greenhouse test, 10% of the F^2 plants, and approximately 7% of the O.S.C. 21 plants were symptomless. When F_3 families from the 20 remaining plants were tested in the field, 15 contained moderately to severely infected plants and were classified as susceptible. Three families contained only one or two plants with very mild infections and were classified as resistant with possible modifier effects. Greenhouse tests of the F^4 generation from these families supported this classification. The final approximate ratio was therefore 196 susceptible to 3 resistant F^2 plants. The chi-square value of .003 with a probability range of .98 to .95 indicates that the data satisfactorily fit a theoretical 63:1 inheritance ratio.

The results of the F^2 test of March, 1954, are presented in Table 4, page 45. Compared to the previous January-1954 test, symptom expressions and the success of the greenhouse inoculations were much improved, but the symptoms again ranged continuously from very severe

Table 4. Results of March-1954 Inoculations of O.S.C. 21 X G.N. 31 F_2 with the W Strain of BYMV

Progeny or Check	Plants Tested	Greenhouse Results		Final Results		X^2	P
		Infected	Free	Infect.	Free	63:1	
O.S.C. 21 X G.N. 31 F_2	476	466	10	467	9	.152	.50 - .20
G.N. 31 X O.S.C. 21 F_2	442	436	6	436	6	.023	.95 - .80
Total for Reciprocals	918	902	16	903	15	.003	.98 - .95
O.S.C. 21	219	210	9	-	-		
G.N. 31	57	0	57	-	-		

to very mild. Only 6.1% of the F_2 plants and 5.9% of the O.S.C. 21 plants were symptomless before the second inoculation. Sixteen plants or approximately 1.7% of the F_2 population remained uninfected at the termination of the greenhouse test. When progenies were tested in the field, one family of O.S.C. 21 X G.N. 31 appeared distinctly susceptible. Of the remaining 15 tested, nine contained one or two plants with very mild infections which could have been caused by aphid transmitted viruses, and six were completely symptomless throughout the season, although the O.S.C. 21 check plots were about 50% severely infected. The chi-square values shown in Table 4 indicate that the ratios for each reciprocal cross, and their combined values, satisfactorily fit a 63:1 inheritance ratio.

These data suggest that the F_2 plants represented by the relatively resistant F_3 progenies were homozygous for two or three major genes which were primarily recessive.

F_3 Generation

Field-1953. The results of the 1953 test are shown in Table 5. The data for O.S.C. 21 and G.N. 31 consist of the total numbers of individual plants from many small plots.

Table 5. Results of the 1953 Field Inoculations of O.S.C. 21 X G.N. 31 F_3 Families with the W Strain of BYMV

F_3 Progeny or Check Variety	Families Tested	Classification		X^2	P
		Susceptible	Resistant	63:1	
O.S.C. 21 X G.N. 31	116	116	0	-	-
G.N. 31 X O.S.C. 21	109	107	2	.085	.80 - .50
Total for Reciprocals	225	223	2	.652	.50 - .20
O.S.C. 21 *	630	314	316	-	-
G.N. 31 *	70	1 **	69	-	-

* Numbers for O.S.C. 21 and G.N. 31, in all tables, refer to the number of individual plants involved.

** Infected with virus that infected young G.N. 31 plants in the greenhouse and was presumed to be a field transmitted strain.

Because of the high escape percentage, the possibility of infection by insect transmitted viruses, the variable number of plants in the field and greenhouse tests, and the use of the W-strain for retesting during the period when this strain infected G.N. 31, it was not possible to make a dependable classification of families. It was only possible to confirm, through retesting, two highly resistant families. These families contained only one and two plants with systemic necrosis and mild mottle in the combined field and greenhouse tests of 51 and 25 plants respectively. The classification of the remainder of the population was questionable. The chi-square value of .085 indicates good agreement with a 63:1 ratio in the case of G.N. 31 X O.S.C. 21, and although no resistant families were identified in the reciprocal, the combined data also fit a 63:1 ratio with a probability of .20 to .50 for the deviation.

Spring-1955. The test of 409 F_3 families made in the spring of 1955 with the Y-strain was relatively good from the standpoint of symptom expression and the degree of inoculation success. The summarized results of the first inoculations are presented in Table 6, page 48, on the basis of presence and severity of systemic symptoms (systemic mottle, dwarfing, and distortion). Various necrotic symptoms, which were abundant in these tests, will be considered later in a discussion of symptom expression in the single large planting of 270 families. The data shown in Table 6 for G.N. 31 and O.S.C. 21 consist of the totals for the several plantings, and are given as the number of individual plants tested. The fact that the escape percentage for O.S.C. 21 was 7.7 does not indicate a similar chance for the escape of F_3 families, which were tested and classified as units of five or six plants.

The results shown in Table 6 were considered to be tentative, because of the possibility of error in classification of families in which symptoms were slight or absent. Because of apparent modifying gene effects, many highly resistant families were found, during the

Table 6. Summarized Results of the Spring-1955 Inoculations of 409 O.S.C 21 X G.N. 31 F_3 Families with the Y Strain of BYMV

Progeny or Check	Families Tested	Infected Families			Uninfected Families	X^2 63:1	P
		Mild Inf.	Severe Inf.	Total Inf.			
O.S.C. 21 X G.N. 31	175	51	121	172	3	.034	.95 - .80
G.N. 31 X O.S.C. 21	234	76	153	229	5	.176	.80 - .50
Total for Reciprocals	409	127	274	401	8	.192	.80 - .50
O.S.C. 21	90	0	83	83	7		
G.N. 31	75	0	0	0	75		

study, to contain a low percentage of plants which were susceptible to mild infection. Table 7, page 49, presents the results of retests of families which were classified as resistant in Table 6, and in addition, a number of other families containing only one or two mild infections. Many of the total number of retested families are not included in Table 7 because of the high susceptibility found in the retests. Also included in Table 7 is the percentage of field infection for those families included in the gladioli test.

Family 2-126 contained no systemically infected plants except one which developed very light systemic vein necrosis without mottle in the first test. Families 2-126, 2-128, 3-34, 4-68, 4-72, and perhaps 2-101, comprised a highly resistant group. Family 4-146 was also quite resistant, and 2-167 should be considered as a possible resistant type because four of the five infected plants developed systemic necrosis

Table 7. Summary of Results for Retested O.S.C. 21 X G.N. 31 F_3 Families of the Spring-1955 Tests with the Y Strain of BYMV

Family	Plants in 1st Test with Severe Infect.	Mild Infect.	No Infect.	Plants in Retest with Severe Infect.	Mild Infect.	No Infect.	% Infection in Gladioli Test
2-101*	0	0	5	1	2	12	2.4
2-126	0	0	5**	0	0	33	0.0
2-128	0	0	5	0	2	52	0.0
2-167	0	0	5	1	4	13	
2-196	0	0	6	5	0	13	
4-34	0	0	5	0	1	34	8.3
4-68	0	0	5	1	0	17	0.0
4-146	0	0	6	2	0	16	
2-66	1	0	4	0	5	10	
4-123	0	1	4	0	4	12	
4-72	1	0	4	0	1	28	
4-57	0	1	4	0	7	8	
2-121	0	1	4	2	3	10	
2-198	0	2	4	0	2	16	
O.S.C. 21	-	-	-	26	0	2	
G.N. 31	-	-	-	0	0	21	

* Prefix designation 2- refers to families from the cross G.N. 31 X O.S.C. 21; 4- refers to the reciprocal cross.

** Number included one plant with very light systemic vein necrosis without mottle.

of the leaves and stems without mottle. The remainder of the families listed in Table 7 appear to be less resistant. Because these data for disease reaction show a continuous variation, a genetic classification of the F_3 families is necessarily arbitrary. It is suggested that the group of eight families listed above are homozygous for the major recessive genes involved, but contain modifiers which condition a low degree of susceptibility. Table 8 presents the resulting experimental ratios.

Table 8. A Classification, Based on Retests of Highly Resistant Families, of 409 O.S.C. 21 X G.N. 31 F_3 Families Tested in the Spring of 1955

F_3 Families of	Families Tested	Susceptible	Resistant	X^2 63:1	P
O.S.C. 21 X G.N. 31	175	171	4	.241	.80 - .50
G.N. 31 X O.S.C. 21	234	230	4	.025	.95 - .80
Total	409	401	8	.192	.80 - .50

The satisfactory fit of the data to a 63:1 ratio indicates that the eight highly resistant families are those which are homozygous for three major genes. Although it is possible that the group of families homozygous for major genes was sufficiently large to fit a 15:1 ratio, and that the remaining effects were due to modifiers, it was not possible to delineate such a group from these data. By arbitrarily including all retested families, except 4-57, in a single group, the ratios presented in Table 9 are obtained.

Table 9. A Classification of O.S.C. 21 X G.N. 31 F_3 Families of the Spring-1955 Tests, Comparing Moderately and Highly Resistant Families Against More Susceptible Families

F_3 Progeny of	Families Tested	Susceptible	Resistant or Near-resist.		X^2	P
O.S.C. 21				15:1	1.894	.20 - .10
X G.N. 31	175	169	6	63:1	2.949	.10 - .05
G.N. 31 X				15:1	2.718	.10 - .05
O.S.C. 21	234	226	8	63:1	3.965	.05 - .01
				15:1	5.134	.05 - .01
Total	409	395	14	63:1	8.002	<.01

It is apparent from the chi-square tests in Table 9, that the addition of only six families to the most resistant class would favor the acceptance of a 15:1 ratio. The families added in Table 9, however, should not logically be considered to have resistance approaching that of G.N. 31.

No substantial differences between reciprocal crosses have been evident in these results.

Symptom expression in the Spring-1955 F_3 tests. The observations of symptoms in the Spring-1955 tests were made to facilitate the genetic classification of families, and as a study of the effect of genetic segregation on the symptom expression of a single virus strain in the progeny of one cross. The family classification presented in Table 10 involves only the single large planting of 270 families made in a ground-bed greenhouse. Descriptions and photographs, however, pertain to the entire group of 409 families tested during this season and apply generally to the progeny of the cross O.S.C. 21 X G.N. 31.

Photographs showing examples of various F_3 symptom types are included as appendix plates.

The symptoms which were observed in the tested families, are classified below according to their type and the part of the plant affected. The numbers of related appendix plates appear following the descriptions.

I. Inoculated leaf symptoms. (Plates 6,7)

A. Discrete necrotic local lesions.

B. Vein necrosis - either as net necrosis spreading from discrete lesions, or occurring alone in various degrees.

II. Systemic symptoms.

A. Necrotic.

1. Pod necrosis - severe reddish necrotic areas in combination with pod distortion. (Plate 10)

2. Necrotic lesions - often appearing as an early symptom on upper non-mottled leaves, or as necrotic flecks on mottled leaves. (Plates 8,9)

3. Vein or net necrosis - as spreading areas from discrete lesions, or appearing in patches or generally over the surface of upper leaves. (Plates 8,9,10)

4. Stem necrosis - occurring at any stage of development, and ranging in severity from a chronic mild condition, to death of the terminal or the entire upper part of the plant.

B. Non-necrotic

1. Systemic mottle - typical mosaic symptoms with degrees of leaf distortion and plant stunting, ranging from very mild to extremely severe. (Plates 11,12,13)
2. Pod distortion - ranging from very slight to severe roughening and twisting. (Plate 13)

The above symptoms appeared in many degrees and combinations, and varied in the stage of plant development at which they were expressed. Individual F_3 families tended strongly to produce uniform symptoms, although genetically segregating families included plants of different types, with the result that classification of families into definite symptom groups was difficult. For simplicity, the 270 families were classified somewhat arbitrarily as presented in Table 10, according to the combination of symptoms observed.

Systemic mottles are divided into three categories, in Table 10, which include extremely severe, severe, and mild. Extremely severe types were strikingly dwarfed and distorted (Plate 13), with leaf diameter reduced to less than one-fifth, and plant height to less than one-tenth of normal. In mildly mottled plants (Plate 11), distortion and reduction of size were slight or absent. The intermediate class, into which all infected plants of O.S.C. 21 were classified, was moderately to severely dwarfed, with considerable leaf distortion. Pod distortion was common in types 1 and 2 (Table 10), and occurred to some extent in type 3 families, but many severely mottled plants, including those of O.S.C. 21, did not show distinct pod distortion.

Types 5 and 6 were variable in respect to systemic mottles,

Table 10. Classification of 270 F_3 Families of O.S.C. 21 X G.N. 31, Inoculated with the Y Strain of BYMV, According to Symptom Expression

General Systemic Symptoms	Systemic Necrosis		Primary Leaf Necrosis			Sub Total
			Slight or Absent	Net Necrosis	Discrete Lesions	
1. Extremely severe systemic mottle.	Present	0	--	--	--	19
	Absent	19	13	4	2	
2. Severe systemic* mottle. Typical of susceptible parent	Present	12	10	0	2	144
	Absent	132	120	7	5	
3. Mild systemic mottle.	Present	32	29	0	3	61
	Absent	29	28	0	1	
4. Severe and mild mottle (segregating.)	Present	22	21	1	0	29
	Absent	7	7	0	0	
5. Lethal systemic necrosis.	Present	6	3	0	3	6
6. Late general necrosis.	Present	6	3	0	3	6
7. Light systemic vein necrosis only.	Present	1	0	0	1	1
8. No systemic** symptoms.	Absent	4	1	0	3	4
Totals	Present	79	66	1	12	270
	Absent	191	169	11	11	

* Of the 20 O.S.C. 21 plants tested, 19 were in this group; one escaped.

** All of the 20 G.N. 31 plants tested were in this final group.

upon which were superimposed severe necrotic reactions.

There was no strong relationship apparent between severity of mottle and the type of inoculated leaf symptoms, although in types 5 through 8 there was a greater tendency for the development of discrete necrotic lesions. Systemic necrosis was apparently related to mottle severity, being absent in type 1, but present in 10% of the type 2 families, and in over 50% of the mild (type 3) and segregating (type 4) families. Types 5, 6, and 7 were classified originally on the basis of systemic necrosis. The necrotic tendency therefore increases from group 1 to group 8, which is resistant, with complete localization of infection in the inoculated leaves. All inoculated plants of G.N. 31 were classified into this final category.

<u>Greenhouse - 1955-56</u>. Results of the tests of 595 F_3 families from the second or repeat crosses of O.S.C. 21 X G.N. 31, are shown in Table 11. In these tests, which were conducted during the fall and winter of 1955 and the winter of 1956, many of the necrotic symptoms described in the preceding section were not observed, or were of reduced severity. Because of such incomplete symptom expression, and a limitation on time and facilities, these families were classified only as uninfected, mildly infected, or severely infected, on the basis of both the original test and retests of negative or questionable families. In Table 11 families with one severe infection or two mild infections in 12 plants tested, have been arbitrarily included in the category "Slightly Susceptible." Nine families did not develop symptoms, although results of earlier F_3 tests would indicate that

Table 11. Results of 1955-1956 Inoculations of O.S.C. 21 X G.N. 31 F_3 Families with the Y Strain of BYMV

Progeny or Check	Families Tested	Distinctly Suscept. Severely Infected	Distinctly Suscept. Mildly Infected	Slightly * Susceptible	Resistant (Non-infected)
O.S.C. 21 X G.N. 31	301	235	53	9	4
G.N. 31 X O.S.C. 21	294	237	43	9	5
Total for Reciprocal Crosses	595	472	96	18	9
O.S.C. 21	191	160	0	0	31
G.N. 31	121	0	0	0	121

* Families containing one or two infected plants - usually mildly infected - in 12 plants tested.

most, if not all, of these families would probably contain an occasional infected plant if inoculated in larger numbers in the spring or summer.

Table 12 shows the statistical test of the ratio of uninfected *versus* infected families. Because the reciprocal crosses were almost identical in behaviour, they have been combined for the application of the chi-square test.

Table 12. The Ratio of Infected *versus* Uninfected Families of O.S.C. 21 X G.N. 31 F_3, from the Results of the 1955-1956 Tests with the Y Strain of BYMV. Reciprocal Crosses Combined.

Families Tested	Infected	Not Infected	X^2 63:1	P
595	586	9	.010	.95 - .80

Table 13 combines resistant and slightly susceptible families and tests the ratio of the resulting group versus the remainder of the susceptible families.

Table 13. Suggested Ratio of Slightly Susceptible and Resistant Families versus Distinctly Susceptible Families of O.S.C. 21 X G.N. 31 F_3, from the Results of the 1955-1956 Tests. Reciprocal Crosses Combined.

Families Tested	Distinctly Susceptible	Resistant or Slightly Susc.	χ^2		P
595	568	27	15:1	2.754	.10 - .05
			63:1	31.810	<.01

The ratio of infected versus non-infected families presented in Table 12 satisfactorily fits a 63:1 hypothesis, according to the chi-square test. The ratio of distinctly susceptible families versus resistant and slightly susceptible families as arbitrarily delineated in Table 13, does not fit a 63:1 hypothesis, but fits a 15:1 ratio with a probability of .10 to .05. This ratio is included and tested to demonstrate that the consideration of only a small number of slightly susceptible families would support a hypothesis that two major recessive genes, with modifier effects, condition the inheritance of resistance. The only classification which appears to delineate the type homozygous for three major genes, in this particular test, is that which compares completely uninfected families with all families which contained infected plants. Direct comparisons cannot be made between this classification and that presented for the spring-1955 F_3 tests, in which no completely uninfected families were observed. Because the previous test involved a large number of plants for the most resistant families, and was conducted under different environmental conditions, the differences in behaviour between the two populations are not necessarily due to genetic differences. If one or several susceptible G.N. 31 plants had been used in the 1953 crosses, from which the F_3 families tested in the spring of 1955 originated, the effect would not have been to eliminate the homozygous recessive class, but rather to change the proportion of resistant to susceptible families by a small percentage. Although such may have occurred, the differences between the 1953 crosses and the 1954 crosses (source of F_3 families tested in 1955-1956) appear to be within the limits of the effect of environment on susceptibility and symptom expression.

In general, the results of the present (1955-1956) F_3 tests corroborate the results of the previous F_3 tests. The large proportion of families falling into a distinctly susceptible category with symptom severity resembling that of the susceptible parent, the wide variation of symptoms, and the occurrence of occasional susceptible plants in almost all families, indicate that resistance is recessive, and is conditioned by two or three major genes with modifiers. Differences between reciprocal crosses were not observed.

F_1 Backcross Generation

Spring-1954. The results of the first generation backcross tests made in the spring of 1954 are shown in Table 14 without chi-square tests of experimental ratios. The data for reciprocal backcrosses of O.S.C. 21 X G.N. 31 F_1 generation X O.S.C. 21 have been combined in this table.

Table 14. Results of the F_1 Backcross* Test Made in the Ground-bed Greenhouse in the Spring of 1954 with the W Strain of BYMV

Backcross Progeny	Plants Tested	Severe Early Infection	Mild Late Infection	Necrotic Symptoms Only	No Visible Symptoms
F_1 X O.S.C. 21**	153	153			
(21 X 31) X G.N. 31	65	51	9	3	2
(31 X 21) X G.N. 31	49	33	8	4	4
G.N. 31 X (21 X 31)	34	27	3	4	0
G.N. 31 X (31 X 21)	37	22	3	10	2
Total for (F_1) X G.N. 31	185	133	23	21	8

* Reciprocal backcrosses of O.S.C. 21 X G.N. 31 F_1 X each parent.

** Combined reciprocal backcrosses to O.S.C. 21.

The classification presented in Table 14 is necessarily somewhat arbitrary. The number of plants classed under Severe Early Infection was the number showing this condition between three and four weeks after inoculation. All tested plants of the backcrosses of the F_1 generation X O.S.C. 21 were in this category, but were generally more severely affected and developed symptoms earlier than the backcrosses to the resistant parent. Systemic vein necrosis was absent in the backcrosses to the susceptible parent, but prevalent in the backcrosses to the resistant parent, especially in plants which developed symptoms late in the test period.

In Table 15 the data for the four separate F_1 X G.N. 31 backcrosses have been combined, and tested against theoretical ratios on the basis of several possible divisions.

Table 15. Tests of Experimental Ratios for the Summarized Results of (O.S.C. 21 X G.N. 31) F_1 X G.N. 31 Backcrosses Tested in the Spring of 1954

Basis for Class Division	Class 1	Class 2		X^2	P
Plants with early severe infection *versus* remainder of tested plants.	133	52	3:1	0.810	.50 - .20
Plants with systemic mottle *versus* plants without systemic mottle.	156	29	3:1	8.046	<.01
			7:1	1.442	.50 - .20
Infected plants *versus* non-infected (symptomless plants).	177	8	15:1	0.884	.50 - .20

The chi-square tests in Table 15 would indicate that two major dominant genes condition early severe systemic infection, and that three major dominant genes may determine susceptibility to systemic mottle of any severity. These data, as tested, would also indicate that the inheritance for the symptomless condition was determined by at least four recessive genes. The tendency for infection to be milder and later in backcrosses to the resistant parent, when compared to backcrosses to the susceptible parent, is probably due to the effect of modifying genes derived from G.N. 31.

Summer-1955. The summer, 1955, inoculations of the 1954 backcrosses, with the Y-strain, produced considerably milder effects than the inoculation with the W-strain described in the past section. Symptom expression in these tests was generally mild, with very little occurrence of systemic necrosis. Such mild effects were possibly due in part to the heavy shading of the greenhouses and the generally high temperatures, which resulted in vigorous, very succulent growth. Differences could also result because the Y-strain is noticebly less infective and severe in effect than the W-strain. Results of these tests are shown in Table 16.

Table 16. Results of Summer-1955 Tests of (O.S.C. 21 X G.N. 31) F_1 X G.N. 31 Backcrosses, with the Y-strain of BYMV.

Backcross or Check	Plants Tested	Plants Infected	Plants not Infected	X^2 3:1	P
G.N. 31 X (31 X 21)	100	70	30	1.080	.50 - .20
G.N. 31 X (21 X 31)	164	118	46	0.658	.50 - .20
(31 X 21) X G.N. 31	273	199	74	0.549	.50 - .20
(21 X 31) X G.N. 31	198	136	62	3.879	.05 - .01
Total for Backcrosses	735	523	212	5.566	.05 - .01
O.S.C. 21	69	61	8		
G.N. 31	41	0	41		

The X^2 value of 5.566, with a probability of .05 - .01 for the deviation, indicates that the combined data for this test do not closely fit a theoretical 3:1 ratio, although ratios for tests of progeny from each reciprocal backcross fit 3:1 ratios satisfactorily in most cases. It should be noted, in considering these ratios, that 12% of the O.S.C. 21 plants escaped infection. The percentage of infected plants observed in this test would compare only with the percentage of early severe infections of the previously described test with the W-strain in 1954. In the case of these backcross plants inoculated with the Y-strain, the effects of modifying factors from the resistant parent appear to suppress, in the particular environment of this test, that segment of the potentially susceptible population conditioned by one major dominant factor.

G.N. 31 X Mont. 43-15

The cross of G.N. 31 X Mont. 43-15 was made in hopes of obtaining information which would clarify or enlarge upon that obtained in crosses of O.S.C. 21 X G.N. 31. In the latter case, the two parents are of entirely different types and are of divergent origin, whereas G.N. 31 and Mont. 43-15 are very similar in general characteristics and probable background. It was considered possible that these varieties have much of their genotype in common. The mode of inheritance of resistance in such a cross might therefore be more simplified and involve fewer modifier effects, which were apparently complicating factors in the O.S.C. 21 X G.N. 31 crosses. The following sections describe the results of this cross.

Parent Varieties

The reaction of the resistant parent G.N. 31 has been described adequately in previous sections. Data for the infection of Mont. 43-15 is included in tables for progeny tests in which it served as the susceptible check variety. This variety developed distinct symptoms, with a very low escape percentage. The first trifoliate leaf, in all cases, was severely affected, but later symptoms tended to be milder

Figure 10. Mont. 43-15 infected with the Y-strain of BYMV. Typical symptoms of fully expanded lower trifoliate leaves (left) and upper trifoliate leaves (right).

Figure 11. Upper leaflet from G.N. 31 X Mont. 43-15 F_1 inoculated with the Y strain of BYMV, showing systemic vein necrosis and chlorotic areas.

than those of other susceptibles, such as O.S.C. 21. Figure 10 shows typical symptoms on infected leaves.

F_1 Generation

Although only a few plants were tested, the intermediate behaviour of the F_1 generation of G.N. 31 X Mont. 43-15 was quite apparent. Of the six plants inoculated in the first test, five developed vein necrosis of upper leaves followed by a mild systemic mottle (in two of these plants the mottle was very indistinct). Figure 11 shows systemic vein necrosis in one of the youngest leaves which did not develop a mottle. The remaining plant in this test developed necrotic lesions of the inoculated primary leaves only, and was considered to possibly be a selfed G.N. 31 plant. These results are included in Table 17 with the tabulated results of the F_2 test.

The symptoms of the F_1 plants were distinctly late in occurrence. When observations were made two weeks after inoculation, Mont. 43-15 plants were severely infected, while the F_1 plants were symptomless. The F_1 behaviour was very similar to that of a substantial portion of the F_2 and F_3 plants.

A second planting of nine F_1 plants was made in March, 1956. The latest available records of these plants indicated a behaviour similar to that described above. Approximately two weeks after inoculation, the F_1 plants were symptomless, while all plants of Mont. 43-15 were severely infected.

Although the F_1 generation of G.N. 31 X Mont. 43-15 is susceptible to systemic infection by the Y-strain, it is clearly intermediate in comparison with the parents. It is probable that it possesses at least one less dominant gene for susceptibility than the F_1 generation of O.S.C. 21 X G.N. 31.

F_2 Generation

Plants of the G.N. 31 X Mont. 43-15 F_2 generation were of three general types which (1) developed systemic mottle in the first trifoliate leaf and subsequent growth, (2) developed discrete lesions or vein necrosis on the first trifoliate or subsequent leaves followed by mild mottle, and (3) remained symptomless during the test. Type 1 was variable in initial severity, but generally symptoms were less severe on the first trifoliate leaves and were apparent a few days later than in Mont. 43-15. In later stages differences between most individuals and Mont. 43-15 were less apparent. Symptoms which resembled those of the F_1 plants, appeared in type 2 individuals after varying periods, with some plants showing the first evidence of infection about one month after inoculation. This indicates that the separation of plants of type 2 from those with G.N. 31 resistance was uncertain. Table 17 shows the results of the F_1 and F_2 inoculations. For purposes of classification and discussion, the term "early-mottle" refers to early, non-necrotic systemic mottle; while "necrotic-mottle" refers to systemic necrosis followed by mild mottle.

Table 17. Results of July-1955 Tests of G.N. 31 X Mont. 43-15 F_1 and F_2 Generations with the Y Strain of BYMV

Progeny or Check Variety	Plants Tested	Early-Mottle	Necrotic-Mottle	No Systemic Symptoms
F_1 Generation	6	0	5	1
F_2 Generation	229	72	52	105
Mont. 43-15	87	86	0	1
G.N. 31	15	0	0	15

The data in Table 17 indicate that the early-mottle plants may represent a homozygous class in a single gene-pair system. On this basis, Table 18 presents and tests the ratio of plants which developed an early-mottle _versus_ the remaining necrotic-mottle and symptomless individuals. The probability of .05 to .01 shown in Table 18 indicates a barely acceptable statistical fit to a monogenic ratio.

Table 18. Experimental Genetic Ratio of Early-mottled Plants versus Necrotic-mottled and Symptomless Plants of G.N. 31 X Mont. 43-15 F_2 from the July-1955 Tests

Plants Inoculated	Early-mottled	Necrotic-mottled or Symptomless	X^2 1:3	P
229	72	157	4.729	.05 - .01

A high general level of resistance, compared to the susceptible parent reaction, was observed in F_2 plants included in the field test with natural infection from gladioli. The average infection of two plots of F_2 plants was 7.7%, while the average for four plots of Mont. 43-15 was 72.25%.

F_3 Generation

Symptom types in the F_3 families were generally the same as were observed in the F_2 generation. Table 19 presents a classification of families on the basis of the original tests, and limited information obtained in retests. All families which were uninfected in the first test were replanted and inoculated in March, 1956. It was not possible to delay the tabulation of data sufficiently long to permit an accurate evaluation of the results of the second inoculations. Two weeks after inoculation, however, one or two early systemic mottles were observed in each of eight of the 55 retested families, which were thus included in category 2 in Table 19.

The incidence of the necrotic-mottle type, within the families where it occurred, was very low. In 61 of the 110 families including this symptom type, it occurred in only one plant, suggesting that the

Table 19. Results of 1955-1956 Tests of G.N. 31 X Mont. 43-15 F_3 Families with the Y Strain of BYMV

Symptom Expression	F_3 Progeny	Susceptible Mont. 43-15	Resistant G.N. 31
1. Early-mottle only	50	111	0
2. Early-mottle and symptomless	159	-	-
3. Early-mottle, necrotic-mottle, and symptomless	65	-	-
4. Necrotic-mottle and symptomless	45	-	-
5. Symptomless only	47	7	70
Total Tested	366	118	70

separation of this symptom type from resistant types may be difficult, and that negative observations are subject to question. It is likewise impossible, from the data available, to accurately determine which families included resistant segregates and which included escaped early-mottle types. It is therefore difficult to make genetic interpretations of these data. A classification is suggested in which families that included early-mottle types (classes 1, 2, and 3 of Table 19), are separated from those which were symptomless or included infected plants of the necrotic-mottle type only (classes 4 and 5). A statistical test of this classification is shown in Table 20.

The classification shown in Table 20 fits a 3:1 ratio with a .95 probability value, and indicates that a single gene pair conditions

Table 20. Experimental Genetic Ratio, for G.N. 31 X Mont. 43-15 F_3, of Families Including Early-mottled Plants *versus* Families Including Only Necrotic-mottled or Symptomless Plants

Families Tested	Families Including: Early-mottled Plants	Families Including: Necrotic-mottled or Symptomless Plants Only	X^2 3:1	P
366	274	92	.004	.95

the occurrence of the early systemic mottle type, or type comparable to the susceptible parent. This hypothesis does not explain the relationship between resistant and necrotic-mottled plants. It is suggested that modifying genes are also involved, and determine the expression of symptoms within this group. Such modifiers might also explain the differences in severity of mottle between the early-mottle segregates, and the susceptible parent.

Phaseolus vulgaris L. X P. coccineus L.

F_1 Generation

The six plants of O.S.C. 22 X 2014 F_1 that were inoculated in the field in 1954 did not develop visible symptoms; no attempt was made to isolate the virus from them.

The results of the small tests made in the greenhouse in 1955 of hybrids between O.S.C. 21 and various *P. coccineus* L. lines, are shown in Table 21. These data are not intended as conclusive results, but are included only to show that the F_1 of *P. vulgaris* X *P. coccineus* L. may be distinctly susceptible. Symptoms of the infected plants were variable, but generally they were mild in effect, though

Table 21. Susceptibility of F_1 Generations of O.S.C. 21 X *P. coccineus* L. Lines Tested in 1955 with the Y and W Strains of BYMV

F_1 Tested	W-Strain Inoculations		Y-Strain Inoculations	
	Tested	Infected	Tested	Infected
O.S.C. 21 X 2012	-	-	1	1
O.S.C. 21 X 2016	1	0	-	-
O.S.C. 21 X 2018	1	1	1	0
O.S.C. 21 X 2019	3	3	1	1
O.S.C. 21 X Scarlet Runner	3	1	2	1

distinctly visible.

F_2 Generation (O.S.C. 22 X 2014)

The resistant parent, *P. coccineus* L. line 2014, was never observed to be infected or proven infected by test inoculations to susceptible plants in any test conducted. This line appears to be immune to the Y and W strains of BYMV. The susceptible parent, O.S.C. 22, was similar in reaction to O.S.C. 21, which has been previously described.

Infections of O.S.C. 22 X 2014 F_2 plants varied in severity from that of the susceptible parent, to a symptomless condition. Symptoms of most F_2 plants were distinct in appearance, but mild in effect, with little distortion and reduction of leaf or plant. The range of symptoms was the same for each virus strain, but the W-strain caused symptoms which were somewhat more severe and more easily recognized in general than those of the Y-strain. General necrosis of leaf blades occurred in many plants during spring or summer, but was only slightly present in winter. Leaf symptoms for the F_2 and O.S.C. 22 plants are shown in Figures 12 through 15.

The results of the test conducted in December, 1954, are shown in Table 22. These data are based primarily on the presence or absence of visible symptoms. The results of the tests made in the spring of 1955 are shown in Tables 23 and 24. These data are based on visible symptoms and inoculations on Dwarf Horticultural for all questionable or apparently uninfected plants.

Ratios of infected _versus_ uninfected plants for each of the three F_2 inoculations are presented and tested statistically in Table 25.

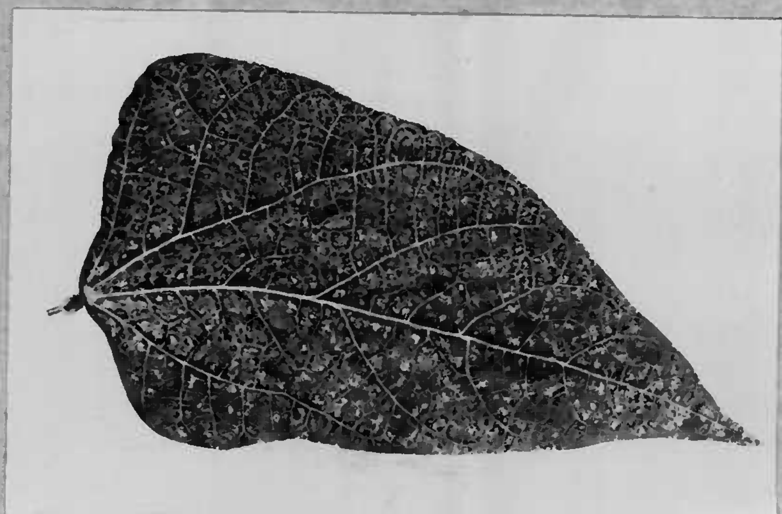

Figure 12. Leaf necrosis of O.S.C. 22 X 2014 F_2 inoculated in June, 1955, with the W Strain of BYMV.

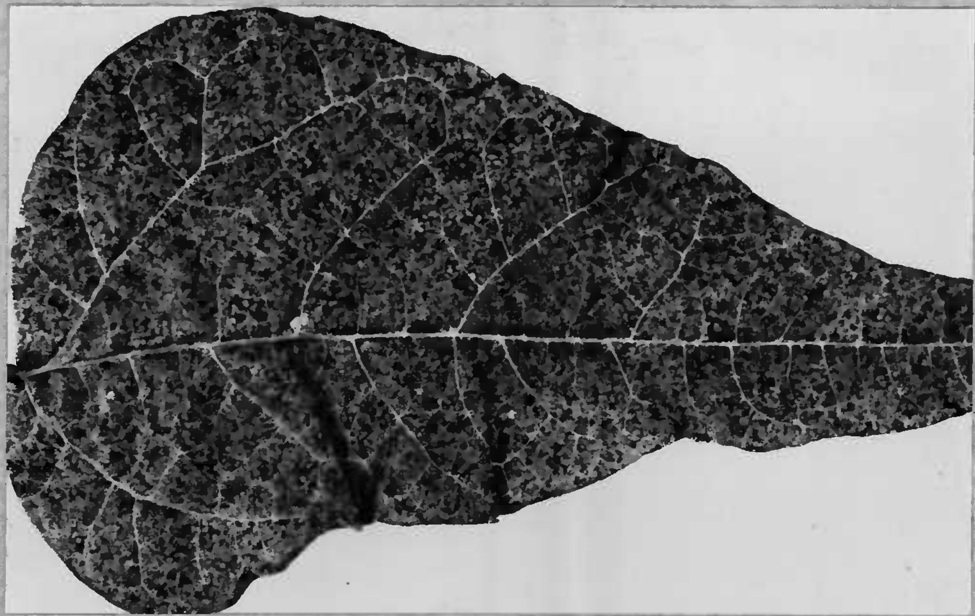

Figure 13. Mild infection of O.S.C. 22 X 2014 F_2 inoculated with the Y strain of BYMV in February, 1955.

Figure 14. Very mild or indistinct symptoms of O.S.C. 22 X 2014 F_2 inoculated with the Y strain of BYMV in February, 1955.

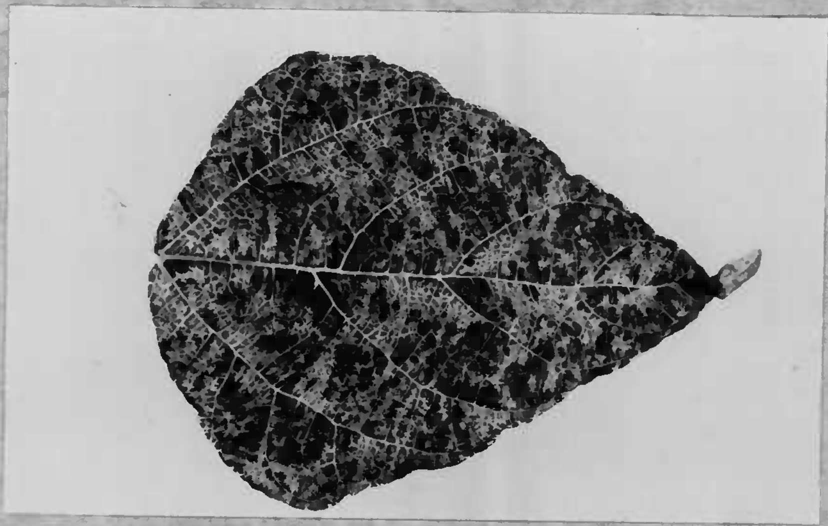

Figure 15. Typical infected leaflet of O.S.C. 22 inoculated with the Y strain of BYMV in February, 1955.

Table 22. Results of the O.S.C. 22 X 2014 F_2 Test Made in December, 1954, with the Y Strain of BYMV

Progeny or Check Variety	Plants Tested	Plants Infected	Plants Not Infected
O.S.C. 22 X 2014 F_2	156	134	22
O.S.C. 21 *	90	89	1
2014	21	0	21

* Tested instead of O.S.C. 22 as susceptible check. O.S.C. 22 and O.S.C. 21 are very similar in susceptibility to the Y and W strains of BYMV.

Table 23. Results of the February, 1955, Test of O.S.C. 22 X 2014 F_2 with the Y Strain of BYMV

Progeny or Check	Plants Tested	Infections				Non Infected
		Severe	Mild	Indistinct	Symptomless	
O.S.C. 22 X 2014 F_2	287	16	132	66 *	40 *	33
O.S.C. 22	9	8	0	0	0	1
2014	10	0	0	0	0	10

* Reaction confirmed by test inoculations on Dwarf Horticultural

Table 24. Results of the June, 1955, Test of O.S.C. 22 X 2014 F_2 with the Y and W Strains of BYMV

Progeny or Check	Plants Tested	Infections				Non Infected
		Severe	Mild	Indistinct	Symptomless	
				W-strain Inoculations		
O.S.C. 22 X 2014 F_2	118	5	108	0	3 *	2 *
O.S.C. 22	34	34	0	0	0	0
2014	9	0	0	0	0	9
				Y-strain Inoculations		
O.S.C. 22 X 2014 F_2	97	3	86	1 *	5 *	2 *
O.S.C. 22	26	26	0	0	0	0
2014	6	0	0	0	0	6
				Total Inoculations		
O.S.C. 22 X 2014 F_2	215	8	194	1	8	4
O.S.C. 22	60	60	0	0	0	0
2014	15	0	0	0	0	15

* Reaction confirmed by test inoculation on Dwarf Horticultural

Table 25. Experimental Ratios of Infected *versus* Uninfected Plants from the F_2 Tests of O.S.C. 22 X 2014

F_2 Test Made in	Plants Tested	Infected	Uninfected	X^2 values	P
December 1954	156	134	22	3:1 9.31 15:1 14.90	<.01 <.01
February 1955	287	254	33	3:1 27.25 15:1 12.70	<.01 <.01
June 1955	215	211	4	63:1 0.003	.98 - .95

Chi-square values indicate that the data of the June-1955 tests fit a 63:1 ratio, while those of previous tests were intermediate between 15:1 and 3:1 ratios. Such differences are possibly due to environmental effects as well as the procedures employed. The low infection percentage of the first test (Table 22) could probably be expected, because the plants were inoculated only once, and because all symptomless plants were not tested by inoculations on a susceptible variety. In addition, this test was conducted during the winter months when symptom expression has been observed to be less distinct. Tables 23 and 24 clearly show that many plants without visible symptoms may carry the virus in a concentration sufficient to infect Dwarf Horticultural. In most such cases, Dwarf Horticultural plants were infected as readily and severely as when visibly infected plants served as an inoculum source. In a few cases, however, the infection in Dwarf Horticultural was sufficiently late and mild in appearance in the early stages to indicate that the F_2 plant contained a very low concentration of virus. The F_2 plants grown in the greenhouse ground beds (Table 23) were large and vigorous, and in many cases developed thick, very dark green leaves which may have tended to mask the virus effects.

The data presented in the tables do not permit exact interpretations of the inheritance of resistance to BYMV in this cross. They do, however, permit certain general conclusions which should be useful in practical application. Resistance is apparently recessive, and conditioned by at least two and possibly three major genes. In either case, the variation of the visible symptoms from severe to indistinct or absent, and the variability of the number of plants infected under different conditions, would seem to indicate the effect of modifying genes. The inheritance of resistance in this cross, therefore, does not vary greatly from that in O.S.C. 21 X G.N. 31.

Grafting Experiments

Of the 16 young G.N. 31 plants approach-grafted to infected plants of susceptible varieties, two developed scattered patches of reddish vein necrosis on leaflets above the graft union. A leaflet from one of these plants is shown in Figure 16, in comparison with a leaf from an O.S.C. 21 X G.N. 31 F_3 family which later developed a mild systemic mottle. The vein necrosis appeared on only two or three leaves, and no further symptoms developed in these plants. When inoculum was prepared from a leaflet with vein necrosis and used to inoculate Dwarf Horticultural plants, typical severe infection resulted, but when the first symptomless leaf above the vein necrosis was used for a similar inoculation, no infection occurred.

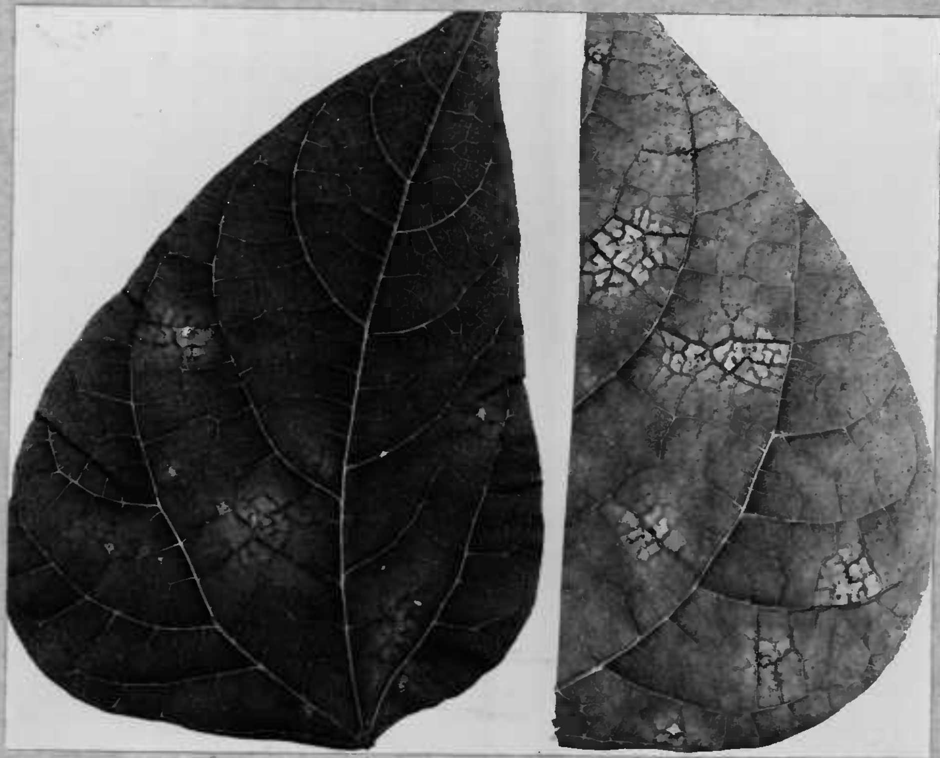

Figure 16. Left: Upper leaflet from a G.N. 31 plant which had been approach-grafted to a Dwarf Horticultural plant infected with the Y strain of BYMV. Right: Upper leaflet from an O.S.C. 21 X G.N. 31 F_3 plant which developed similar patches of vein necrosis following rub-inoculation with the same virus strain, but which later developed a mild systemic mottle.

Although the percentage of grafted plants producing symptoms was low, it is improbable that such infection represented a chance situation which could occur in rub-inoculated G.N. 31 plants. Hundreds of plants of this variety were rub-inoculated with the Y-strain during the same season without the occurrence of a single symptom above the inoculated leaves.

The fact that it is possible to produce a necrotic symptom in the upper parts of G.N. 31 plants by grafting them to a heavily infected plant, is an indication that hypersensitivity, or restriction of the virus to the area of inoculation, is an important mechanism of resistance in this variety. It is possible that other mechanisms, perhaps controlled by modifying genes, do exist in this variety and explain the occurrence of mild mottles in infected plants of progenies with G.N. 31 as resistant parent.

No symptoms were observed in the four 2014 plants which were grafted to infected O.S.C. 21 plants.

Natural Infection with Gladioli as the Source of Inoculum

The natural infection of varieties and lines planted near gladioli was generally comparable to infection of the same materials inoculated with the Y-strain in the greenhouse. The results for each variety or progeny line are given in Table 26 in terms of numbers and percentages of plants infected. The row number for each plot is also given to show the distance of the plot from the gladiolus rows.

The most important susceptible check variety, O.S.C. 21, was 83.9 to 96.6% severely infected. Mont. 43-15 and Dwarf Horticultural were also heavily infected but with more variation between plots. Some effect of position or distance from the source of infection was apparent in the plots of these three varieties, in which infection generally decreased from row 1 to row 4.

G.N. 31 plots contained no distinct systemic infections, although near the end of the season, three plants developed a necrotic-blotch

Table 26. Natural Infection of Bean Varieties and Breeding Lines Grown Near Gladioli in the Field in 1955

Variety or Line Grown	Row	Plants Grown	Plants Infected	% Infected
Check Varieties				
O.S.C. 21	1	30	29	96.6
O.S.C. 21	2	29	28	96.6
O.S.C. 21	3	31	26	83.9
O.S.C. 21	4	31	26	83.9
Mont. 43-15	1	26	22	84.6
Mont. 43-15	2	28	24	85.7
Mont. 43-15	3	27	21	77.8
Mont. 43-15	4	28	14	50.0
G.N. 31	1	21	0	0.0
G.N. 31	2	39	0	0.0
G.N. 31	3	30	0	0.0
G.N. 31	4	34	0	0.0
Dwarf Horticultural	1	36	31	86.1
Dwarf Horticultural	2	29	15	51.7
Dwarf Horticultural	3	33	15	45.4
Dwarf Horticultural	4	29	14	48.3
Progeny Lines - Greenhouse Reaction				
II-103 (F5) Resistant	4	32	1	3.1
II-84 (F5) Resistant	3	29	0	0.0
2-101 (F3) Resistant	4	41	1	2.4
2-128 (F3) Resistant	3	34	0	0.0
4-34 (F3) Resistant	1	36	3	8.3
4-68 (F3) Resistant	2	34	0	0.0
2-126 (F3) Resistant	2	31	0	0.0
II-77 (F5) Mild mottle	3	35	0	0.0
II-79 (F5) Mild mottle	1	41	0	0.0
2-123 (F3) Mild mottle with systemic necrosis	2	36	9	25.0
2-119 (F3) Severe mottle	1	33	28	84.8
4-108 (F3) Severe mottle	3	38	10	26.3
4-142 (F3) Severe mottle and lethal necrosis	1	36	19	52.8
4-89 (F3) Lethal necrosis (predominately)	4	34	17	50.0
4-70 (F3) Extremely severe mottle	2	26	5	19.2

Table 26. continued

Variety or Line Grown	Row	Plants Grown	Plants Infected	% Infected
Progeny Lines				
4-27 (F_3) Extremely severe mottle	4	30	10	33.3
G.N. 31 susceptible selection*	1	35	33	94.3
G.N. 31 X Mont. 43-15 F_2	2	27	3	11.1
G.N. 31 X Mont. 43-15 F_2	4	23	1	4.3
Phaseolus coccineus L. Lines				
2019	1	45	0	0.0
2018	2	28	0	0.0
2012	3	29	0	0.0
2016	3	23	0	0.0
2014	4	25	0	0.0
Scarlet Runner	2	40	0	0.0
Miscellaneous Varieties				
Pencil Pod Black Wax	1	29	17	58.6
Processor	1	49	17	34.7
Red Kidney	1	32	18	56.3
Kentucky Wonder	2	36	35	97.2
F.M. 1 Blue Lake	3	36	29	80.6
Bountiful	3	30	19	63.3
Tendergreen	3	45	22	48.9
Topcrop	4	32	9	28.1
Puregold**	3	47	1	2.1

* Progeny of susceptible mixtures observed in G.N. 31 early in the inheritance study, before uniformly resistant lines of this variety were established.

** The variety Puregold was also highly resistant to the Y strain of BYMV in greenhouse tests.

condition in the top which appeared to be associated with heavy aphid infestations. During the season, small patches of vein necrosis developed at apparent infection points on several G.N. 31 plants.

No symptoms were observed in the 223 plants of <u>Phaseolus coccineus</u> L.

The behaviour of most O.S.C. 21 X G.N. 31 progeny lines was very similar to respective greenhouse reactions to the Y-strain, although the percentage of infection in most susceptible lines was generally low compared to that of O.S.C. 21. Of the seven lines which were classified as resistant in greenhouse tests, three contained a low percentage of mild infections, and four contained no infected plants. Two families susceptible to mild mottle in the greenhouse, II-77 and II-79, were also quite resistant in this test.

The relative resistance of G.N. 31 X Mont. 43-15 F_2 generation tended to support the results of the greenhouse tests of this progeny in that susceptibility was not found to be strongly dominant in this cross.

Among the miscellaneous bean varieties, Kentucky Wonder and F.M. 1 Blue Lake (the source of the O.S.C. 21 and 22 selections) were heavily infected, and the dwarf wax variety Puregold was very resistant. In a test of this kind, it appears that pole varieties are more likely to give high infection readings because of their indeterminate growth habit which lengthens the period of susceptibility to infection and effective symptom production.

Tests of this nature appear to have considerable promise as an aid in breeding programs for resistance to BYMV.

DISCUSSION

Genetics of Resistance in Phaseolus vulgaris L. Crosses

The variety Great Northern U.I. 31 has proven consistently resistant to systemic infection by the Y strain of BYMV. The nature of resistance appears to be related to the localization of infection through the formation of necrotic lesions in the inoculated leaf, although the complexity of the genetics of resistance would suggest that other mechanisms may be involved. When this variety was crossed with Montana Great Northern 43-15, a variety very similar in characteristics but susceptible to systemic-mottle infection by the Y-strain, the F_1 generation was intermediate in disease reaction in that plants were symptomless on the lower trifoliate leaves, but developed necrotic lesions followed by a mild mottle in subsequent growth. This reaction suggested that the heterozygous F_1 gene combination modified the G.N. 31 tendency for localization sufficiently to allow delayed movement of the virus and ultimate mottle production. The F_2 and F_3 progenies included plants similar to the F_1, plants similar to the susceptible parent but developing slightly later and milder symptoms, and symptomless individuals. The F_1 type, for convenience termed the "necrotic-mottle" type, was difficult to distinguish from plants resistant to systemic infection, because of a great variability in the occurrence of symptoms. Experimental ratios, however, were derived on the basis of the occurrence of types similar to the susceptible parent - the "early-mottle" types. Chi-square tests indicated that the F_2 consisted of early-mottle types versus necrotic-mottle or resistant types in a ratio of 1:3, and the F_3 consisted of families homozygous or segregating for the early-mottle type, versus families including only necrotic-mottles or resistants, in a ratio of 3:1.

The low expressivity of the necrotic-mottle type and the inseparability of this class from the resistants, as well as the mild reaction of early-mottle plants in comparison with the susceptible parent, might be explained on the basis of modifying genes.

These results indicate that G.N. 31 and Mont. 43-15 differ at a single major gene locus, designated for purposes of discussion as R_1R_2, for reaction to the Y strain of BYMV. The genotype of Mont. 43-15 may be considered R_1R_1, that of G.N. 31 as R_2R_2, and the F_1 generation as R_1R_2. The F_2, therefore, included 1 R_1R_1 (early-mottle): 2 R_1R_2 (necrotic-mottle): 1 R_2R_2 (resistant), and the F_3 included three families containing early-mottle types to one which contained only necrotic-mottles or resistant types.

Different results were obtained when G.N. 31 was crossed with O.S.C. 21, a Blue Lake type variety that differs greatly from G.N. 31 in general characteristics, and that develops non-necrotic systemic infection which is considerably more severe than that observed in Mont. 43-15. The F_1 of this cross was almost identical to O.S.C. 21 in the development of severe systemic mottles. The F_2 and F_3 generations included a wide range of symptom types, from plants or families which were almost as resistant as G.N. 31, through mildly infected types, to those which were much more severely infected than the susceptible parent. Systemic necrotic symptoms similar to those produced in G.N. 31 X Mont. 43-15 were present in most classes based on mottle severity, but were more common in plants with mild-mottle infections.

The major F_3 studies, in which only the Y-strain was used, and which included tests of 1,004 random families, generally supported the hypothesis that resistance was recessive and differences were due to two or three major gene pairs. F_2 tests and a smaller F_3 test with the W-strain also supported this hypothesis. The predominance, in all tests, of the susceptible type similar to O.S.C. 21 but varying somewhat in severity of symptoms, also supported the hypothesis that at least one and possibly two strongly dominant genes for susceptibility, and possibly a third gene of lesser effects, were derived from the susceptible parent.

It was not possible to evaluate the effects of gene interactions, or of modifier genes, in either the susceptible or resistant types. Such effects were clearly indicated, however, especially in the more resistant F_3 families. In the spring-1955 tests of 409 random families, it was not possible to identify a single family which was as uniformly resistant as G.N. 31. Some families appeared to represent only very slight genetic modifications from G.N. 31, producing only one or two mild or necrotic infections in as many as 40 or 50 plants tested. It was necessary to base the genetic interpretations of this F_3 population, in terms of major gene effects, on such highly resistant but modified genotypes. In the 1955-1956 F_3 tests of 595 additional families, the behaviour of the more resistant families was such that slight degrees of susceptibility were not expressed. In this case, probably because of the small number of plants tested and different environmental conditions, the G.N. 31 genotype was apparently present and was used as a basis of genetic ratios, although the actual genetic situation in these families was likely the same as in the previous test.

Backcross data did not exhibit clear-cut classical inheritance ratios as was hoped would be the case, but never-the-less provided information helpful in interpreting the genetics of the cross. The 100% severe infection of the backcross F_1 X O.S.C. 21 confirmed the strong dominance of susceptibility. Backcrosses to the resistant parent, however, are less easily interpreted. In tests with the Y-strain in the summer of 1955, the ratio of infected versus uninfected plants was approximately 3:1, although the chi-square value was large (5.566), indicating at least two major dominant genes for susceptibility. The differences between these results and those of the F_1 and F_2 tests, as well as the generally mild symptoms of infected plants, were probably due to modifying genes, 75% of which would be derived from the resistant parent in the case of every backcross plant, and which suppressed the expression of symptoms in certain genotypes. The particular environment, which included possible excessive heat and sub-optimal light in the shaded summer greenhouse, may have strengthened these modifier effects. In the case of similar plants inoculated with the W-strain in the spring of 1955 in ground beds, the additional severity and infectivity of this virus strain, in combination with a more favorable environment, could explain the greater numbers of plants producing symptoms. The data obtained in this second test fit a 3:1 ratio satisfactorily only if early severe infections are classed against late and necrotic-mottle infections. It would appear that the ratios obtained are the result of the interaction of genotype, environment, and the virus strain used, and that combinations of these factors which are sub-optimal for infection and the expression of symptoms, allow the detection of only the strongest gene effects.

In summary, for the O.S.C. 21 X G.N. 31 reciprocal crosses, resistance tests indicate that susceptibility is highly dominant, with no reciprocal differences, and that differences between parents are due to two and possibly three major gene pairs, plus modifying genes whose combined effects may, in combination with the environment, be important in the modification of symptom expression and susceptibility to infection.

The relation of genes involved in this cross to those which determine differences in G.N. 31 X Mont. 43-15 could not be determined in the present study. It is suggested, however, that the previously postulated R_1R_2 gene pair is involved, and that at least one additional dominant allele for susceptibility is present in O.S.C. 21 but absent in both G.N. 31 and Mont. 43-15. Such an allele, which may be designated as S for discussion purposes, would be included in the variety genotypes as follows, with X representing a second possible dominant for susceptibility:

O.S.C. 21 - SS XX $R_?R_?$

Mont. 43-15 - ss xx R_1R_1

G.N. 31 - ss xx R_2R_2

The allele at the R locus in O.S.C. 21 may be R_1 or some other allele different from R_2, which could result in the occurrence, in the F_3 progeny types, of systemic necrosis similar to that of the F_1 of G.N. 31 X Mont. 43-15. Evidence for this is actually lacking, however, and any number of major gene or modifier combinations might result in such symptom expression. The strong effects of S and possibly X, probably preclude the possibility of clarifying the effects of the R locus in this cross.

Symptom Expression

Although it was not possible to adequately relate genotypes to the great variety of symptom types which occurred in the progenies of these crosses, observations and limited classification of such symptom types served to emphasize the general effects of genetic segregation on symptom expression.

While each parent was uniform in the production of a single symptom type in a particular environment, the progeny included a range of symptoms which equaled or exceeded the usual criterion for the classification of strains of BYMV in ordinary varieties. The symptoms observed included virtually every type described for the reactions of all reported strains of BYMV in large lists of bean varieties, even though such reported strains include a number of distinctly different types. The possibility that such a diversity of symptom expression is due to chance or a heterogeneous environment is discounted by the marked tendency for a particular symptom type to occur as an F_3 family characteristic. Although the effects of environment on the expression of a particular symptom were observed to be considerable during the study, the test in which symptoms were observed in detail was made in an environment that was quite uniform and favorable for the comparison of different genetic materials.

As previously mentioned, the susceptibility of a plant to virus infection and the expression of a particular set of symptoms are determined by an interaction involving the virus strain, the environment, and the inherant characteristics of the host plant. The observations made in this study have emphasized the importance of the plant genotype in this interaction. Further studies, under controlled environments, are needed to determine more exactly the relationships of these various factors.

Genetics of Resistance in the Interspecific Cross Phaseolus vulgaris L. X P. coccineus L.

Although the extent of the inheritance data obtained for the cross P. vulgaris L. X P. coccineus L. is limited, its practical importance might well be greater than that for the previously described crosses because of the high level of resistance carried by the P. coccineus L. lines. Observations made in connection with the present study would indicate that resistance in the tested lines of this species is not of the G.N. 31 type, but is actually immunity in the case of many virus strains. In breeding for resistance to BYMV and other viruses of beans, it may eventually be necessary to exploit as much as possible the virus resistance of this species, even though the use of the interspecific cross involves many problems not encountered in the usual hybridization within *P. vulgaris* L.

In this study it has been possible to obtain sufficient information on the inheritance of resistance to form a basis for a practical breeding program. F_2 progeny tests of O.S.C. 22 Blue Lake X *P. coccineus* L. line 2014 have clearly indicated that resistance (immunity) is recessive, but the number of genes involved can only be arbitrarily stated. Degrees of symptom expression in infected F_2 plants were observed to range from a severe condition similar to that of O.S.C. 22, to a completely symptomless condition with the virus present systemically in the plant, and detectable only by inoculations on susceptible varieties. In the test which was most successful, in terms of infecting the largest percentage of the inoculated plants, the ratio obtained (211:4) indicates that susceptibility to systemic infection by the Y strain of BYMV may be conditioned by three dominant genes. On the basis of the symptom variation in this test, and the ratios of 134:22 and 254:33 from previous tests, it is perhaps more logical to propose two major dominant genes for susceptibility with an undetermined number of modifiers. It is recognized that possible irregularities in genetic segregation caused by the combination of two species may have affected the results obtained.

From the breeder's standpoint the occurrence of symptomless infected plants must be considered in the formulation of breeding objectives, and imposes problems of technique. Although such symptomless or masked conditions may possibly be related to the tendency of segregates from this cross to be thick-leaved, vigorous, and dark green in color, they may never-the-less occur in advanced generations in a breeding program. It would therefore be necessary to make extensive test inoculations to susceptible varieties in order to avoid the development of tolerant varieties.

Virus Strains in Relation to Resistance

In an inheritance study of this kind, the importance of virus strain relationships should not be overlooked, because the inheritance data and their practical significance depend largely upon the number and nature of strains used for testing.

It was previously known that G.N. 31, while resistant to several reported strains of BYMV (14, p.45), (13, p.11), is susceptible to strains found in Idaho (7, p.621). This does not preclude the possibility of studying the genetic differences between G.N. 31 and those varieties which are more susceptible to a given strain or group of strains. Because only two strains of BYMV were employed in this study, it was important to learn something of the general level of resistance to natural infection in the variety and progeny material. In the field planting with gladioli as a source of natural infection, it was found that the reaction of varieties and progeny lines was generally similar to the reaction of the same materials in greenhouse inoculations with the Y-strain. While it is recognized that our knowledge concerning viruses carried in gladioli is incomplete, the results of this test, in which G.N. 31 and the most resistant progeny lines developed little or no infection in comparison with the severely infected Blue Lake strains, suggest that practical resistance to BYMV is available for breeding work in Oregon. As previously discussed, however, the resistance or immunity of the P. coccineus L. varieties may be more valuable for widespread usage in breeding programs.

SUMMARY

Studies of the inheritance of resistance to two strains of bean yellow mosaic virus in the Great Northern U.I. 31 bean variety were conducted from 1953 to 1956, employing primarily greenhouse testing with the rub-inoculation technique. The resistance of G.N. 31 apparently lies, to a large extent, in the tendency for localization of the infection in the inoculated leaf.

In crosses of G.N. 31 with Mont. 43-15, a variety highly susceptible to moderately severe systemic mottle, the F_1 was intermediate in reaction, developing systemic necrosis followed by a mild mottle. The F_2 and F_3 data supported the hypothesis that the occurrence of the susceptible parental type was conditioned by a single homozygous gene pair. The symptom expressions of the F_1 type and resistant parental type, which were difficult to separate, were apparently influenced by modifying factors. The parent varieties were concluded to differ by a single major gene, without dominance, and modifying genes.

Reciprocal crosses between G.N. 31 and O.S.C. 21 Blue Lake, a variety susceptible to severe systemic infection, produced an F_1 generation which was very similar in susceptibility to the susceptible parent. The F_2 and F_3 generations included a wide range of symptom types, from highly resistant to extremely severe, with the types similar to the susceptible parent predominating. Data from tests of 1,117 F_2 plants, 1,229 small F_3 families, and 1,073 first generation backcross plants supported the hypothesis that resistance is recessive

in inheritance, and conditioned by two or three major genes, with modifiers which influence susceptibility to infection and the type and severity of symptoms. Differences in behaviour between reciprocal crosses were not observed.

Observations of symptoms were made, and the importance of the effect of plant genotype on symptom expression was discussed.

Interspecific crosses were made between Phaseolus vulgaris L. (O.S.C. 22 Blue Lake) and P. coccineus L. (line 2014) which is resistant or immune to the strains of BYMV employed for testing. Variable results of three separate F_2 tests indicated that resistance to infection may be conditioned by three, but more logically two recessive gene pairs, and modifiers. Systemic infection in progeny plants ranged from severe to a symptomless condition in which virus presence could be detected only by test inoculations on susceptible varieties.

A field test was conducted to determine whether G.N. 31, resistant progeny lines, and P. coccineus L. lines were resistant to insect transmitted viruses from gladioli. The behaviour of varieties and progeny lines was very similar to the greenhouse reactions of the same varieties and lines inoculated with the Y strain of BYMV. All six lines of P. coccineus L. were apparently immune.

It is apparent from the results of this inheritance study that the number of genes involved, and the recessive nature of resistance, would require large populations of segregating plants for the isolation of resistant individuals. The use of backcross programs and careful greenhouse testing with several virus strains as well as field elimination with natural infections, would likely be required for the ultimate development of horticulturally acceptable resistant varieties. Lines of _P_. _coccineus_ L. appear to offer a superior source of resistance to BYMV strains.

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APPENDIX

PLATES

The photographs on the following pages show various symptoms observed in F_3 and F_4 progenies of reciprocal crosses between O.S.C. 21 Blue Lake and Great Northern U.I. 31. All symptoms shown are the result of inoculations with the Y strain of bean yellow mosaic virus.

Plate 1. F_3 families of O.S.C. 21 X G.N. 31, and check varieties two weeks after inoculation with the Y strain of BYMV.

Top: Severely affected 2-153, compared with O.S.C. 21 on the right.

Bottom: G.N. 31 without symptoms on the left; severely infected 2-140 on the right.

Plate 2. F_3 families two weeks after inoculation (from the same planting as the families shown in Plate 1).

Left: 4-152, which developed systemic mottle on the first trifoliate leaves later than the more severely infected families shown in Plate 1, and which was ultimately classified as mildly infected.

Right: 2-145, which was symptomless at this time, but which later developed a mild systemic mottle.

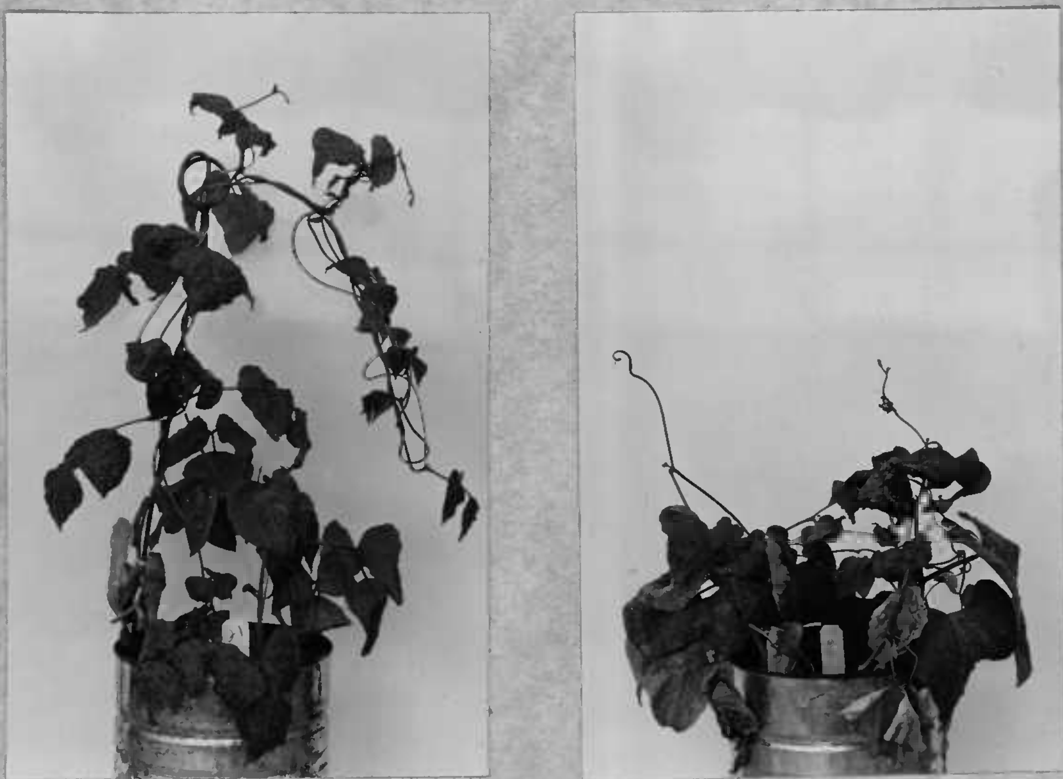


Plate 3. Plants (from the same planting as those in Plates 1 and 2) five weeks after inoculation.

Left: O.S.C. 21 with symptoms typical of inoculated can-grown plants of this variety in spring or summer.

Right: F_3 family which was ultimately more severely affected than O.S.C. 21, with almost complete restriction of growth and death of several plants. Symptoms in this family two weeks after inoculation were similar to those of O.S.C. 21.

Plate 4. F_3 family in which most plants usually died when inoculated, shown four weeks after inoculation.

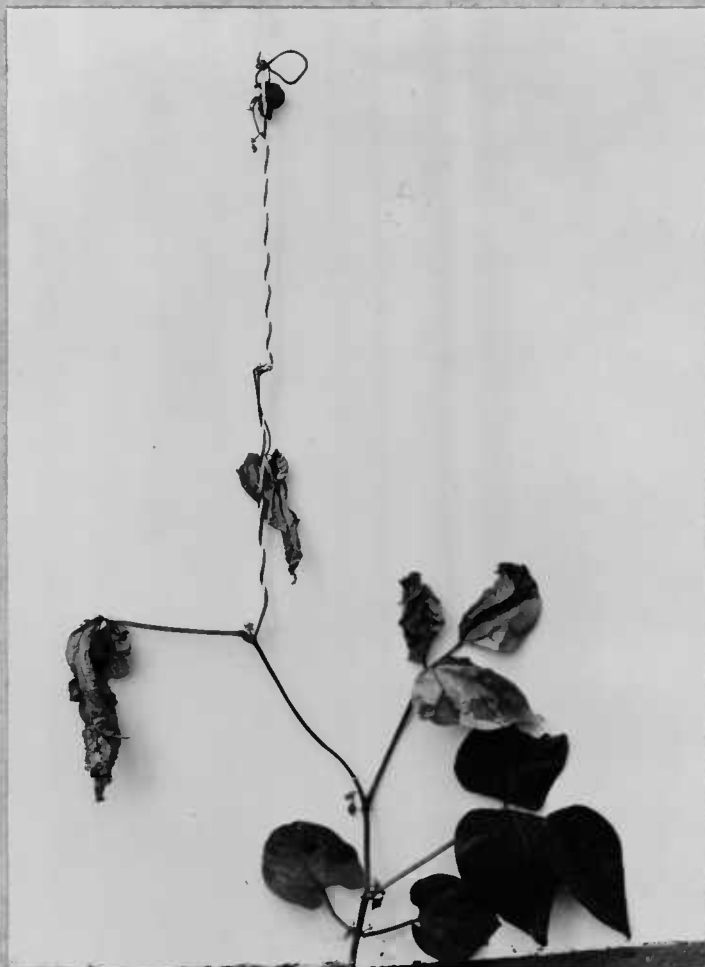


Plate 5. An F_3 plant, eight weeks after inoculation, showing the symptomless first trifoliate leaf, the second trifoliate leaf which developed systemic vein necrosis before being killed, and the dead upper stem and leaves.

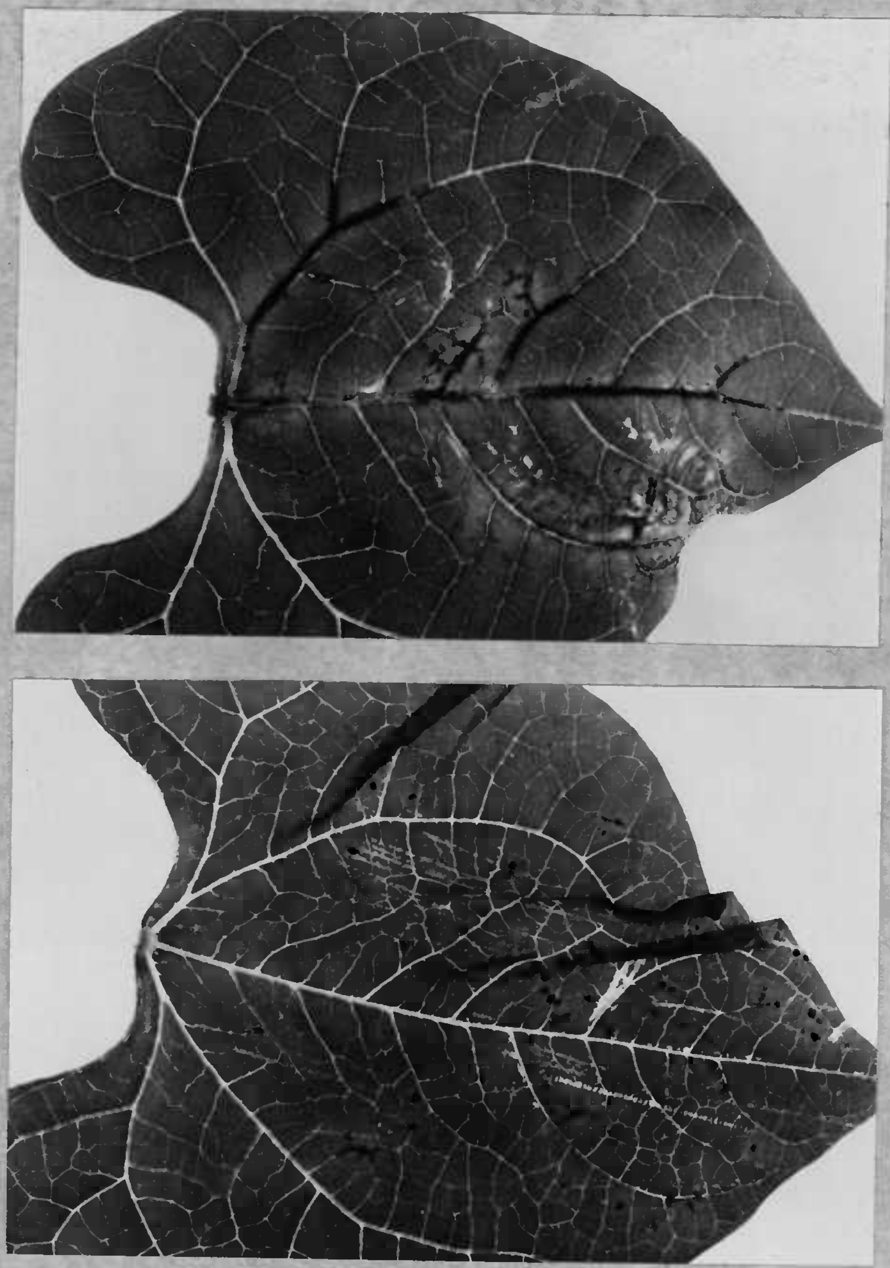


Plate 6. Necrotic symptoms of the inoculated primary leaves.

Top: Necrosis of midrib and main veins, most common in families that developed severe systemic mottles.

Bottom: Small discrete lesions, about one week after inoculation, which usually developed into the condition shown in Plate 7.

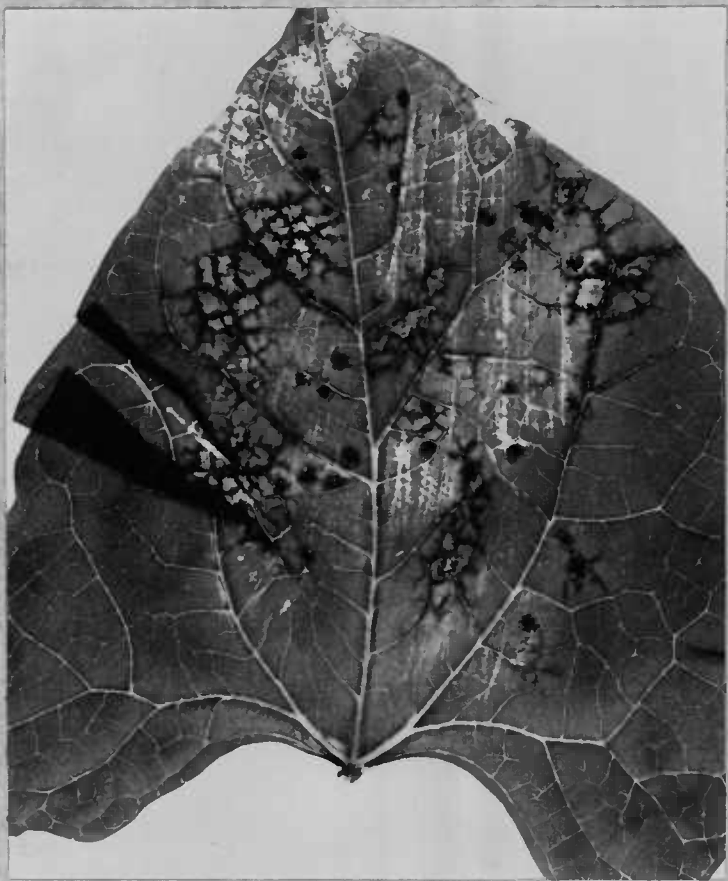


Plate 7. Necrotic symptoms of the inoculated primary leaf of an F_3 plant, about two weeks after inoculation, showing original lesions and spreading vein necrosis.

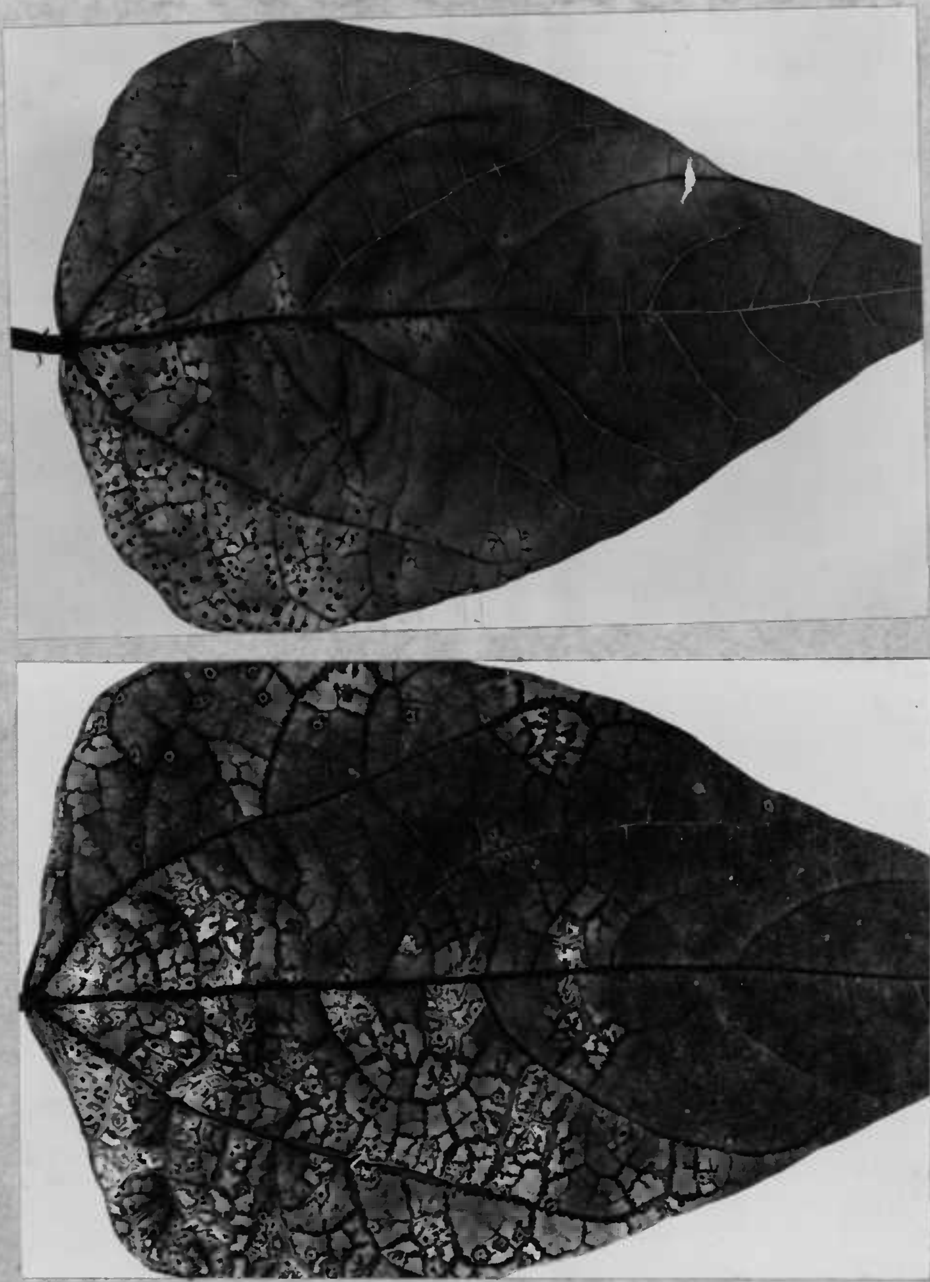


Plate 8. Leaflets with systemically occurring necrosis.

Top: Third trifoliate leaflet showing necrotic lesions and vein necrosis of a plant on which the upper leaves, but not the terminal, were killed.

Bottom: Leaflet with distinct ring-like lesions and vein necrosis with a vein banding effect, from a plant which later developed a severe mottle.

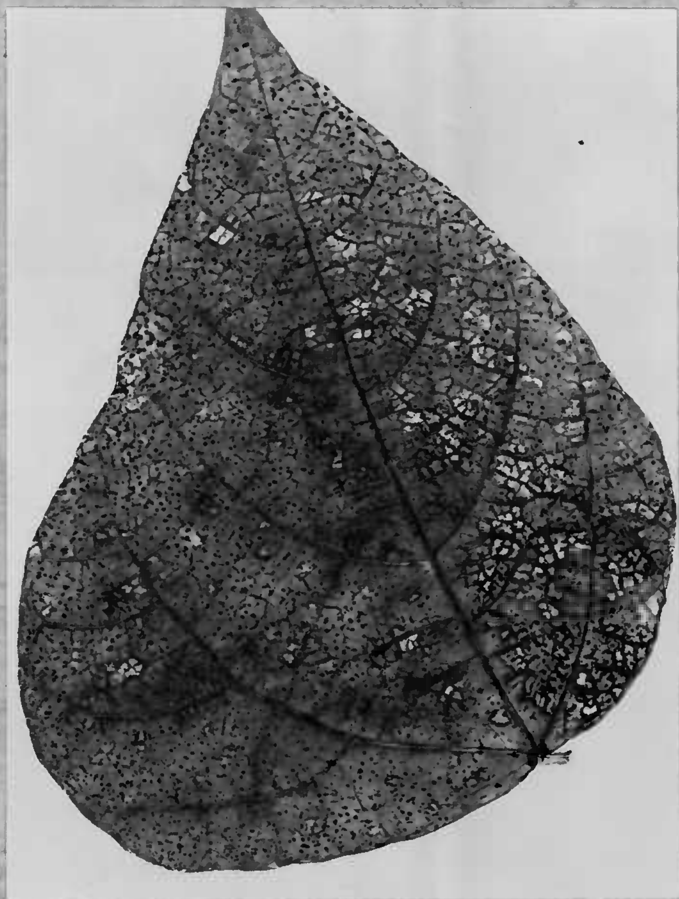


Plate 9. Systemically occurring necrosis. Upper leaflet from a plant which developed general necrosis of leaves and stems.

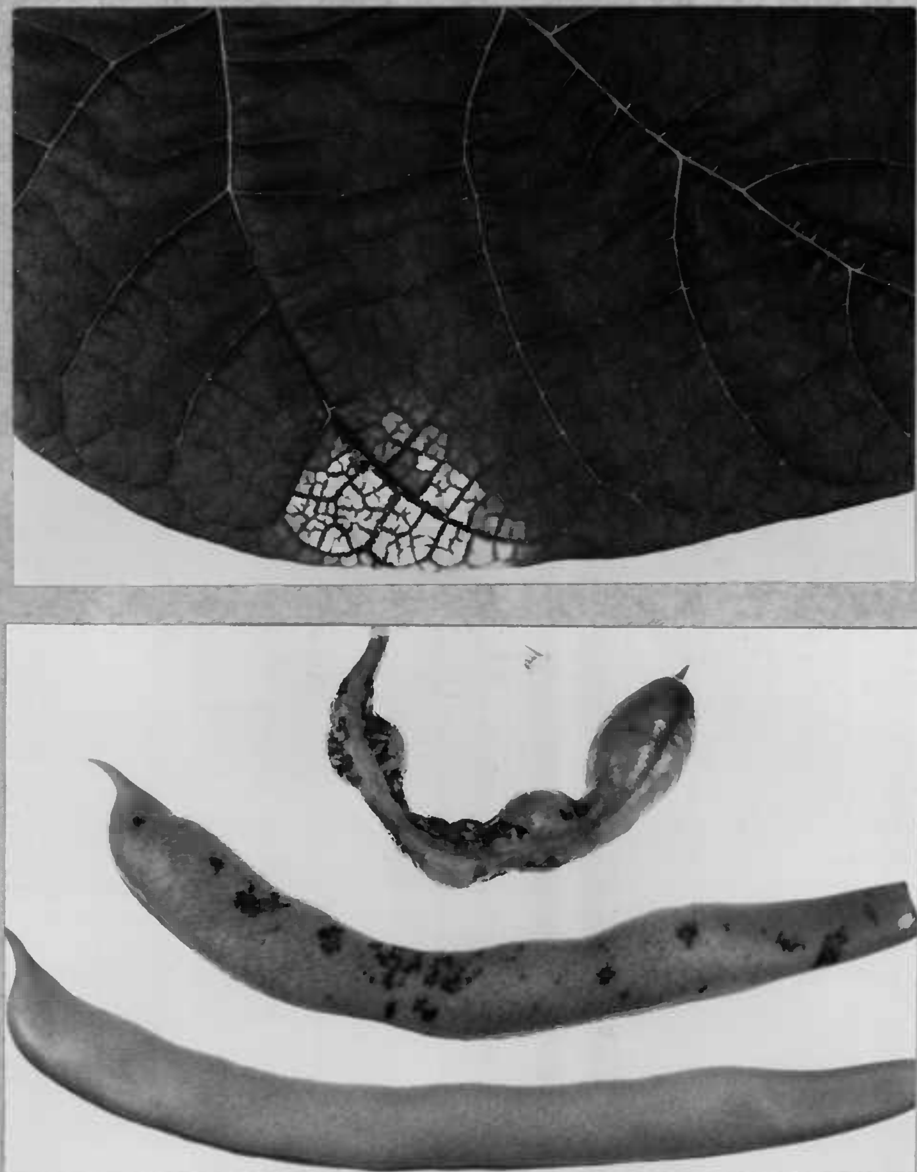
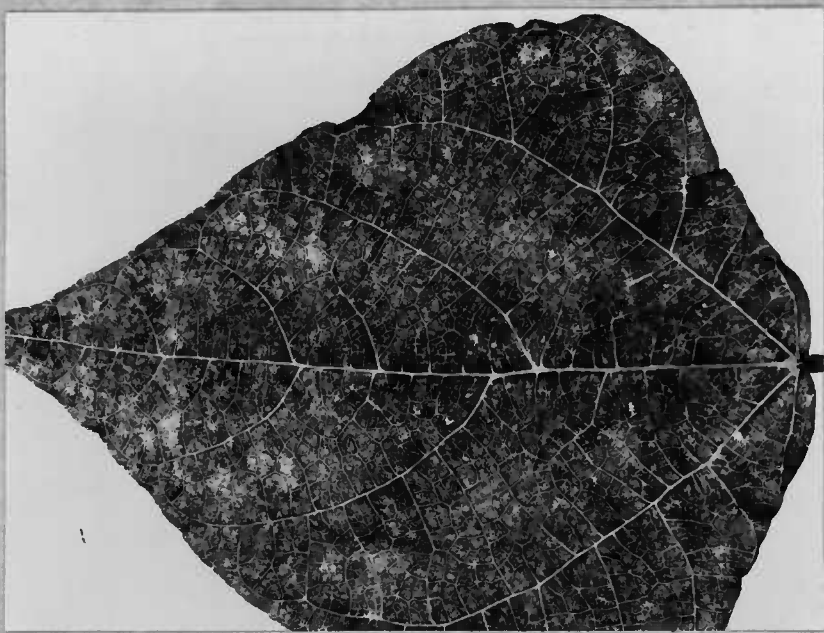


Plate 10. Symptoms from the single infected plant of highly resistant family II-103.

Top: Isolated patch of vein necrosis on the eighth trifoliate leaf. No systemic symptoms had appeared prior to this necrosis, which was proven infective, and which was followed by additional leaf, stem, and pod necrosis, and a mild mottle in the top growth.

Bottom: Distorted and necrotic pods from the upper parts of the plant, with an unaffected pod for comparison.



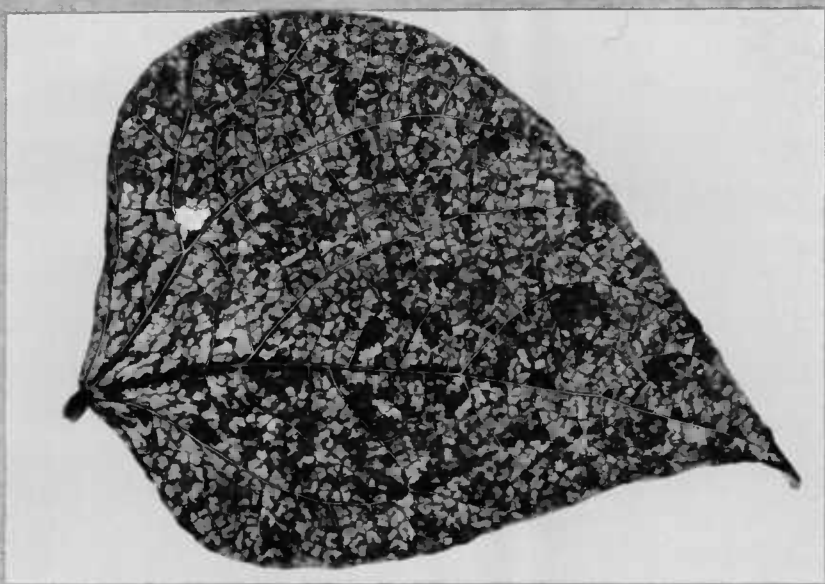
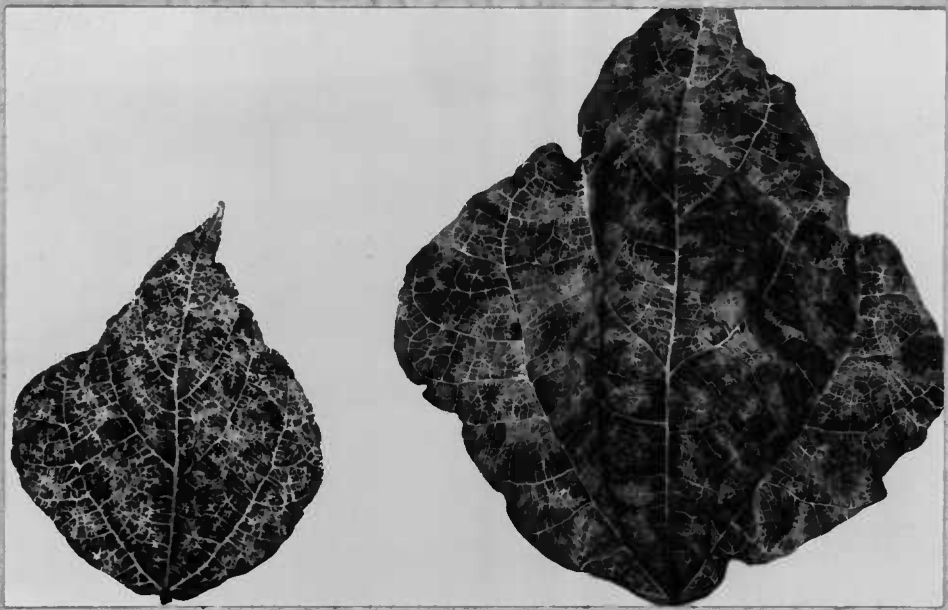


Plate 11. Mild systemic mottles.

Top: Very mild mottle of II-79, with little distortion of the leaf surface.

Bottom: F_3 leaflet showing mild but sharply defined mottle with little distortion or reduction of the leaf surface.



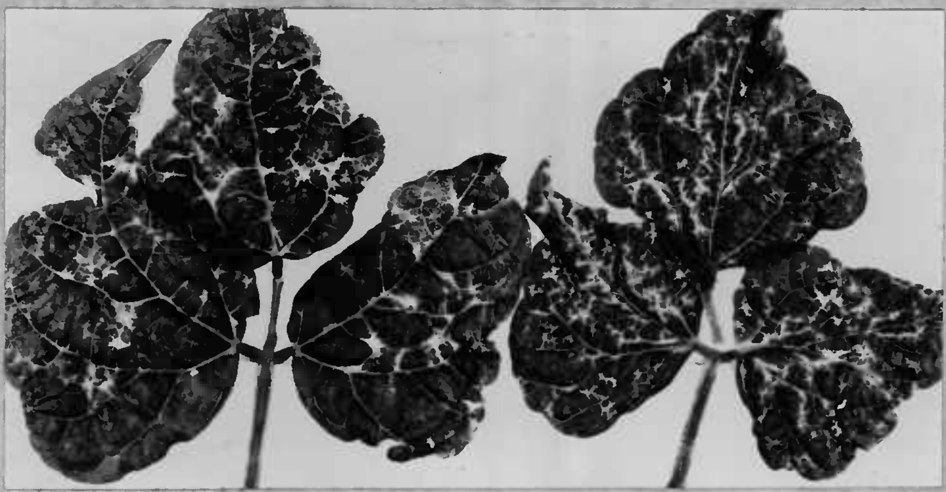


Plate 12. Leaves showing systemic mottles.

Top: Severe mottle of O.S.C. 21 on the left; F_3 leaflet showing coarse, moderately severe mottle, with cupping and moderate reduction of the leaf surface, on the right.

Bottom: Very severe mottle with strong leaf cupping and distortion of the leaf surface.

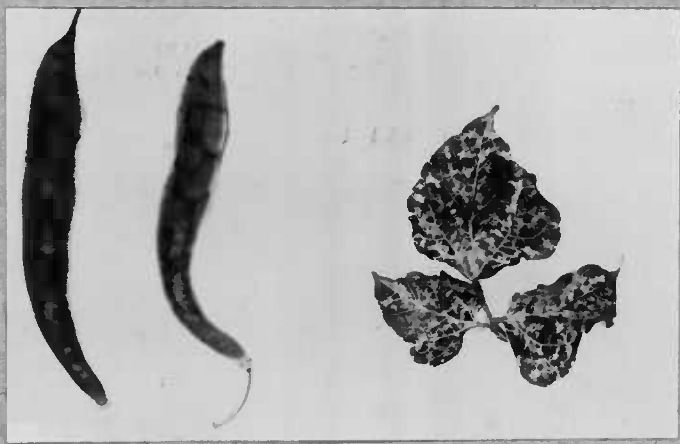


Plate 13. Extremely severe systemic mottles.

Top: Extreme dwarfing and mottle of an F_3 plant 60 days old and about 11-12 inches in height. Average uninfected plants in this test were 10 feet in height, while infected O.S.C. 21 was from $3\frac{1}{2}$ to $4\frac{1}{2}$ feet in height at this time.

Bottom: Leaf and pods from a plant with extremely severe infection. The photograph is about 3/4 actual size.