A POD-DISTORTING STRAIN OF THE YELLOW MOSAIC VIRUS OF BEAN[1]

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INTRODUCTION

During the summer of 1946 in an experimental plot near Madison, Wis., plants of Idaho Refugee and other varieties of bean (*Phaseolus vulgaris* L.) highly resistant to bean virus 1 appeared to be affected with yellow mosaic (bean virus 2), but were more severely stunted than typical and bore severely distorted pods. Preliminary inoculations to bean in the greenhouse indicated that the disease was incited by a virus and that plants of certain varieties developed severe top necrosis while others were highly resistant. In parallel inoculation tests with the typical strain of bean virus 2, mottle and slight stunting occurred, but no varieties were highly resistant and in only one variety did the top necrosis develop. The inoculations indicated that this virus was not the typical strain of bean virus 2, but similarity of symptoms on certain bean varieties suggested that it might be a strain of that virus. This paper is a report of the results of a comparative study of this apparently new virus strain with other viruses of bean.

MATERIALS AND METHODS

The greenhouses in which studies were conducted were kept free of insects by frequent fumigation and spraying. All inoculations and other tests were made in greenhouses in which the temperature was maintained between 24° and 28° C.

A culture of typical bean virus 2 was secured from naturally infected plants in the vicinity of Madison, Wis. This virus is the same as that used in studies reported recently (*2*, *3*),[3] and it is believed to be similar to the strain described by Pierce (*7*). A culture of the pod-distorting virus was obtained from naturally infected Idaho Refugee plants also in the vicinity of Madison. Stock cultures of these viruses were maintained in Sensation Refugee 1066 plants, which variety is highly resistant to bean virus 1. The possibility of contamination with the latter virus was thereby practically eliminated. Sources of several other viruses and virus strains used in cross-protection studies will be given later.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 314.

Lot Z–1 and the variety Rival were supplied by W. J. Zaumeyer. The UI selections were obtained from Leslie Dean of the University of Idaho. The other bean varieties used were obtained from reliable commercial sources. Seeds of the various legumes used were secured from Roland McKee of the United States Department of Agriculture.

All greenhouse plants were inoculated in early stages of growth by rubbing with carborundum as an abrasive. At least 10 plants were used for each inoculation test. Where symptoms on inoculated plants were mild or completely absent, the presence or absence of the viruses was determined by extracting the juice and inoculating healthy Idaho Refugee plants.

The properties of the pod-distorting virus were determined on greenhouse-grown plants by the methods described by Johnson and Grant (*4*).

EXPERIMENTAL RESULTS

INOCULATIONS TO BEAN VARIETIES

Symptom reactions of 37 varieties or strains of bean to the pod-distorting virus and the common strain of bean virus 2 are presented in table 1. They can be grouped with respect to the pod-distorting virus into four classes: (1) No symptoms and no recovery of the virus; (2) stunt, mottle, and leaf deformation but no necrosis (fig. 1); (3) top necrosis followed by partial recovery or general necrosis followed by death of the entire plant (fig. 2 and 3); (4) a modified

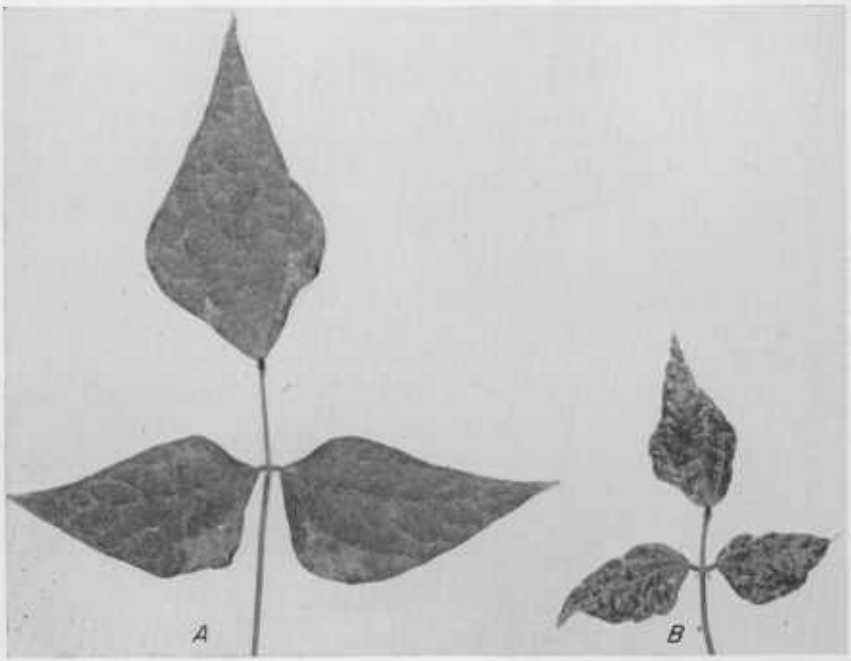

FIGURE 1.—Symptoms on leaves of Idaho Refugee bean plants infected with typical yellow mosaic virus (*A*) and the pod-distorting virus (*B*).

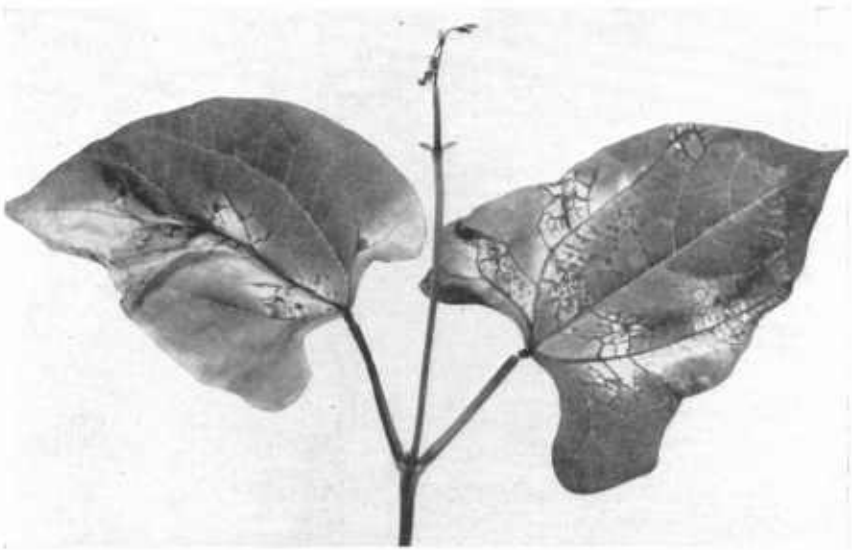

FIGURE 2.—Necrosis on inoculated leaves and on the growing point of a bean plant of the UI 15 variety.

type of necrotic reaction in which the tip leaves turned yellow and were abscised leaving a bare stem at the extremities but not killing the growing point (fig. 4).

Pods which were set on plants of any group with mottle or other symptoms were severely warted and misshapen (fig. 5). The typical strain of yellow bean mosaic virus was infectious to all varieties of bean tested, usually causing a stunt and mottle but no necrosis. However, it caused a necrotic reddening of stem and petiole at the nodes of the plants of Stringless Blue Lake (fig. 6) which resembled the disease described by Virgin (*9*). On McCaslan it caused top necrosis which resembled the symptoms produced by the pod-distorting virus on Michelite (fig. 7), Pinto, and several other varieties listed in table 1. However, in no case did it produce malformed and warted pods. The varieties UI 59, UI 81, and UI 123 were highly resistant to the pod-distorting virus and attempts to recover the virus from them were unsuccessful.

RANGE OF LEGUMINOUS HOSTS

Nineteen leguminous hosts listed in table 2 were inoculated with typical bean virus 2 and the pod-distorting virus to determine whether the latter virus would infect the same hosts as the former, and, if so, whether any differences existed between the two in symptom expression. A study of table 2 will reveal that 7 of the hosts were infected by both of the viruses, symptoms appearing either as a mottle or a vein-clearing mottle. Symptoms incited by typical bean virus 2 and the pod-distorting virus on Canadian field pea, fenugreek, and crimson clover are shown in figure 8. None of the other 12 hosts were infected by the pod-distorting virus but 6 of them were infected by typical bean virus 2. The pod-distorting virus did not infect any host which was

FIGURE 3.—Top necrosis on a plant of the UI 1 variety of bean infected with the pod-distorting virus.

TABLE 1.—*Symptoms produced on plants of 37 varieties or strains of bean inoculated with typical bean virus 2 and on plants inoculated with the pod-distorting virus*

Variety or strain	Symptoms on plants inoculated with—	
	Typical bean virus 2	Pod-distorting virus
U I 59	Faint mottle	None.
U I 81	do	Do.
U I 123	do	Do.
Michelite	Distinct mottle, slight stunt	Top necrosis.
U I 1	do	Do.
U I 15	do	Do.
Pinto	do	Do.
Stringless Blue Lake (black-seeded)	Distinct mottle, slight stunt, and red nodes.	General necrosis of old plants.
Stringless Blue Lake (white-seeded)	do	Top necrosis.
Sensation Green Pod	Distinct mottle and moderate stunt.	Do.
Bountiful	do	Do.
Potomac	do	Necrosis of main stem of old plants but no top necrosis.
Kentucky Wonder	do	Do.
Keystonian	do	Top necrosis.
Golden Cluster Wax	do	Do.
Scotia or Striped Creaseback	Severe stunt, mottle	All plants dead.
Lazy Wife	do	Top necrosis.
McCaslan	Mottle and top necrosis	Do.
Resistant Kentucky Wonder	Moderate stunt, mottle	Necrosis of main stem in old plants.
Robust	do	Mild top necrosis.
New Stringless	do	Leaves twisted and deformed; young leaves abscised.
Full Measure	do	Do.
Pencil Pod Black Wax	do	Do.
Tenderpod	do	Do.
Stringless Black Valentine	do	Do.
Burpee Brittle Wax	do	Do.
Tendergreen	do	Do.
Rival	do	Do.
Giant Stringless Green Pod	do	Severe stunt, mottle, and leaf distortion.
Dwarf Horticultural	do	Moderate stunt, deformed leaves, mottle.
Rust Proof Golden Wax	do	Do.
Plentiful	do	Do.
Improved Golden Wax	do	Do.
Florida Belle	do	Do.
Z-1	do	Do.
Sensation Refugee 1066	do	Do.
Idaho Refugee	do	Do.

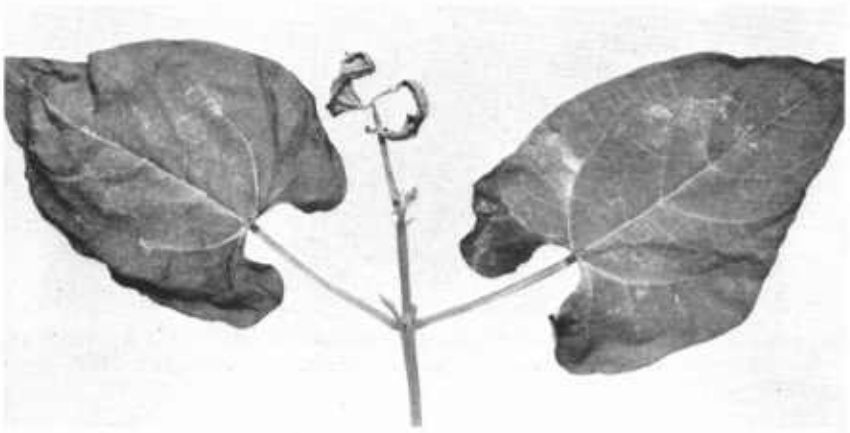

FIGURE 4.—A plant of the Rival variety of bean infected with the pod-distorting virus; one leaflet of the youngest trifoliate leaf has dropped, the other two are dead, but the growing point is not necrotic.

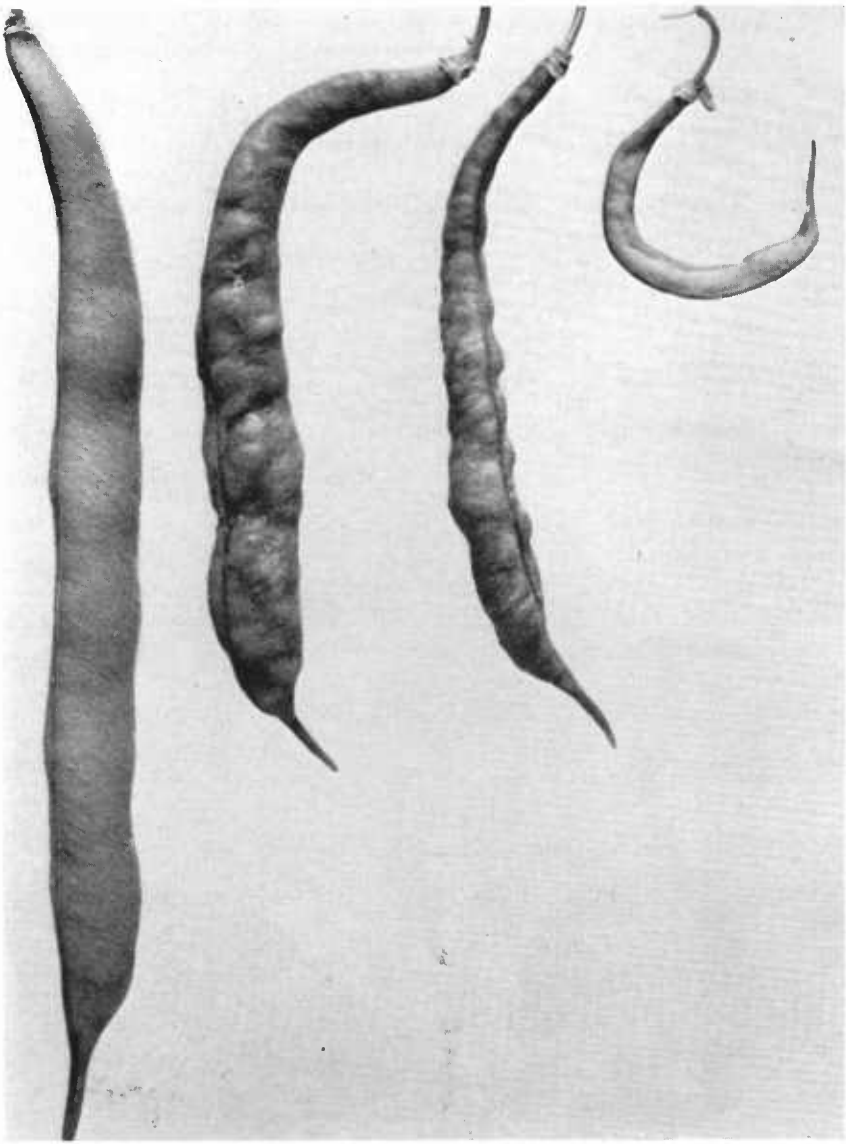

FIGURE 5.—A pod from a healthy Idaho Refugee bean plant (left) compared with severely distorted pods from a plant of the same variety infected with the pod-distorting virus.

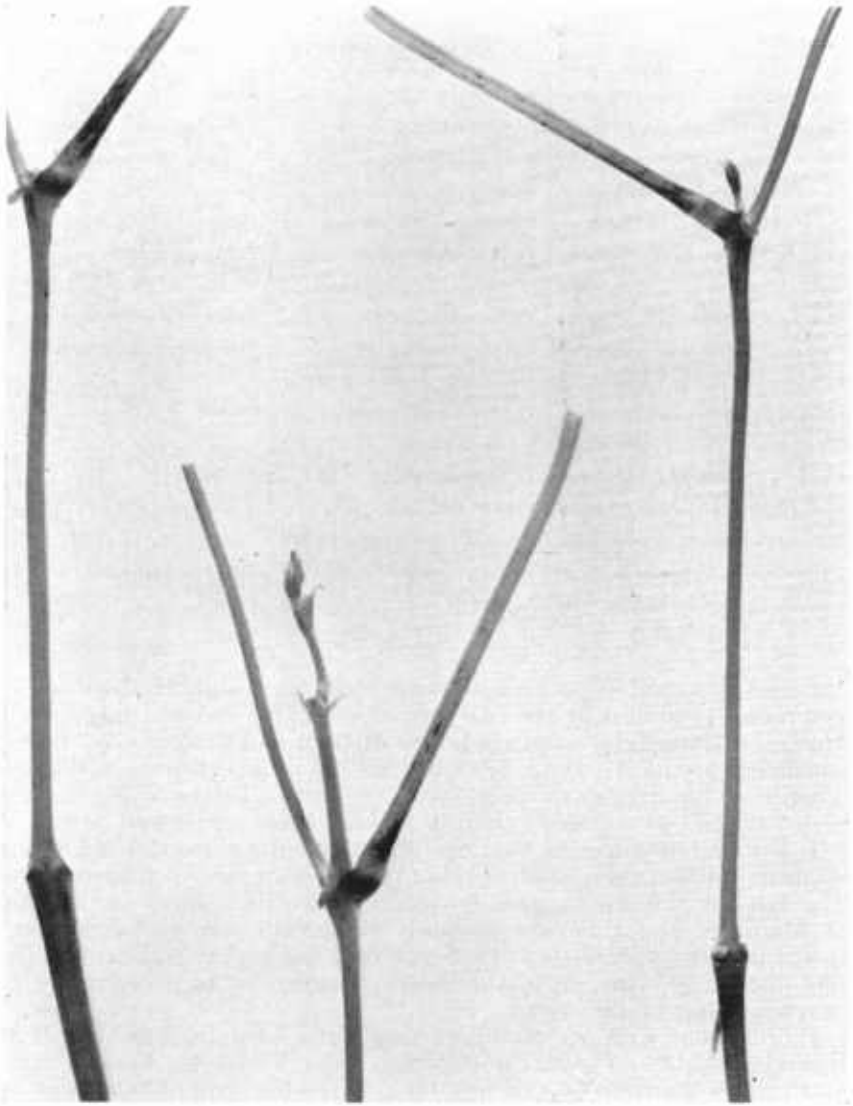

FIGURE 6.—Necrosis at the juncture of stem and petiole in a plant of the Stringless Blue Lake variety of bean infected with typical bean virus 2.

TABLE 2.—*Symptoms produced by typical bean virus 2 and the pod-distorting virus on 18 leguminous hosts other than bean*

Host	Symptoms produced by—	
	Typical bean virus 2	Pod-distorting virus
Pisum sativum L. (pea var. Wisconsin Perfecton).	None	None.
Pisum sativum L. (pea var. Perfected Wales).	Distinct yellowish mottle	Yellowish mottle and slight vein clearing.
Pisum sativum var. *arvense* Poir. (Canadian field pea).	Yellowish mottle	Yellowish mottle and vein clearing.
Phaseolus lunatus L. (Henderson Bush lima).	None	None.
Trifolium incarnation L. (crimson clover).	Reduction in leaf size and vein clearing.	Yellowish-green mottle with vein clearing.
Trifolium hybridum L. (alsike clover)	Faint yellowish mottle	Faint green mottle.
Trifolium pratense L. (red clover)	None	None.
Trifolium repens L. (Ladino white clover).	do	Do.
Vicia monantha (L.) Desf. (Monantha vetch).	Yellow-spotted mottle	Yellow to white-spotted mottle.
Vicia villosa Roth. (hairy vetch)	Faint greenish mottle	Very faint greenish mottle.
Vicia faba L. (Broadbean)	Light greenish-yellow mottle	None.
Vicia sativa L. (spring vetch)	Leaves light green with darker green islands.	Do.
Vicia atropurpurea Desf. (purple vetch).	Yellowish-green mottle	Do.
Melilotus alba Desr. (white sweetclover).	Yellowish mottle	Do.
Melilotus officinalis (L.) Lam. (yellow sweetclover).	Yellow-green mottle	Do.
Medicago hispida Gaertn. (Bur clover).	None	Do.
Soja max (L.) Piper (soybean)	Crinkled upper leaves with yellow mottle.	Do.
Trigonella foenum-graecum L. (fenugreek).	Leaves yellow green with darker green islands.	Yellowish mottle and slight vein clearing.
Arachis hypogaea L. (peanut var. Jumbo).	None	None.

not also susceptible to typical bean virus 2. Slight differences in symptoms produced by the two viruses on several hosts are recorded. However, these differences are not as distinct and probably are not as consistent as the reactions described on the bean varieties in table 1. Therefore for diagnostic purposes, it would be better to use several bean varieties as test hosts than to use the hosts considered here.

It is of interest to note that the pod-distorting virus did not infect white or yellow sweetclover, but that typical bean virus 2 infected both. The latter virus was isolated frequently from these hosts in the field at Madison, and they are probably important sources of primary inoculum for it (*7*, *8*, *14*, *15*). Since they are highly resistant to the pod-distorting virus, some other host or hosts must be concerned with overwintering of this virus.

Inoculations with the pod-distorting virus were made to four nonleguminous hosts, *Nicotina glutinosa* L., *N. tabacum* L., *N. rustica* L., and *Cucumis sativus* L. (variety Ohio 31). No symptoms developed and attempts to recover the virus by inoculating bean failed in all cases.

PROPERTIES OF THE POD-DISTORTING VIRUS

Expressed juice from Idaho Refugee plants recently infected with the virus was treated and then used to inoculate young healthy Idaho Refugee plants. Five plants were used as a test unit and each experiment was repeated three times. The properties of the pod-distorting virus as shown in table 3 were as follows: Thermal inactivation point,

after heating for 10 minutes, 58° to 60° C.; inactivation in vitro at 20°, 32 hours; dilution end point, 1-2,000. These properties conform to those reported by Pierce (*7*) for typical bean virus 1 and typical bean virus 2.

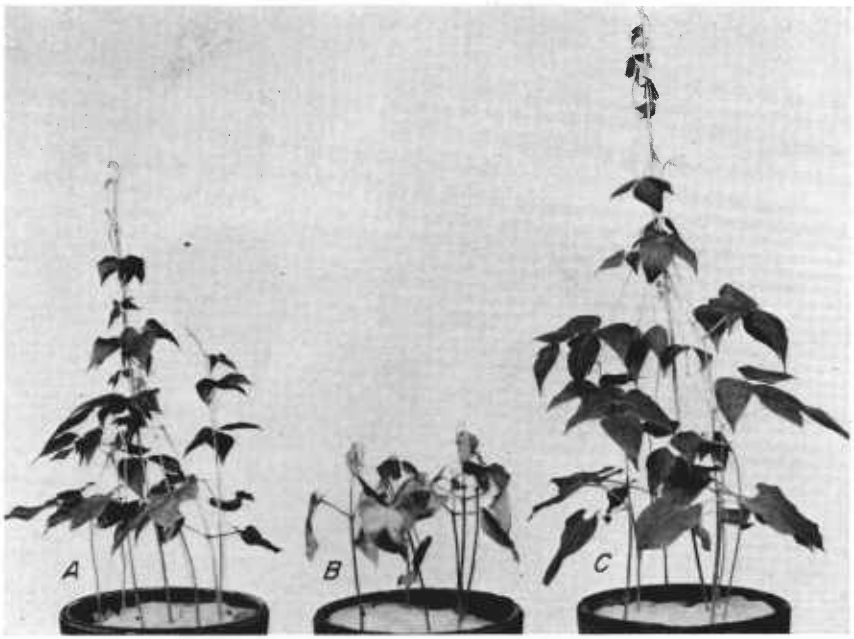

FIGURE 7.—*A*, Plants of the Michelite variety of bean infected with typical bean virus 2; stunting and mottle occur but there is no necrosis. *B*, Plants of the same variety infected with the pod-distorting virus and severely affected with top necrosis. *C*, Uninoculated plants of Michelite variety.

TABLE 3.—*Properties of the pod-distorting virus*

Thermal inactivation			Tolerance to dilution			Longevity in vitro		
Temperature (°C.)	Total plants	Infected plants	Dilution	Total plants	Infected plants	Hours	Total plants	Infected plants
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
Untreated	15	15	Undiluted	15	15	0	15	15
45	15	14	1–10	15	15	12	15	15
50	15	15	1–100	15	15	24	15	3
52	15	15	1–1,000	15	7	32	15	0
54	15	11	1–1,200	15	6	48	15	0
56	15	6	1–1,400	15	3	60	15	0
58	15	3	1–1,600	15	4			
60	15	0	1–1,800	15	1			
65	15	0	1–2,000	15	0			
70	15	0	1–2,200	15	0			
80	15	0	1–10,000	15	0			

SEED TRANSMISSION

Pierce (*7*) reported no infection in 6,532 seedlings grown from seed of plants infected with bean virus 2, while seed transmission of bean virus 1 was readily demonstrated. When 285 seeds from Idaho Refugee plants artificially infected with the pod-distorting virus in the

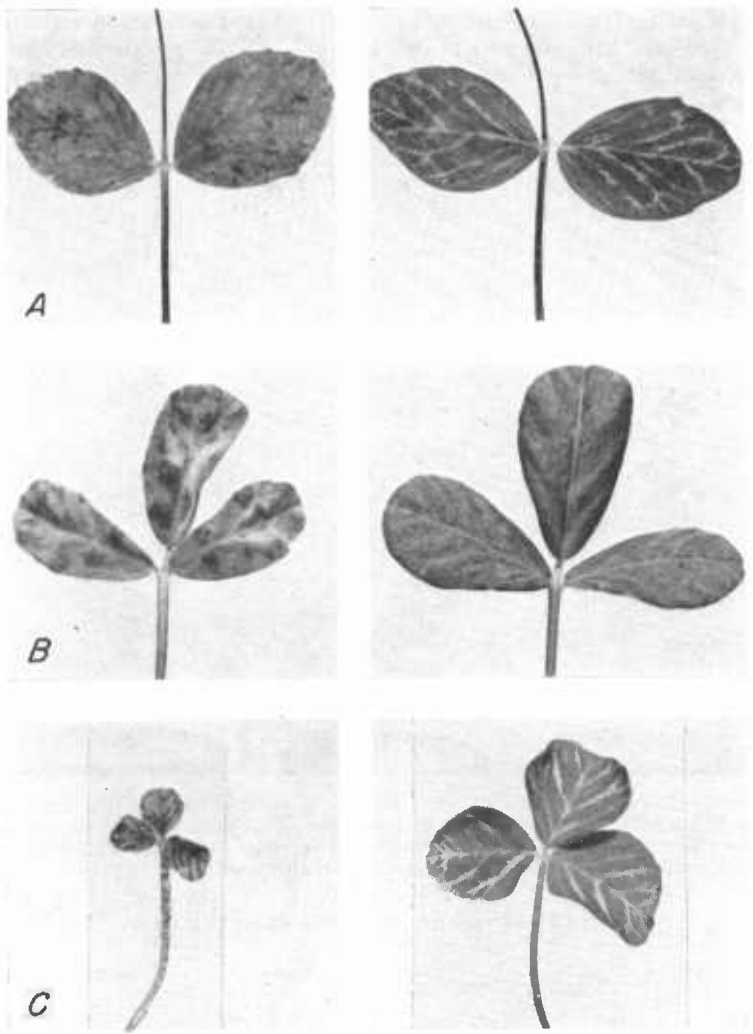

FIGURE 8.—Leaves from bean plants infected with typical bean virus 2 (left) and the pod-distorting virus (right): *A*, Canadian field pea; *B*, fenugreek; *C*, crimson clover.

greenhouse were planted in flats in the greenhouse, no disease occurred. When 160 seeds from naturally infected Sensation Refugee 1066 plants infected in the field were tested in the same manner, no plants developed disease. Although further trials are needed to determine conclusively whether this virus is ever transmitted through bean seeds, these preliminary tests indicate strongly that it is not.

INSECT TRANSMISSION

The typical strain of bean virus 2 is transmitted by several species of aphids (*7*). Two trials were conducted to determine whether the pod-distorting virus is insect-transmitted. Green peach aphids

(*Myzus persicae* (Sulz.)) were raised on healthy cabbage plants, removed to a clean, dry test tube, and starved for 3 hours. After this interval they were allowed to feed on plants infected with the pod-distorting virus for 15 to 20 minutes. Five or more aphids were then transferred to each healthy test plant and allowed to feed until the insects died. In one test two plants out of six inoculated became infected with the pod-distorting virus; in a second test three plants out of six inoculated were infected.

CROSS PROTECTION BY SEVERAL VIRUSES OF BEAN AGAINST THE POD-DISTORTING VIRUS

The pod-distorting virus caused severe top necrosis on several varities of bean, two of which were Michelite and Pinto. This distinctive reaction provided a test to determine whether or not several other viruses of bean which cause systemic infection but no necrosis could prevent or decrease the incidence of necrosis caused by the pod-distorting virus if they were applied to the test plants several days in advance of the latter. When strains of bean virus 2 and Burkholder's strain of bean virus 1 were being tested, plants of the Michelite variety were used for the test. When the typical strain of bean virus 1 was used it was necessary to use Pinto plants since Michelite is not susceptible to that strain.

The viruses used were typical bean virus 2, greasy-pod virus, Burkholder's strain of bean virus 1, and typical bean virus 1 from stock cultures described previously (*2*, *3*). In addition to these, there was included a culture of southern bean mosaic (*11*, *12*) obtained from W. C. Price and a culture of cucumber virus 1 (strain 14), which is infectious to bean and pea (*10*). This latter virus was obtained from a stock culture maintained in this laboratory. These viruses were inoculated to the test plants 15 days in advance of the pod-distorting virus and final readings on necrosis produced were made 1 month after the second inoculation, a period sufficient to permit the maximum development of necrosis. Results are presented in table 4.

TABLE 4.—*Cross protection by 5 viruses of bean against the pod-distorting virus*

Treatment	Test variety	Total plants	Necrotic plants
Typical bean virus 2 followed by the pod-distorting virus [1]	Michelite	41	3
Cucumber virus 1 (strain 14) followed by the pod-distorting virus	do	18	18
Greasy-pod virus followed by the pod-distorting virus	do	42	38
Southern bean mosaic virus followed by the pod-distorting virus	do	17	14
Pod-distorting virus only	do	40	38
Greasy-pod virus followed by the pod-distorting virus	Pinto	30	2
Typical bean virus 1 followed by the pod-distorting virus	do	30	1
Pod-distorting virus only	do	30	29

[1] All second inoculations were made 15 days after the first.

When susceptible plants were inoculated with typical bean virus 2, greasy-pod virus, typical bean virus 1, or Burkholder's strain of bean virus 1, 15 days before inoculation with the pod-distorting virus, there was a marked reduction in the amount of top necrosis produced by the pod-distorting virus. However, similar inoculations with southern bean mosaic virus or cucumber virus 1 (strain 14) caused no reduction in top necrosis. When Michelite plants were inoculated

with the greasy-pod virus and later inoculated with the pod-distorting virus, no reduction in the amount of top necrosis resulted. In contrast to this, when similar inoculations were made to plants of the Pinto variety a marked reduction in the amount of top necrosis occurred. This is explained by the fact that Michelite is highly resistant to the greasy-pod virus while Pinto is susceptible. Therefore when Michelite was inoculated with the greasy-pod virus no infection resulted and the subsequent inoculation with the pod-distorting virus was made to healthy plants which developed top necrosis to as great an extent as the control plants (pod-distorting virus alone).

In order to determine whether or not the suppression of the top-necrosis symptom described above was due to a complete or nearly complete inhibition of development of the pod-distorting virus, the following experiment was carried out. Fifteen Stringless Green Refugee plants (susceptible to both bean virus 1 and the pod-distorting virus) were inoculated with bean virus 1 at the two-leaf stage and 15 days later 10 of these plants were inoculated with the pod-distorting virus. Five plants were thereby left with only the bean virus 1 inoculation. At this same time five healthy plants of the same age were inoculated with the pod-distorting virus and five plants were left uninoculated to serve as controls. Observations made 20 days later indicated that the pod-distorting virus was not present in the plants which were infected with bean virus 1 at the time of inoculation with the former virus, since the plants thus treated could not be distinguished from those which had been inoculated with only bean virus 1. However, those healthy plants which had been inoculated with the pod-distorting virus at the time of the second inoculation were severely diseased. In order to determine whether the pod-distorting virus was entirely absent, or present and masked, a composite inoculum was made by taking a small trifoliate leaf from each plant, grinding them together, and inoculating five plants each of Michelite and Stringless Green Refugee. These inoculations indicated that bean virus 1 had completely inhibited the development of the pod-distorting virus since the Michelite plants remained symptomless while the Stringless Green Refugee plants were severely affected with common mosaic.

COMPARISON WITH OTHER STRAINS OF BEAN VIRUS 2

Several strains of bean virus 2 have been described. Virgin (*9*) described a disease which occurs on bean in some of the Western States the incitant of which he believed to be a strain of bean virus 2. A culture of this virus was received from Virgin and inoculations to several varieties of bean indicated that it was identical with or at least very similar to the virus referred to in this paper as typical bean virus 2. Both of these strains caused the necrotic joints or "red node" symptoms described by Virgin (*9*). McWhorter et al. (*6*) described a severe virus disease of bean in Oregon which usually occurred in fields near plantings of gladiolus, which were thought to be the source of inoculum in nature. Two isolates were furnished by McWhorter under numbers 231 and 250. Isolate 231 caused death of pea plants (Prince of Wales variety) but caused no necrosis in any of the bean varieties inoculated. It failed to infect any of the Refugee varieties

(Idaho Refugee, Stringless Green Refugee, Sensation Refugee 1066, and Sensation Refugee 1071) tested and is therefore different from any of the viruses considered in this paper. Isolate 250 was not recovered from the infected plant tissue received. LeBeau (*5*) reported a virus-induced top necrosis of bean in Mississippi. However, his virus infected tobacco and cucumber and had a heat inactivation point of 68° C. It is therefore different from the pod-distorting virus described in this paper.

DISCUSSION

On the basis of symptoms, modes of transmission, physical properties, and cross-protection studies the pod-distorting virus found on bean in Wisconsin appears to be closely related to typical bean virus 2 as described by Pierce (*7*). It has been shown in a previous paper (*3*) that bean virus 1 is closely related to bean virus 2. Cross-protection studies presented in this paper indicate that the pod-distorting virus is also related to bean virus 1. Since the former virus is not transmitted in bean seed, however, it is probably more closely related to bean virus 2 than to bean virus 1. The reactions of the bean varieties inoculated with typical bean virus 2 and pod-distorting virus also show a close relationship between the two viruses. Varieties UI 59, UI 81, and UI 123 which were most tolerant to the typical strain, were highly resistant to the pod-distorting virus. Varieties Stringless Blue Lake, Striped Creaseback, and McCaslan, which were the most susceptible to the typical strain, developed severe necrosis, and were sometimes killed when affected with the pod-distorting virus. Yellow bean mosaic is a very important disease in western canning areas where Stringless Blue Lake is the variety used most commonly. It is not known whether the pod-distorting strain occurs in these areas, but if it should become prevalent it would probably cause as much or more damage than has been reported for the typical strain.

Since the white and yellow sweetclovers are immune or highly resistant to the pod-distorting virus they cannot serve as overwintering hosts as they do commonly for typical bean virus 2. Further study is needed to determine the hosts in which the pod-distorting virus overwinters.

Several other viruses which might be strains of bean virus 2 were studied to determine their similarity to or difference from the pod-distorting virus. All of these viruses were shown to differ in one or more ways and are therefore distinct from the virus considered here. More work is needed to characterize them.

SUMMARY

A pod-distorting virus isolated from naturally infected bean plants at Madison, Wis., was studied in comparison with the typical strains of bean virus 1 and bean virus 2 and several other bean viruses. Evidence is presented to show that this virus is closely related to bean virus 1 and bean virus 2. Because of symptoms on certain hosts and because it is probably not seed-transmitted, it is thought to be a strain of bean virus 2.

The typical strain of bean virus 2 infected all varieties of bean tested, while the pod-distorting virus infected all but UI 59, UI 81, and

UI 123. The latter virus caused severe top necrosis on several varieties which when infected with typical bean virus 2 developed only a slight stunt and mottle.

Of 20 other leguminous varieties and species tested, 7 were susceptible to both viruses, 6 were susceptible to typical bean virus 2 and highly resistant to the pod-distorting virus, and 7 were not infected by either virus.

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