Effects of Soybean Mosaic Virus and/or Bean Pod Mottle Virus Infection on Soybean Nodulation

J. C. Tu, R. E. Ford, and S. S. Quiniones

First and third authors, Postdoctoral Research Associates, and second author, Professor, Department of Botany and Plant Pathology, Iowa State University, Ames 50010. Present address of senior author, Plant Pathology Department, University of Alberta, Edmonton, Alberta, Canada.

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ABSTRACT

Nodulation was studied in soybeans infected with soybean mosaic virus (SMV), with bean pod mottle virus (BPMV), or with both viruses. Nodule differences were marked between soybean varieties, but were small within soybean varieties infected with different SMV isolates. Number, size, and wt of nodules were reduced by SMV infection, and the earlier the infection, the greater the reduction. Maximum reduction (81%) occurred when Bansei soybeans were inoculated 2 weeks after sowing, but none occurred 9 weeks or later. Marked decreases in nodule numbers often resulted in an increase in nodule sizes in virus-infected plants.

A mixed infection of soybeans by SMV and BPMV reduced nodules more than single infections. Frequent fertilizer applications reduced nodule production in both healthy and SMV-infected plants. Decreases in plant fresh wt in SMV-infected soybeans has been attributed primarily to decreased photosynthesis and increased respiration. Reduced nodulation may also be an important factor. Phytopathology 60:518-523.

Soybeans infected with both soybean mosaic virus (SMV) and bean pod mottle virus (BPMV) show yield reduction, growth retardation (16), delayed seed maturation, and more severe symptoms than those infected with either virus alone. Dwarfing is an apparent feature in the mixed (SMV-BPMV) infection that is not generally obvious in SMV- or BPMV-infected soybeans, but sometimes we observed elongation of internodes in diseased plants.

Chlorophyll and photosynthesis is reduced in many virus-infected plants (2, 3, 7, 8, 9, 10, 19). This seems to occur also in SMV-infected soybeans. Fewer nodules have been observed consistently in SMV-infected soybeans in the field and greenhouse, but the effect of SMV infection on nodule development has not been studied.

This investigation was conducted to study the effects of SMV and BPMV each alone and in combination on various parameters of nodule formation at different stages of growth of soybeans.

MATERIALS AND METHODS.—*Field experiment.*—Seed of five cultivars of soybean (*Glycine max* [L.] Merr. 'Bansei', 'Bethel', 'Granger', 'Kanrich', and 'Richland') harvested in 1967 were planted 1 July 1968. The seeds were not artificially inoculated with rhizobia, but the field was known to be infested with *Rhizobium japonicum* (Kirchner) Buchanan. Each variety was planted in 10-ft rows, 40 inches apart. Some soybeans were naturally infected with SMV, developing typical mosaic symptoms by mid-September.

A random sample of 10 SMV-infected plants and 10 healthy soybeans was taken by digging each plant in a soil ball of 5-inch radius 1 ft deep 3 months after planting. The plants were still green. The plants with soil ball enclosed in a plastic bag were stored overnight at 4 C.

Nodules of each plant were harvested by removing the soil in running water through a 30-mesh screen and collecting the nodules from the screen. Those still adhering to the roots were picked off individually. Nodules were surface-dried with paper towels, sealed in a small plastic bag, and stored at 4 C.

Nodules from each group of plants, healthy and SMV-infected, were weighed and counted within 3 days. Measurements (width × length) were made of 200 randomly selected nodules from healthy and infected soybeans for each variety.

Greenhouse experiments.—All greenhouse plantings were made in unsterilized sand:peat:loam soil mixture (1:1:2). The soil used was previously tested for presence of rhizobia. Two seeds were planted/6-inch clay pot. The greenhouse temperature was 24 ± 3 C. Seedlings without symptoms due to seed-transmitted SMV were mechanically inoculated 2 weeks after seeding. Inoculum was prepared by expressing crude sap from virus-infected leaves and diluting 10 times with 0.01 M phosphate buffer, pH 7.0. A small amount of 600-mesh Carborundum was also added. The inoculum was then rubbed on leaves previously dusted with Carborundum. All control plants were rubbed with neutral phosphate buffer and Carborundum. Leaves were rinsed with tap water immediately after rubbing. Supplemental light supplied by a bank of four cool-white fluorescent lights provided a minimum of 800 ft-c for a 14-hr day. Nodules from each pot were collected 10 weeks after seeding as described. Details of specific experiments preface the results.

RESULTS.—*Effect of SMV infection on nodules.*—Twenty pots of each cultivar (Bansei, Granger, Kanrich, and Richland) were planted in the greenhouse. Each cultivar planting was divided into four groups of 5 plants each. One group was the noninoculated control, and the other 3 were inoculated with SMV-O, SMV-NC, or SMV-M (15), respectively. Two inoculations were made, one at the unifoliate stage and another 1 week later. The experiment was done twice.

All 5 field-grown cultivars infected with SMV produced nodules that were reduced in wt, size, and num-

TABLE 1. The effect of soybean mosaic virus infection on nodulation of field-grown soybean plants, Ames, Iowa

Var.	Avg wt/plant			Avg no./plant		Avg size of nodule	
	Diseased	Healthy	Reduction	Diseased	Healthy	Diseased	Healthy
	g	*g*	*%*	*no.*	*no.*	*mm*	*mm*
Bansei	1.06	1.37	23	41.8	47.1	2.9 × 3.5	3.0 × 3.5
Bethel	0.47	0.59	20	25.5	30.4	2.5 × 2.9	2.6 × 3.1
Richland	0.45	0.62	27	22.2	31.9	2.7 × 3.1	2.8 × 3.2
Kanrich	1.21	1.56	22	45.2	47.5	3.2 × 3.7	3.3 × 3.9
Granger	1.13	1.52	26	58.4	64.3	2.6 × 3.0	3.1 × 3.6

ber (Table 1). Nodule wt was reduced 20 to 27% in SMV-infected soybeans.

The four different soybean cultivars in the greenhouse varied in their nodulation ability as compared with the healthy control (Table 2). The variance may be due to genetic differences. Nodule reduction appeared correlated with differences in varietal susceptibility. Bansei is highly susceptible to SMV, and shows severe mosaic and leaf rugosity. Granger is least susceptible, exhibiting mild leaf rugosity with very indistinct mottling. Richland and Kanrich show an intermediate symptom pattern. Although varieties differed in their responses to infection by SMV isolates, the reaction of each cultivar was consistent for a given isolate (Table 2). For example, the range of nodule yield reduction (wt) in Bansei infected with SMV-O, -M, and -NC was very narrow (78-81%), while that for Kanrich was wider (45-54%), and those for Richland and Granger were widest (34-50% and 18-33%, respectively). Variations in nodule size, wt, and numbers caused by different SMV isolates are shown in Table 2 for soybeans inoculated 2 weeks after seeding and fertilized at 4-week intervals; the nodule differences for SMV-O-infected Bansei and Kanrich are illustrated in Fig. 1 and 5. Nodule reduction in Bansei infected with SMV isolates are arranged in order of decreasing symptom severity: SMV-O, SMV-M, and SMV-NC (Table 2).

Effect of time of infection on nodulation.—Bansei and Kanrich soybeans and the SMV-O isolate were used. One hundred pots were seeded to each variety and divided into groups of 10. In the first group of 10 pots, all plants in 5 pots were inoculated at the unifoliate stage 2 weeks after seeding with SMV-O; the other 5 pots were controls. The remaining groups of 10 pots were treated identically at 1-week intervals.

The influence of time of infection of SMV-O on the nodulation of both Bansei and Kanrich soybeans fertilized at 2-week intervals was similar (Fig. 1, 2). Fresh wt of nodules was reduced 55% in SMV-O-infected Kanrich, which expressed milder mosaic symptoms, when compared with a reduction of 81% in Bansei (Fig. 2). For example, nodule wt was least when soybeans were inoculated with virus 2 weeks after seeding. Similarly, the adverse effect of SMV-O infection on size and numbers of nodules and fresh wt of plant shoots was less in Kanrich than in Bansei (Fig. 1, 2).

Nodule wt (Fig. 1-A) variation in the Bansei controls occurred in a range of 0.85-0.95 g/plant. The maximum deviation from the mean (0.90 g/plant) in the controls was 0.05 g, which was roughly ±5%. Nodule wt in SMV-infected Bansei plants, on the other hand, varied from 0.16 to 0.86 g/plant, and was inversely related to growth stage when the soybeans were inoculated. There was little effect of virus infection during the last 3 weeks of the experiment on final nodule wt.

The maximum variation in nodule numbers among controls was an average of ±2 nodules/plant, a ±3% deviation from the mean of 82 nodules/plant (Fig. 1-B). Soybean plants inoculated in early stages of growth showed least numbers of nodules, while those inoculated in later stages were unaffected (Fig. 1, 6).

Size variation of nodules among the controls was small enough to draw a straight line (Fig. 1-C), while size of nodules varied greatly between SMV-infected groups. Plants infected in 4th to 7th weeks after seeding produced larger nodules than their respective controls.

Variations in fresh wt of plants, not including roots, ranged from 18 to 23 g/plant in the controls (Fig. 1-D). In general, the earlier the infection, the greater reduction in fresh wt. SMV-infected soybeans were slightly taller than healthy ones, due to stem elongation

TABLE 2. The effect of three soybean mosaic virus isolates on nodulation of soybeans in the greenhouse

Cv.	Noninoculated	SMV isolate		
		O	M	NC
	Avg wt nodules/plant	Reduction of nodule wt in infected plants		
	g	*%*	*%*	*%*
Bansei	1.19	80.5	77.6	79.2
Richland	0.61	50.5	39.5	34.2
Kanrich	1.24	54.4	51.4	45.4
Granger	1.27	32.5	21.5	17.6
	Avg no. nodules/plant			
	no.	*no.*	*no.*	*no.*
Bansei	57.3	22.5	33.1	30.6
Richland	32.1	18.5	20.3	19.2
Kanrich	52.6	28.0	35.4	41.5
Granger	68.0	35.4	46.6	49.3
	Avg size nodules/plant			
	mm	*mm*	*mm*	*mm*
Bansei	2.6 × 2.9	1.7 × 1.9	1.8 × 2.0	1.9 × 2.0
Richland	2.6 × 3.1	2.3 × 2.5	2.5 × 2.7	2.6 × 2.7
Kanrich	2.7 × 3.1	2.4 × 2.8	2.6 × 3.1	2.7 × 3.1
Granger	2.8 × 3.2	2.5 × 2.9	2.7 × 3.2	2.7 × 3.2

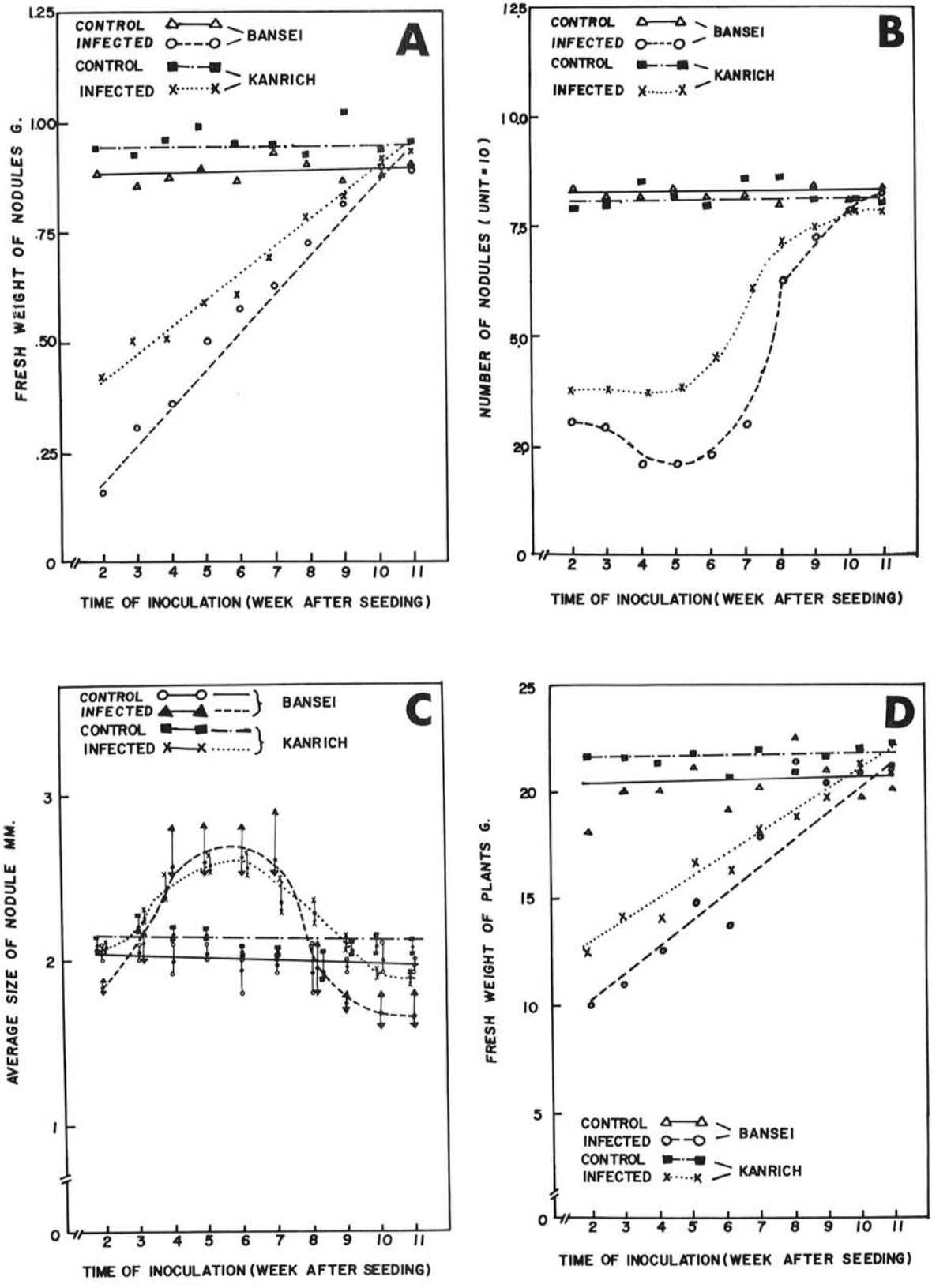
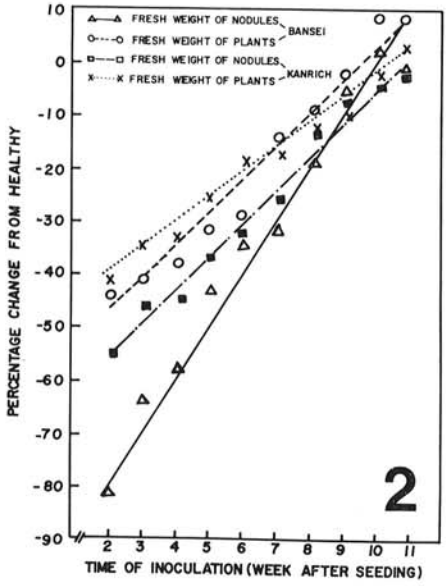
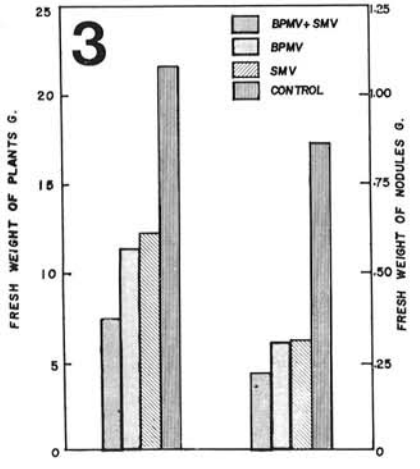
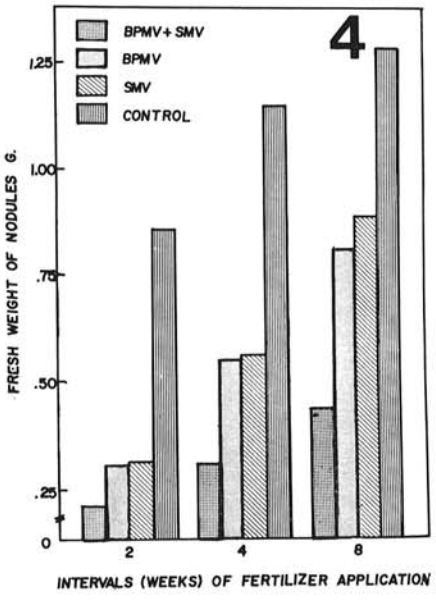


Fig. 1. Effect of soybean mosaic virus isolate O (SMV-O) infection on nodulation of Bansei and Kanrich soybeans. Plants were inoculated at weekly intervals starting 2 weeks after seeding. All plants were harvested 2.5 months after seeding. **A)** Weight. **B)** Number. **C)** Size of nodules per plant (each bar represents the maximum variation of nodule measurements at the dot). **D)** Fresh wt of plant (root not included).







(Fig. 5). Nodule wt was reduced relatively more by SMV infection than was plant fresh wt (Fig. 2).

Effect of infection on nodule expression.—Sixty pots each of four cultivars of soybean, Bansei, Kanrich, Richland, and Granger were seeded in 6-inch pots, 3 seeds/pot. Two weeks after seeding, when the soybeans reached the unifoliate stage, at which time no nodules were observed, plants in 30 pots were inoculated with SMV-O. Plants in 30 comparable pots served as buffer-rubbed controls. Observations on the first visible nodule were made at 2-day intervals from plants in three pots each of the noninoculated soybeans to determine the effect of infection on the formation of nodules.

Nodules appeared on healthy and inoculated seedlings at approximately the same time. They were observed first on Bansei and Granger 18 days after seeding, but not until 22 days after seeding on Kanrich and Richland.

Effect of mixed infection on nodules.—Twenty pots each of Bansei and Kanrich soybeans were planted. Plants in one group of 5 pots were buffer-rubbed controls, and those in the other three groups were inoculated with either SMV-O, BPMV, or SMV-O + BPMV. The experiment was conducted three times, each experiment at a different level of fertilizer application. The three fertilizer applications were made at 2-, 4-, and 8-week intervals. Results were recorded 2.5 months after seeding. The first fertilizer application in each experiment was made 1 week after inoculation. Vigoro (Swift & Co., Agriculture Division, Baltimore, Md., N:P:K ratio = 6:10:4) was watered in at the rate of 0.5 g to each 6-inch pot.

BPMV-infected soybeans were not dwarfed, but dwarfing did occur in SMV-BPMV mixed infections. Mosaic and rugose were more severe in SMV-BPMV mixed infections than that caused by either virus alone in plants fertilized at 2-week intervals. Reduction of nodule fresh wt resulting from virus infection was relatively greater than reduction of plant fresh wt (Fig. 3).

Increased frequency of fertilizer application decreased the nodule formation in both healthy and diseased soybeans (Fig. 4).

DISCUSSION.—Numerous factors such as environment, soil acidity, nutrition, rhizobial population, and C-compound availability, etc., affect nodulation (20). One report (12) on the effect of a virus (clover phyllody virus) on nodulation of white clover probably involved a mycoplasma rather than a virus.

SMV infection in soybeans reduced the size, number, and wt of nodules, and most pronounced reductions occurred in early infections. Nodule reductions are

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Fig. 2-4. 2) Percentage reduction in fresh wt of plants and nodules from healthy and soybean mosaic virus isolate O (SMV-O)-infected plants. **3)** Effect on nodule production and fresh wt (roots not included) of Bansei soybeans infected with soybean mosaic virus isolate O (SMV-O) and bean pod mottle virus (BPMV) in combination or each virus alone. **4)** Effect of the frequency of fertilizer application (0.5 g Vigoro/6-inch pot) on the nodulation of healthy, soybean mosaic virus isolate O (SMV-O)-, bean pod mottle virus (BPMV)-, and SMV-O- and BPMV- (mixture) infected Bansei soybeans.

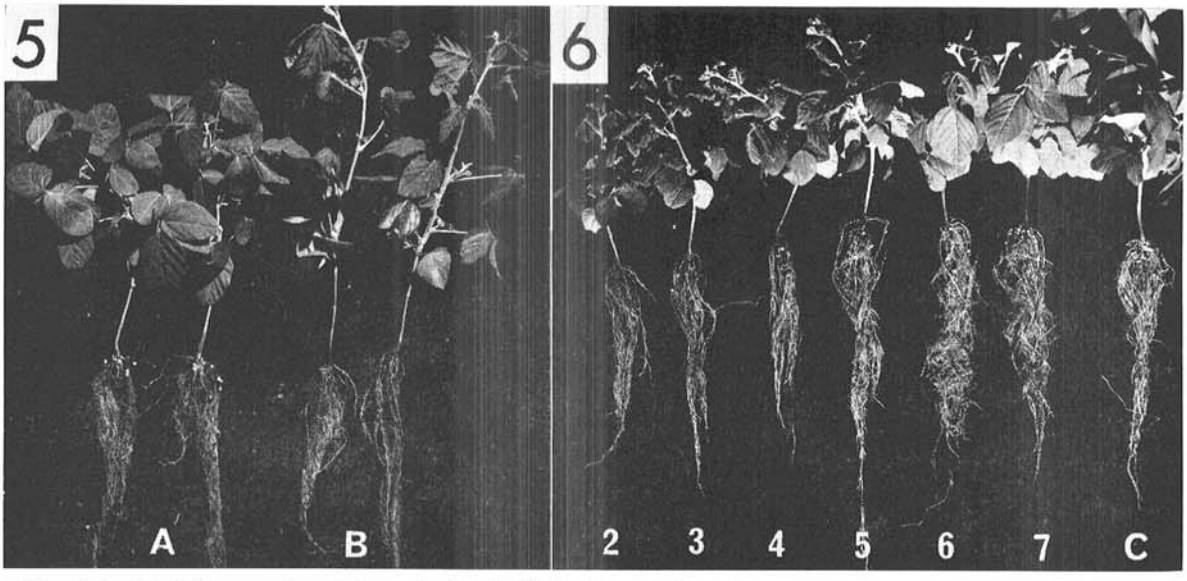

Fig. 5-6. **5)** Differences in nodule production in **A)** healthy and **B)** soybean mosaic virus isolate O (SMV-O)-infected Bansei soybean. **6)** Effect of weekly inoculation of Bansei soybeans with soybean mosaic virus isolate O (SMV-O) 2-7 weeks after seeding. Inoculations 8 and 9 weeks after seeding were omitted since they were similar to 7. C is a buffer-rubbed control. Four fertilizer applications were made at 2-week intervals during the 2.5-month growing period, starting 1 week after virus inoculation.

probably caused by virus replication causing physiological changes of reduced photosynthesis or increased respiration (2, 3, 8, 19), imbalance of auxins (18), and enzyme levels, etc., which directly or indirectly affect the *Rhizobium*-soybean symbiotic relationship. Shading pea plants reduced nodule size, and subsequent spray applications of sucrose improved nodule size and efficiency of N fixation, but nodule numbers were not reduced by shading (21). Since SMV reduced both size and numbers of nodules on soybeans, the process is probably a more complicated physiology than a simple reduction of photosynthesis. If time of shading was manipulated, as was time of SMV infection, there may have been some influence on nodule numbers. Limiting CO_2 and light levels that affect photosynthesis influence nodule formation and function (13, 20, 21).

Ineffective symbioses are characterized by small nodules that fail to grow to normal size because the degeneration that starts in the bacterial region quickly spreads to the nodule meristem and stops its growth (14). Rhizobia probably entered the soybean roots in this study before SMV inoculation; thus, SMV infection had little effect on preventing or delaying nodule formation. SMV affected nodule growth immediately, according to our data, and possibly had an adverse effect on rhizobial establishment in newly formed roots.

Although results were similar from both field and greenhouse studies, nodulation was generally more profuse in the greenhouse, especially on secondary roots, probably because of better soil aeration in potted plants.

Greater soybean nodule numbers were always accompanied by smaller nodule size. A similar inverse relationship of average nodule size and abundance occurs in red clover and other legumes (14). The most prominent relationship between sequential infection and nodulation was reflected in the linear nodule wt increases with delayed inoculation (Fig. 1-A).

Auxin and enzyme balances may have contributed to the indirect influence of soybean viruses on nodules, since considerable stem elongation, curving, and rugosity of leaves occurred (Fig. 5). A high auxin content accumulating in foliage and stems is suggested, with a possible concomitant-reduced auxin translocation to roots. This might have retarded nodule growth. Nodule fresh wt was reduced relatively more than plant fresh wt when virus-infected, suggesting that simple decrease in fresh wt of plant tissue was not the sole factor responsible for fresh wt decrease of nodules. SMV probably has a more complex influence on the symbiotic relationship and its N-fixing efficiency. The amount of N fixed in a nodule is closely related to total viable bacterial population and length of time of their association with host tissue (4, 6).

The inverse relationship of nodulation and frequency of fertilizer application, independent of virus infection, is attributable to increased mineral N which partially negates the requirement for the symbiotic *Rhizobium*-soybean relationship.

The mechanism of synergistic reduction of nodules in SMV-BPMV-infected soybeans, which correlates with other observations on synergistic reactions (15), is not elucidated by our data. However, the severe competition for basic building materials invoked by the synergistic viral pathogens must create a balance more favorable for the viruses and less favorable for the symbiotic rhizobial association.

Nodulation was reduced by SMV infection in all soybean varieties tested, although there were variations

in varietal reactions (Tables 1, 2). Likewise, there are varietal and specific genetic differences in symbiotic effectiveness of *Rhizobium* (1, 5, 11, 17). Nodule differences are perhaps due to varietal differences in disease severity.

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