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Effects of Virus Infection on Nitrogen Fixation in Cowpea [*Vigna unguiculata* (L.) Walp][1]

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Abstract. **The effects of the cowpea strains of southern bean mosaic (SBMV-CS), cucumber mosaic (CMV-CS), and tobacco mosaic (TMV-CS) viruses on cowpea [*Vigna unguiculata* (L.) Walp] plant growth and N fixation parameters were evaluated in greenhouse and field experiments. TMV-CS infection resulted in the most severe symptoms and was associated with significant reductions in nitrogenase activity, total nodule weight, nodule number, and plant dry weight, in susceptible cultivars. Infection with CMV-CS was associated with significant reductions in nitrogenase activity, nodule number, and plant dry weight in field-grown 'Mississippi Silver'. SBMV-CS infection was least severe and was associated with a significant reduction in nitrogenase activity and nodule weight in only 1 susceptible cultivar under field conditions.**

Cowpea (southernpea), an important crop in the southern United States, is a host for several virus diseases. The effects of infection may vary from complete death of the plant to no symptom expression. The cowpea strains of southern bean mosaic (SBMV-CS) (10), cucumber mosaic (CMV-CS) (1), and tobacco mosaic (TMV-CS) (14) viruses are among those that have been observed in the United States.

Virus infection in legumes has been associated with changes in various plant processes, including N fixation. In soybeans, Tu (15, 16) reported that soybean mosaic virus reduced the number, size, and weight of nodules. Rajogopalan (9) suggested an interaction between *Dolichos* enation mosaic virus and culture method on *Rhizobium* infection of field beans. An increase in nodule number was observed on plants grown in N-free sand culture, and a decrease was noted when soil culture was used. A similar reduction in plant growth as well as nodule number, weight, and size was reported in pulse crops following infection with 2 arhar mosaic virus strains (12). The purpose of this investigation was to evaluate the effect of virus infection on plant growth and N fixation in cowpea, under both greenhouse and field conditions.

Materials and Methods

Two seedborne viruses, SBMV-CS and CMV-CS, and a highly infectious TMV-CS were selected for use in the greenhouse experiment. On May 14, 1977, seeds harvested from virus-free plants of virus-susceptible (3) and moderately high N-fixing 'California Blackeye No. 5' and virus-tolerant (2) and relatively low N-fixing 'Mississippi Silver' were planted 3 to a 1.5-liter pot, utilizing a randomized complete block design with 23 replications. The growing medium was an N-free, porous mixture consisting of 1 builder's sand:1 coarse vermiculite (v:v). Seeds were inoculated at planting with a commercial *Rhizobium* inoculant. Seedlings were thinned to 1 per pot. Plants were watered as needed and fertilized on alternate waterings with an N-free nutrient solution (13). Supplemental lighting was not used; however, pots were covered with aluminum foil to prevent excessive pot temperature and reflect light back to plants.

When the primary leaves were fully expanded, 12 days after planting, inoculum of each virus was prepared following the procedure described previously (8). An artist's airbrush was used to make the inoculations (7). Local lesion hosts were utilized to verify virus presence in both studies (4, 5). Seven weeks after planting, the plants were removed from the pots for evaluation of growth and nitrogenase activity.

Harvesting took place during mid-afternoon. Plant tops were severed at the point of cotyledonary attachment and placed in paper bags for drying. Nodule development and nitrogenase activity determinations were made following the procedure outlined by Zary et al (18).

Viruses and cultivars selected for use in the field trial were the same as in the greenhouse experiment with the addition of SBMV-CS tolerant 'Clay' (4), and 'Chinese Red' and 'Knuckle Purple Hull', both susceptible (3, 6). Again, cultivar selection was based on differential N-fixation potential (18) and virus reaction (Table 1). On June 20, 1977, *Rhizobium*-inoculated seeds of each cultivar were planted in 7-m-long and 1-m-wide plots. A post-planting application of Aldicarb [2-methyl-2-(methylthio) propionaldehyde *O*-(methylcarbamoyl) oxime] 4.5 kg/ha was made to control aphid movement. Seedlings were thinned to 15 cm between plants and inoculated with virus treatments when the primary leaves were fully expanded. Inoculum of each virus was prepared and all inoculations were made as previously described. To minimize the possibility of contamination, a split-plot design with 3 replications was utilized for this experiment. The cultivars were established as the whole plots, and subplots were the inoculated and non-inoculated plants. Plants were watered with overhead irrigation and sprayed with malathion (*O,O*-dimethyl phosphorodithioate of diethyl mercaptosuccinate) as needed. Visual observations were used to note virus spread to non-inoculated plants. At 8 weeks, 5 plants were harvested from each plot and evaluated for nitrogenase activity, nodule number, nodule weight, and plant top dry weight.

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Table 1. Cultivars evaluated for the influence of viral infection on nitrogen fixation, $N_2(C_2H_2)$.

Cultivar	Nitrogenase activity rating	Virus reaction
California Blackeye No. 5	High	Susceptible
Clay	Low	SBMV tolerant
Chinese Red	Low	Susceptible
Knuckle Purple Hull	High	Susceptible
Mississippi Silver	Low	Tolerant

Results and Discussion

The symptoms of CMV-CS and TMV-CS infection were very prominent in the greenhouse-grown plants of both 'California Blackeye No.5' and 'Mississippi Silver'. The prominent symptom expression was attributed to the N-free growing medium and nutrient solution, since these symptoms were not observed on virus maintainer-inoculum source plants growing in sand-vermiculite-peat mix supplemented with a complete fertilizer. Symptoms of SBMV-CS were not as obvious. Control plants developed no virus symptoms.

Nitrogenase activity in virus-free plants of 'California Blackeye No. 5' was more than double that recorded for virus-free plants of 'Mississippi Silver' (Table 2). A significant reduction in plant nitrogenase activity was observed in CMV-CS and TMV-CS infected plants of both cultivars, with TMV-CS infected plants exhibiting the greatest reduction. SBMV-CS infection was associated with a significant increase in nitrogenase activity in 'California Blackeye No. 5'. SBMV-CS had no effect on nitrogenase activity in 'Mississippi Silver'.

Virus infection had little effect on total nodule weight/plant, except for a significant reduction in the total weight of TMV-CS infected 'California Blackeye No. 5' (Table 2). This coincides with the observation that TMV-CS infection in 'California Blackeye No. 5' was associated with the greatest reduction in nitrogenase activity.

Although there were trends toward reduced nodule number in virus-infected 'California Blackeye No. 5' plants, these trends were not significant (Table 2). Virus infection had no effect on nodule number in 'Mississippi Silver'.

A significant reduction in plant dry weight was observed in both 'California Blackeye No. 5' and 'Mississippi Silver' infected with CMV-CS and TMV-CS (Table 2). Again, TMV-CS infection in 'California Blackeye No. 5' was associated with the greatest reduction in dry weight of the stems and leaves. This coincides with the reduction in nitrogenase activity associated with these same viruses. Dry weight reduction was not found in plants infected with SBMV-CS. These variations in dry weight follow the pattern of nitrogenase activity in 'Mississippi Silver'; however, they did not coincide with a significant increase in nitrogenase activity observed in SBMV-CS infected plants of 'California Blackeye No. 5'.

Virus symptoms of SBMV-CS and CMV-CS infection under field conditions were mild, while those of TMV-CS were very noticeable. No virus symptoms were observed in the control plants. Nitrogenase activity in the virus-free control plants appeared to be highest in 'California Blackeye No. 5' and lowest in 'Chinese Red' (Table 3). SBMV-CS infection was associated with a significant increase in nitrogenase activity in 'Chinese Red' and 'Mississippi Silver', and a nonsignificant trend toward reduced nitrogenase activity. CMV-CS infection was associated with a significant increase in nitrogenase activity in 'California Blackeye No. 5' and a significant reduction in 'Mississippi Sil-

Table 2. The influence of 3 cowpea viruses on plant specific activity, nodule weight, nodule number, and plant dry weight, greenhouse experiment.

Cultivar	Virus treatment				Mean
	Control	SBMV	CMV	TMV	
Plant specific activity (μmoles C_2H_4/plant·hr)					
California Blackeye	11.13b[z]	14.76a	6.15c	3.75c	9.12
Mississippi Silver	5.01a	5.59a	3.10b	3.27b	4.24
Mean	8.13b	10.16a	5.08c	3.51c	
Nodule wt (mg/plant)					
California Blackeye	546a	490a	478a	359b	468
Mississippi Silver	315a	310a	313a	283a	305
Mean	430a	400a	395a	321a	
Nodule number					
California Blackeye	71a	63a	65a	62a	66
Mississippi Silver	33a	35a	30a	34a	33
Mean	54a	48a	47a	48a	
Plant dry wt (g)					
California Blackeye	1.9a	1.8a	1.4b	1.1c	1.6
Mississippi Silver	1.2a	1.2a	0.9b	1.0b	1.1
Mean	1.5a	1.5a	1.2b	1.0b	

[z]Mean separation within rows by Duncan's multiple range test, 5% level.

Table 3. The influence of 3 cowpea viruses on plant specific activity and nodule weight, field experiment.

Cultivar	Virus treatment				Mean
	Control	SBMV	CMV	TMV	
Plant specific activity (μmoles C_2H_4/plant·hr)					
California Blackeye	5.98b[z]	4.25b	11.35a	2.13c	5.93
Clay	3.16a	1.14a	—	—	2.15
Chinese Red	1.82b	5.29a	3.82ab	0.10c	3.00
Knuckle Purple Hull	2.81ab	1.94b	4.65a	3.00a	3.10
Mississippi Silver	3.43b	4.72a	0.68c	0.70c	2.13
Mean	3.24b	3.85b	5.54a	1.55c	
Nodule wt (g/plant)					
California Blackeye	2.24a	0.69b	1.81a	0.59b	1.33
Clay	0.91a	0.36b	—	—	0.64
Chinese Red	0.70a	0.48a	0.57a	0.07b	0.46
Knuckle Purple Hull	0.55ab	0.16b	0.58ab	0.72a	0.50
Mississippi Silver	0.35a	0.36a	0.58a	0.35a	0.41
Mean	0.95	0.41	0.88	0.43	

[z]Mean separation within rows by Duncan's multiple range test, 5% level.

ver', as compared to the uninoculated control plants. TMV-CS infection was associated with significant reductions in nitrogenase activity in 'California Blackeye No. 5', 'Chinese Red', and 'Mississippi Silver'. This follows similar reductions observed in the greenhouse study. 'California Blackeye No. 5' infected with SBMV-CS showed an increase in nitrogenase activity when grown in the greenhouse (Table 2) but tended toward reduced activity in the field (Table 3). When the same cultivar was infected with CMV-CS, there was a decrease in nitrogenase activity in the greenhouse and an increase in the field. Such contradictions were not observed in 'Mississippi Silver'. This may indicate a cultivar-virus-environment interaction.

Nodule weight/plant was affected more by the virus treatments in the field (Table 3) than in the greenhosue (Table 2). A significant reduction in nodule weight was observed in SBMV-CS infected 'California Blackeye No. 5' and 'Clay', and trends of reduction were observed in SBMV-CS infected 'Chinese Red' and 'Knuckle Purple Hull' (Table 3). Nodule weight of 'Mississippi Silver' was not affected by SBMV-CS infection. Little change in nodule weight was observed in CMV-CS infected plants. Significant reductions in nodule weight were found in 'California Blackeye No. 5' and 'Chinese Red' infected with TMV-CS. This reduction appears to be associated with premature death and decay of the nodules.

Nodule number was not significantly influenced by infection with SBMV-CS in any of the cultivars (Table 4). CMV-CS infection was associated with a significant reduction in nodule number in 'Mississippi Silver', and TMV-CS infection was associated with a significant reduction in both 'California Blackeye No. 5' and 'Mississippi Silver'. It has been postulated that viruses interfere with Rhizobia recognition sites on the root hairs, thus reducing nodulation (11, 12, 17). In this experiment, most plants were nodulated by the time that virus infection took place. Thus, reductions in nodulation due to viral interference would not be expected. The observed reductions could be attributed to premature death of the nodules.

Table 4. The influence of virus infection on nodule number and dry weight, field experiment.

Cultivar	Virus treatment				Mean
	Control	SBMV	CMV	TMV	
Nodule number					
California Blackeye	113a[z]	114a	130a	51b	102
Clay	63a	33a	—	—	48
Chinese Red	51ab	64a	63a	26b	51
Knuckle Purple Hull	32a	38a	29a	31a	32
Mississippi Silver	49a	44ab	22c	41b	39
Mean	62a	59a	61a	37b	
Plant dry wt (g)					
California Blackeye	9.2b	12.5ab	16.5a	9.1b	11.8
Clay	9.8a	12.5a	—	—	11.2
Chinese Red	12.9ab	17.3a	9.1bc	4.1c	10.8
Knuckle Purple Hull	7.4a	7.7a	8.4a	5.5b	7.2
Mississippi Silver	10.0a	11.0a	5.0b	7.4ab	8.6
Mean	9.9a	12.2a	10.0a	6.5b	

[z]Mean separation within rows by Duncan's multiple range test, 5% level.

SBMV-CS infection was associated with a trend toward an increase in plant dry weight in all field-grown cultivars (Table 4). A significant increase in dry weight was observed in CMV-CS infected 'California Blackeye No. 5', and a significant reduction was found in 'Mississippi Silver' infected with CMV-CS. These correspond with similar differences in nitrogenase activity for the same cultivars with respect to CMV-CS infection. TMV-CS infection was associated with a significant reduction in plant dry weight in 'Chinese Red' and 'Knuckle Purple Hull'. 'Chinese Red' had reduced nitrogenase activity; however, 'Knuckle Purple Hull' did not (Table 3).

Results from this investigation demonstrated that virus infection can have a significant effect on N fixation, as well as plant growth. Significant reductions in N fixation were generally associated with similar reductions in plant growth and were most severe in plants that exhibited strong virus symptoms. Unexpected increases in N fixation were observed in virus-infected plants that otherwise appeared healthy. It is not known if these were ephemeral increases or if they were long-lasting; results of nodule weight and number suggest that the former is the case. These findings suggest the presence of unexplained interactions similar to those observed by Rajogopalan (9). Of the viruses evaluated, TMV-CS and SBMV-CS produced the most visible and least visible effects, respectively.

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