Restricted Systemic Movement of Cowpea Chlorotic Mottle Virus in Soybean with Nonnecrotic Resistance

B. J. Goodrick, C. W. Kuhn, and R. S. Hussey

Department of Plant Pathology, University of Georgia, Athens 30602.
This research was supported by state and Hatch funds allocated to the Georgia Agricultural Experiment Stations.
We thank H. R. Boerma (University of Georgia) for seeds of soybean genotypes.
Accepted for publication 15 April 1991 (submitted for electronic processing).

ABSTRACT

Goodrick, B. J., Kuhn, C. W., and Hussey, R. S. 1991. Restricted systemic movement of cowpea chlorotic mottle virus in soybean with nonnecrotic resistance. Phytopathology 81:1426-1431.

Resistance in soybean PI 346304 to cowpea chlorotic mottle virus was related to restricted virus movement. When unifoliolate leaves were mechanically inoculated, both PI 346304 and susceptible cultivar Davis developed local chlorosis and accumulated similar quantities of virions in inoculated leaves. However, PI 346304 greatly restricted virus movement and subsequent accumulation in uninoculated sections of inoculated leaves and in uninoculated leaves and roots. Nonnecrotic resistance in PI 346304 was shown not to involve induced resistance, inactivation of virions, or resistance to symptom development. In immunocytochemical studies, virus antigen was found in most cells of mechanically inoculated leaves of both Davis and PI 346304, indicating that cell-to-cell movement was largely unrestricted. However, antigen was rarely found within vascular tissue of PI 346304 but occurred frequently in similar Davis tissue. We suggest that restriction of virus entry and/or exit from the vascular tissue is responsible for nonnecrotic resistance in PI 346304.

Additional keyword: Glycine max.

Cowpea chlorotic mottle virus (CCMV), a beetle-transmitted bromovirus, is economically important because it causes the reduction of seed quality and yield in both cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) (8) and soybean (*Glycine max* (L.) Merr.) (5,6). Recently, Bijaisoradat and Kuhn (1) discovered a source of nonnecrotic resistance to CCMV in soybean PI 346304. Resistance is characterized by high virus concentration in mechanically inoculated leaves, limited systemic accumulation of virus, and no or few symptoms. Paguio et al (11) found PI 346304 to be highly resistant under field conditions expressing no symptoms, having limited virus concentration in uninoculated tissue, and causing no yield reduction. It is not clear whether restricted virus accumulation is due to reduction of virus multiplication or restriction of local or systemic virus movement.

The objective of the current research was to compare the general pattern of virus movement, symptom development, and location of viral antigen in susceptible soybean cultivar Davis and resistant PI 346304.

MATERIALS AND METHODS

Virus and hosts. The soybean strain of CCMV (CCMV-S) (5) was cultured in soybean cultivar Davis or cowpea cultivar California Blackeye. Fully expanded unifoliolate leaves on test plants were mechanically inoculated (cheesecloth pad) with 100 μg/ml of purified CCMV in 0.01 M potassium phosphate buffer (pH 7.0) containing 1% Celite. Plants were grown in a mixture of soil/sand/vermiculite (2:1:1, v/v/v) previously fumigated with methyl bromide in 10-cm-diameter plastic pots. An N-P-K (20-20-20) fertilizer was applied weekly.

Environmental conditions. Test plants were maintained under greenhouse conditions or in growth chambers at 24, 28, and 32 C. Results did not greatly vary between these conditions. The growth chamber photoperiod was 16 h and illumination (6,000–10,000 lx) was with incandescent and fluorescent lights. In the greenhouse, temperature ranged from 18–35 C, and fluorescent lights extended the photoperiod to 16 h.

Serology. Antibodies were raised in a New Zealand white female rabbit by six weekly intramuscular injections, 1 mg each, of highly purified CCMV-S (1). The rabbit was exsanguinated when the titer reached 1:512 as determined by a microprecipitin test. The antiserum was used in immunocytochemistry and in double antibody sandwich enzyme-linked immunosorbent assay (ELISA) tests (2).

Virus quantification. Virus concentration in infected tissue usually was determined by analyzing purified virions, following the procedure of Bijaisoradat and Kuhn (1). Inoculated tissue usually was unifoliolate leaves, while uninoculated tissue consisted of the fourth and/or fifth expanded trifoliolate leaves. Treatments were replicated four times, and each replication consisted of tissue (1–5 g) from three plants growing in the same pot. The final

virus pellets were suspended in 1.5 ml of 0.1 M sodium acetate (pH 5.0) containing 0.01 M $MgCl_2$.

A purification procedure to quantify relatively small and large amounts of CCMV in soybean in the same experiment was described previously by Bijaisoradat and Kuhn (1). In most tests, virus concentration was determined spectrophotometrically ($E = 5.8$ $[mg/ml]^{-1}$ cm^{-1} at 260 nm) after one cycle of ultracentrifugation. A correction (reduction) of 30 $\mu g/g$ of tissue was made because some ultraviolet-absorbing material (260 nm) occurred in tissue samples from uninoculated soybean plants that had been subjected to the same purification procedure. In some experiments when test samples had less than 100 $\mu g/g$ of tissue, the presence and quantity of virions was determined by density gradient centrifugation. Samples from four replications were combined, 4 ml was layered on a 10–40% sucrose column, and virus quantity was analyzed as described previously (1).

In a few tests, ELISA was used to compare the relative concentration of CCMV antigen in Davis and PI 346304 tissue. Twelve days after inoculation (DAI), inoculated unifoliolate and uninoculated trifoliolate leaves from five individual plants were ground with a mortar and pestle and serially diluted 1:10 to 1:2,160. ELISA absorbance values at 405 nm were recorded with an EIA Reader, model 2550 (Bio-Rad Laboratories, Richmond, CA).

Temperature study. The effect of temperature on symptom development and virus concentration was studied by incubating inoculated Davis and PI 346304 plants at constant temperatures of 24, 28, and 32 C. Virus concentration within inoculated and uninoculated leaves and roots was analyzed 14 DAI. A randomized complete block design was used with four replications (combined tissue from three plants per pot constituted a replicate).

Induced resistance. The following treatments were used to determine if PI 346304 resistance is induced in uninoculated leaves by primary inoculation. Unifoliolate leaves of Davis and PI 346304 were inoculated with 100 $\mu g/ml$ of CCMV-S. Control plants were buffer-rubbed, or the rubbing was omitted. Fourteen days later, newly developed trifoliolate leaves were inoculated with the same virus. Virus concentration within trifoliolate leaves was analyzed 14 DAI. A randomized complete block design was used with four replications (combined tissue from three plants per pot constituted a replicate).

Movement within leaves. To determine if PI 346304 restricts virus movement within inoculated leaves, various sections of unifoliolate leaves were inoculated and virus concentration was analyzed within inoculated and uninoculated sections. In one study, a centrally located 5-mm-wide strip that was perpendicular to the midvein was inoculated with a cotton applicator dipped in inoculum. Virus concentration within the middle inoculated strip and the uninoculated tip and petiole portions of leaves was analyzed 5, 10, and 20 DAI. In another study, either the petiole or tip half of unifoliolate leaves was inoculated and virus concentration was analyzed within the inoculated and uninoculated halves 5, 10, and 20 DAI. In all studies, new leaf buds were removed to decrease leaf senescence, and plants were incubated in a greenhouse. A randomized complete block design was used with two pots per replication, three plants per pot, and four replications.

Biological quality of virions. Virus-specific infectivity was evaluated to determine if the biological quality of virions was altered as a component of resistance in PI 346304. Virus was purified from inoculated unifoliolate leaves of Davis and PI 346304 7 DAI, equalized at 2–18 $\mu g/ml$, and inoculated onto the unifoliolate leaf halves of hypersensitive Bragg soybean. Treatments were replicated eight times with paired treatments on each leaf being randomly selected. Necrotic lesions were counted 4 DAI.

Immunocytochemistry. In study 1, unifoliolate leaves of PI 346304 and Davis were inoculated with 100 $\mu g/ml$ of CCMV-S. Twelve DAI, a strip of tissue, including a major vein, was cut from an inoculated unifoliolate leaf and uninoculated trifoliolate leaflets of two plants.

A second study was performed to determine if veinal chlorosis in uninoculated leaves of PI 346304 is associated with an increase in systemic accumulation of virus antigen. Fully expanded unifoliolate leaves of PI 346304 were inoculated with CCMV-S and incubated in a greenhouse. Two weeks later, all trifoliolate leaves were removed. Newly emerging leaves that developed veinal chlorosis were evaluated.

In both studies, the tissue was fixed, dehydrated, infiltrated with a graded series of London resin white, polymerized, and sectioned as previously described by Van Lent and Verduin (14), with the following modifications. Tween 20 (0.01%) was added to the fixative, polymerization was performed at 50 C, and sections were floated off of the glass knife with water and placed on a microscope slide in a drop of 30% acetone.

Immunolabeling was performed as previously described by Van Lent and Verduin (14) but goat-antirabbit IgG conjugated to 5-nm-diameter gold was used as the secondary antibody. CCMV-S antiserum was cross-absorbed with extracts from healthy soybean (3). Antiserum was diluted (1:1,000) in PBS-BSA-Tween (0.01 M Na_2HPO_4, 0.150 M NaCl, 0.02% Tween 20, 0.02% NaN_3, and 1% bovine serum albumen). Thick sections were silver enhanced (IntenSE Kit, Janssen Life Sciences Products, Piscataway, NJ) and stained with toluidine blue (1% toluidine blue in 1% sodium borate). Observations were made with a light microscope. Controls consisted of CCMV-S-infected tissue initially incubated with an irrelevant antiserum (antitobacco etch virus) or healthy tissue of similar age treated as described above.

RESULTS

Disease reaction. One to 4 DAI, inoculated unifoliolate leaves of Davis and PI 346304 developed similar intensities of chlorosis. This reaction usually began as discrete chlorotic lesions that coalesced to yield a general chlorosis that faded within 2 wk.

Three to 7 DAI, the first or second set of uninoculated trifoliolate leaves of susceptible Davis plants developed mosaic, rugosity, distortion, and stunt (Fig. 1). This reaction continued with new growth, but symtomatology tended to ameliorate within 1 mo and new growth was less severely affected. Uninoculated trifoliolate leaves of infected PI 346304 plants were usually symptomless. In approximately 5% or fewer of PI 346304 plants, one trifoliolate leaf developed a localized chlorosis around a few veins (Fig. 1). When this veinal chlorosis occurred, usually all leaflets

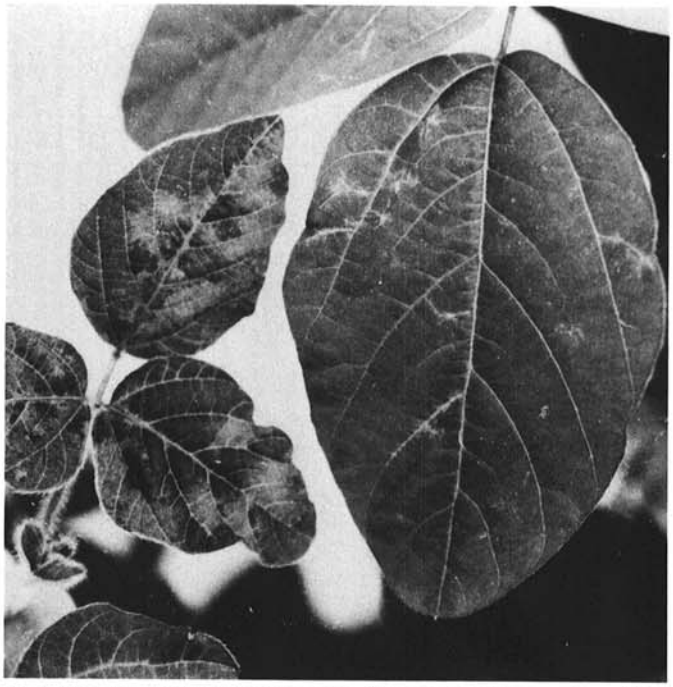

Fig. 1. Uninoculated trifoliolate leaves of susceptible Davis soybean (left) displaying mosaic, rugosity, and stunting, and of resistant PI 346304 (right) displaying limited veinal chlorosis.

of that trifoliolate leaf had small patches of chlorotic tissue, but all other trifoliolate leaves remained symptomless and the chlorosis became less intense within 1 wk of the onset of symptoms. Veinal chlorosis was not observed on Davis plants and was therefore a unique characteristic of PI 346304.

Virus concentration. Over the course of several experiments conducted in the greenhouse during all seasons of the year, virus concentration, as determined after purification, in inoculated unifoliolate leaves was usually high and did not differ between Davis and PI 346304 at 15 DAI (Table 1).

Virus accumulation within uninoculated trifoliolate leaves of Davis was only 22% as much as that in inoculated unifoliolate leaves of the same plants, but it was 3.5-fold higher than uninoculated trifoliolate leaf tissue of PI 346304 (Table 1). Virus accumulation in uninoculated trifoliolate leaves of PI 346304 was only 7% as much as that in inoculated unifoliolate leaves of the same plants. Both virion presence and low virus concentration in PI 346304 uninoculated trifoliolate leaves was confirmed by density gradient centrifugation.

Viral antigen concentration, as determined by ELISA, was similar in inoculated unifoliolate leaves of Davis and PI 346304. In uninoculated leaves of infected Davis plants, however, a 216-fold dilution of plant sap was required to obtain ELISA absorbance values similar to those (0.78) for sap of PI 346304 uninoculated leaves.

Concentration of virus in roots of Davis was only 35% as much as that in inoculated leaves of the same plants, but it was 7.9× greater than virus concentration in roots of PI 346304 (Table 1). Virus concentration in roots of PI 346304 was only 5% as much as that in inoculated leaves of the same plants.

Although virus concentration in uninoculated trifoliolate leaves of both Davis and PI 346304 varied from experiment to experiment, the difference between the two hosts was always significant (Table 1). Furthermore, Davis symptomatology was always severe, while PI 346304 remained symptomless or only a few plants showed limited veinal chlorosis.

Temperature study. Initial systemic symptoms of mosaic developed on Davis plants 3–4 DAI at 28 and 32 C. Similar symptoms were delayed 2 to 3 days at 24 C, a delay that also occurred when plants were incubated in the greenhouse. In general, systemic symptoms did not develop on PI 346304 plants. CCMV-S concentration was similar in inoculated unifoliolate leaves of Davis and PI 346304 at the three temperatures. However, in uninoculated trifoliolate leaves, concentration was less than 10 μg/g of tissue in PI 346304 as compared with 70, 95, and 303 μg/g of tissue in Davis plants at 32, 28, and 24 C, respectively (Table 2).

Induced resistance. In induced resistance tests, trifoliolate leaves of uninfected and systemically infected plants were inoculated with CCMV-S. Chlorosis developed on inoculated trifoliolate leaves of both previously infected and uninfected PI 346304 plants and on previously uninfected Davis plants. However, chlorosis was not observed on the trifoliolate leaves of previously infected Davis plants, perhaps because these leaves already had mottle symptoms. A systemic mottle developed on new trifoliolate leaves of previously uninfected Davis plants. No systemic symptoms developed on either previously infected or uninfected PI 346304 plants.

Challenge-inoculation of trifoliolate leaves on PI 346304 caused a dramatic increase in virus concentration, in two tests, from less than 10 and 28 to 286 and 572 μg/g of tissue, respectively (Table 3). In a similar comparison, challenge-inoculation of trifoliolate leaves on previously infected Davis plants caused only an average twofold increase in virus concentration.

Movement within leaves. High quantities of virus accumulated in the middle inoculated sections of both Davis and PI 346304 at 5 DAI, and accumulation increased at 10 and 20 DAI (Table 4). In the first test, at 5 and 10 DAI, Davis and PI 346304 had similar levels of virus in inoculated tissue, but at 20 DAI, Davis accumulated more virus than PI 346304. In test 2, Davis and

TABLE 1. Concentration of cowpea chlorotic mottle virus in inoculated and uninoculated tissue of Davis and PI 346304 soybean

	Virus concentration (μg/g)[a]		
		Uninoculated tissue	
Genotype	Inoculated unifoliolate leaves[b]	Trifoliolate leaves[c]	Roots[d]
Davis	617 ± 420	136 ± 47	214 ± 107
PI 346304	596 ± 246	39 ± 15	27 ± 16

[a] Micrograms of purified virus per gram fresh weight of tissue (± SE).
[b] Data derived from eight experiments with four replications each. Tissue harvested an average of 15 days after inoculation (DAI). Inoculated leaves of the two genotypes had similar intensities of chlorosis; infrequently, no symptoms occurred on either genotype.
[c] Data derived from three experiments with four replications each. Tissue harvested an average of 15 DAI. Davis leaves were mottled and stunted while PI 346304 leaves had limited veinal chlorosis on about 5% of the plants.
[d] Data derived from four experiments with four replications each. Tissue harvested an average of 15 DAI.

TABLE 2. Temperature effect on cowpea chlorotic mottle virus concentration in uninoculated trifoliolate leaves of infected Davis and PI 346304 soybean

Temperature (C)	Virus concentration (μg/g of tissue)[a,b]	
	Davis	PI 346304
24	303 ± 191	< 10
28	95 ± 28	< 10
32	70 ± 31	< 10

[a] Virus concentration (± SE) determined 14 days after inoculation of unifoliolate leaves. Davis leaves were mottled and stunted at all temperatures; PI 346304 had no symptoms at 24 C and limited veinal chlorosis occurred on 5% or less of the plants at 28 and 32 C.
[b] Virus in treatments with less than 100 μg/g determined by density gradient analysis.

TABLE 3. Test for induced resistance in symptomless uninoculated trifoliolate leaves of infected Davis and PI 346304 soybean plants

	Virus concentration (μg/g of tissue) (± SE)					
			Challenge-inoculated leaves[b]			
	Single inoculated plants[a]		Davis		PI 346304	
Test number	Davis	PI 346304	Inf[c]	Not inf[d]	Inf[c]	Not inf[d]
1	303 ± 191	< 10[e]	616 ± 131	786 ± 54	286 ± 79	445 ± 151
2	424 ± 129	28[e] ± 14	812 ± 327	1,112 ± 219	572 ± 184	689 ± 311

[a] Fourteen days after inoculation of unifoliolate leaves (presence or absence of chlorosis on inoculated leaves had no effect on virus concentration in trifoliolate leaves).
[b] Fourteen days after challenge-inoculation of trifoliolate leaves.
[c] Unifoliolate leaves inoculated on day 0. Infected (Inf) trifoliolate leaves challenge-inoculated 14 days after primary inoculation.
[d] Unifoliolate leaves buffer-rubbed on day 0. Not infected (Not inf) trifoliolate leaves inoculated 14 days later.
[e] Virus analyzed by density gradient analysis (less than 5% of the plants had trifoliolate leaves with limited vein chlorosis).

PI 346304 had similar levels of virus in inoculated tissue at all harvest dates with accumulation nearly doubling at 10 and again at 20 DAI (Table 4).

At 5 DAI, virus concentration was very low and similar in the uninoculated tip and base leaf portions of both Davis and PI 346304 (Table 4). By 20 DAI, however, virus concentration in the uninoculated tip and base leaf portions increased at least 5- to 30-fold more in Davis tissue than in PI 346304 tissue. The amount of virus was considerably lower in the uninoculated tissue than in the inoculated tissue of both hosts. A bias in acropetal virus movement and accumulation was observed in the first test, but movement was directionally neutral in the second experiment. A small quantity of virus always could be detected in uninoculated tissue of PI 346304 by density gradient analysis.

In the second type of movement study, where either the tip or base half of unifoliolate leaves was inoculated, low virus concentration occurred in uninoculated tissue of both Davis and PI 346304 at 5 and 10 DAI. But by 20 DAI, virus accumulation in uninoculated portions of Davis tissue had increased from less than 60 to 147 and 216 μg/g of tissue in the tip and base tissue, respectively. Very little accumulation of virus (< 10 μg/g of tissue) occurred in PI 346304 in either of the uninoculated halves of leaves, but virus could be detected.

Biological quality of virions. There was no difference ($P = 0.05$, Student's t test) in specific infectivity of virions purified from Davis and PI 346304 at each concentration used for inoculum. The average number of lesions per half leaf was 31, 87, 82, and 111 at 2, 6, 9, and 18 μg/ml of virus, respectively.

Immunocytochemistry. Virus antigen was detected in various leaf cells. Distribution and intensity of silver label was similar in most types of cells in inoculated unifoliolate leaves of Davis and PI 346304: top and bottom epidermal, palisade, spongy and paraveinal mesophyll, and bundle sheath (Fig. 2). In contrast, antigen was rarely detected within vascular tissue of inoculated unifoliolate leaves of PI 346304 (Fig. 2B) but was commonly, though not always, found in vascular tissue of Davis tissue (Fig. 2A).

In uninoculated diseased trifoliolate leaves of infected Davis plants, labeling was intense and similar to that occurring in inoculated Davis tissue (Fig. 2C). Uninoculated, symptomless trifoliolate leaves of PI 346304 resembled control leaves from uninfected plants, with little or no detectable antigen (Fig. 2D). This was in sharp contrast to antigen observed in PI 346304 uninoculated leaf tissue near veins that developed chlorosis (Fig. 2E). Labeling was intense in most cells, similar to that in Davis tissue, but only rarely present in vascular tissue.

DISCUSSION

Host response to CCMV-S infection in inoculated tissue of Davis and PI 346304 was similar both in terms of symptomatology and virus concentration. Typical of a susceptible reaction, CCMV-S accumulated in inoculated tissue to a relatively high concentration and chlorotic symptoms developed. However, when Davis and PI 346304 were studied on a cellular level with immunogold silver staining, local infection differed in one respect: little or no virus antigen was found within PI 346304 vascular tissue, whereas antigen was prevalent in such Davis tissue.

In uninoculated tissue, systemic infection of Davis and PI 346304 differed in several respects. Virus accumulation in uninoculated tissue (leaves and roots) of PI 346304 was only 13–29% as much as that in Davis, and symptoms were absent or mild. In fact no symptoms on PI 346304 were observed under field conditions (11) or in about 95% of plants grown in the greenhouse. This is in contrast to Davis, which developed severe mosaic, rugosity, distortion, and stunt. Immunocytochemistry results supported these observations with minimal virus antigen detected in symptomless PI 346304 tissue and high levels of virus antigen detected in Davis tissue.

Restriction of virus movement appears to be the best explanation for the mechanism of resistance to CCMV-S in PI 346304. There was no evidence to support restriction or alteration of any step in the replication cycle, biological activity of virions, events that induce pathogenesis, or susceptibility of uninoculated leaves on infected plants. In general, two types of virus movement in plants are believed to occur: cell-to-cell via plasmodesmata and long distance via vascular tissue (7). CCMV-S cell-to-cell movement in PI 346304 appeared to be largely unrestricted; it seems unlikely that high levels of virus (350–800 μg/g of tissue) in whole inoculated leaves could have been produced if virus were restricted to inoculated upper epidermal cells. Moreover, direct evidence showed the prevalence of virus antigen from upper to lower epidermal cells in inoculated leaves. Since virus antigen was rarely present in vascular tissue of PI 346304, virus entry into the vascular tissue and subsequent distal movement appears far less efficient than cell-to-cell movement.

CCMV-S can move through vascular tissue in PI 346304 since it is detected in low quantities in uninoculated leaves and roots. Therefore, a partial restriction of virus movement possibly could explain the nature of resistance in the following manner. After CCMV-S is delivered mechanically to epidermal cells, virions or a complex of viral genomic RNA plus coat protein, movement protein(s), and other essential proteins move to adjacent cells (spongy and palisade mesophyll and bundle sheath) via plasmodesmata and replicate (7). Cell-to-cell movement continues for short distances until an obstacle (vascular tissue) is encountered. Perhaps the CCMV-S movement protein interacts inefficiently with a "molecular gate" between the bundle sheath and vascular tissue. In a mechanically inoculated leaf, most of the tissue becomes infected and virus accumulates because inoculum is introduced to most areas between the network of vascular tissue. With incomplete inoculation, networks of vascular tissue may com-

TABLE 4. Movement of cowpea chlorotic mottle virus within inoculated unifoliolate leaves of Davis and PI 346304 soybean

Portion of leaf[c]		Virus concentration (μg/g)[a,b]					
		Day 5[d]		Day 10		Day 20	
Inoculated	Analyzed	Davis	PI 346304	Davis	PI 346304	Davis	PI 346304
Test 1							
	Tip	< 10	< 10	98 ± 18	17 ± 9	308 ± 146	< 10
Middle	Middle	251 ± 44	300 ± 52	976 ± 114	733 ± 147	$1{,}471 \pm 299$	722 ± 98
	Base	< 10	< 10	41 ± 7	< 10	142 ± 30	< 10
Test 2							
	Tip	50 ± 41	30 ± 15	71 ± 22	26 ± 9	200 ± 48	37 ± 7
Middle	Middle	176 ± 19	173 ± 11	326 ± 67	294 ± 41	566 ± 85	593 ± 61
	Base	20 ± 13	21 ± 8	69 ± 13	$< 15 \pm 5$	219 ± 53	48 ± 22

[a] Micrograms of purified virus per gram fresh weight of tissue ($\pm$ SE).
[b] Virus in all treatments with less than 100 μg/g of tissue was determined by density gradient analysis.
[c] A middle strip (5 mm wide), perpendicular to the midvein, of unifoliolate leaves was inoculated. Virus concentration in tissue within and adjacent to the inoculation site was analyzed. The inoculated tissue usually was chlorotic; no symptoms were observed on the uninoculated tissue.
[d] Days after inoculation.

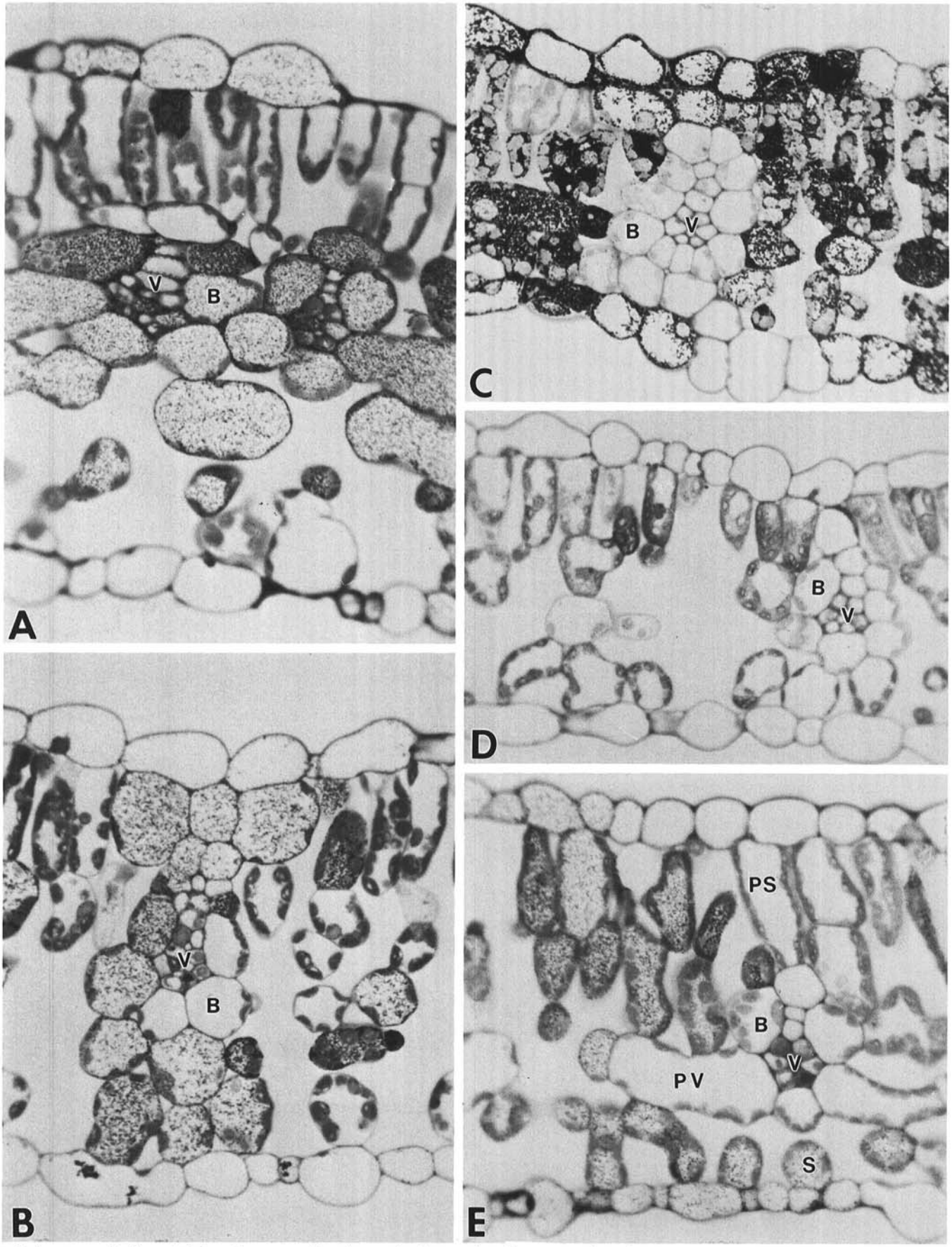

Fig. 2. Immunogold silver staining of cowpea chlorotic mottle virus antigen in cross section of soybean leaves (×590). Mechanically inoculated unifoliolate leaves of **A,** Davis, and **B,** PI 346304 soybean. Uninoculated trifoliolate leaflets on **C,** infected susceptible Davis leaf with mosaic and rugosity, **D,** resistant PI 346304 leaflet with no symptoms, and **E,** tissue from a chlorotic area of a resistant PI 346304 leaflet. Leaflet in **E** was approximately 10 days older than those in **C** and **D.** B = bundle sheath, PS = palisade mesophyll, PV = paraveinal mesophyll, S = spongy mesophyll, V = vascular bundle.

partmentalize areas of high virus accumulation that could explain the distinct border of high virus accumulation in inoculated strips of leaf tissue (within leaf movement study) and restrict virus accumulation in distal tissues. Therein, CCMV-S may be confined mainly to vascular tissue because it would face the same "molecular gate" in exiting vascular tissue. This hypothesis also may explain the occurrence of limited veinal chlorosis in PI 346304 plants. Perhaps infectious CCMV-S occasionally moves from the confines of vascular tissue into trifoliolate mesophyll and establishes an island of virus accumulation via cell-to-cell movement and replication. However, typical of inoculated leaves, compartmentalization again occurs and virus is limited to the chlorotic area near veins. Future ultrastructural studies might reveal specific sites of blockage.

A similar but less effective restriction of virus movement may be operating in cultivar Davis. When mechanically inoculated, both unifoliolate and trifoliolate leaves accumulate similar levels of CCMV-S. However, the trifoliolate leaves have less virus than inoculated ones when virus inoculum is delivered through the vascular system.

Similar types of nonnecrotic resistance have been observed in other virus-host pathosystems. Very similar to our results are the findings of Tu and Ford (13) with the maize dwarf mosaic virus (MDMV) strain A corn (*Zea mays* (L.)) pathosystem. Lei and Agrios (10) had similar results with MDMV strain B in resistant corn inbreds Pa405 and Bsq, but unlike the CCMV-soybean interaction, restriction of movement appeared complete with no virus detected in uninoculated tissue. Law et al (9) detected MDMV-A in the inoculated leaf, stem, and pith below the inoculated leaf, and in roots of resistant corn genotype PB3187, but upward transport was restricted. Dufour et al (4) found resistance to cucumber mosaic virus in pepper related to restriction of virus translocation into or outside the vascular system.

Current findings support the work of Bijaisoradat and Kuhn (1) and Paguio et al (11). Resistance in PI 346304 is of agronomic value due to its durability as evidenced by failure of nine strains of CCMV to break resistance (12), two of which (strains D and N) can overcome the hypersensitive resistance in Bragg and Williams soybean and one (strain R) that can overcome nonnecrotic resistance in PI 186465 cowpea.

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