

Effect of a Soybean Genotype Resistant to Soybean Mosaic Virus on Transmission-Related Behavior of Aphid Vectors

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ABSTRACT

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The probing behavior of *Myzus persicae* (Homoptera: Aphididae) on soybean cultivar Clark 63 (susceptible to infection by soybean mosaic virus) and its isoline L78-434 (similar to Clark except resistant to infection by soybean mosaic virus) was observed under a dissecting microscope. No differences were observed in the number of probes, the duration of each probe, or probing time within a 10-min period. Landing rates of aphid vector species on the cultivar and its isoline were monitored in a field experiment. No significant differences in landing rates were observed.

Soybean mosaic virus (SMV) is transmitted in a nonpersistent manner by aphid vectors and through seeds of infected parent plants (12). Infected seeds provide the initial inoculum in the field (12). Because aphids do not colonize soybeans in the Western Hemisphere (13), transient alate aphids that land and probe on soybeans are responsible for plant-to-plant transmission of the virus.

Transmission of SMV from an infected host depends on vector species, virus isolate, and soybean cultivar (5-7). Under field conditions, spread of SMV depends on the landing rates of aphid vectors, on the respective propensities of the vector species to transmit SMV, and postlanding vector activity, especially probing within the crop (11,12,16,17). Aphid landing rates depend heavily on cropping practices that lead to differences in canopy cover and color (10,15).

Use of resistant cultivars is a potentially important method for controlling SMV epidemics. Two kinds of resistance to this virus have been identified: resistance to infection, which restricts the virus replication in the host plant, and resistance to seed transmission, which inhibits the process of viable virus infecting seedlings through seeds of the parent plant (3-6).

The effect of an SMV-resistant soybean genotype on aphid landing and probing behavior has not yet been established. In this study, landing rates and probing activity of aphid vectors on

cultivar Clark 63 were compared with those on the SMV-resistant isoline L78-434 to determine if differences in aphid behavior on these isolines might lead to differences in SMV transmission and thus also play a role in resistance.

MATERIALS AND METHODS

Soybean isolines and their pubescence characteristics. Seeds of soybean cultivar Clark 63 and its SMV-resistant isoline (L78-434), both with normal pubescence (2), were obtained from the USDA Northern Soybean Germplasm Collection at the University of Illinois, Urbana. L78-434 was developed by R. L. Bernard at the University of Illinois by transferring gene *Rsv* (14) for SMV resistance from PI 96983 to Clark 63 through five backcrosses. PI 96983 is a cultivar introduced from Korea in 1932 by USDA agricultural explorers P. H. Dorsett and W. J. Morse. The isoline L78-434 is resistant to SMV strains G1, G2, G3, G4, G5, and G6 but gives a necrotic reaction to strain G7 (6).

To quantify their pubescence, 10 leaf disks 4 mm in diameter were cut with a cork borer from fully expanded trifoliolates of each isoline. The number of hairs on the upper surface of each leaf disk was counted under a dissecting microscope.

Virus strain and mechanical inoculation. SMV strain G5 (5), obtained from the Department of Plant Pathology at the University of Illinois, was used throughout the study. Inoculum for mechanical inoculation was prepared by grinding 1 g of SMV-infected soybean leaves in 5 ml 0.05 M sodium phosphate buffer (pH 7.4) in a mortar with pestle. Carborundum (600-mesh) was added to the inoculum, and the mixture was rubbed with a cotton applicator onto primary leaves of soybean plants at the unifoliate stage of development. Plants were held in a greenhouse at 23 ± 3 C for development of symptoms. Observations were made to

determine the susceptibility and symptom expression of both cultivars. Inoculated plants were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of SMV antigen to confirm visual diagnosis.

ELISA procedure. The ELISA procedure used was that described by Clark and Adams (8). Leaf samples were stored in the freezer at -40 C until enough samples were assembled to occupy a full ELISA plate minus the edge wells. Frozen Immulon I flat-bottomed plates (Dynatech Laboratories, Inc., Alexandria, VA) were obtained from C. J. D'Arcy, Department of Plant Pathology, University of Illinois at Urbana-Champaign. The plates were presensitized with SMV IgG at the rate of 1 μ g of globulin per milliliter of coating buffer. Leaf samples were ground individually in phosphate-buffered saline (0.02 M-phosphate plus 0.15 M NaCl) with 0.05% Tween 20 (PBS-Tween) at pH 7.4 in a mortar with pestle. Defrosted plates were rinsed five times with PBS-Tween. After the final rinse, plant sap samples were added in 200- μ l aliquots to the appropriate wells; several wells with buffer, plant sap from a healthy plant, and plant sap from a known SMV-infected plant were used as controls. The plate containing the plant sap samples was incubated at 4 C overnight, incubated at least 3 hr at room temperature with an alkaline phosphatase conjugate of the same antiserum diluted 1:300 with PBS-Tween, and then incubated for 30 min at room temperature with a substrate of 1.5 mg *p*-nitrophenyl phosphate dissolved per 5 ml of 10% diethanolamine buffer (pH 9.8). The plate was rinsed five times between each step. To estimate relative virus titers of mechanically inoculated isolines, an ELISA reader (Bio-Tek Instruments, Inc., Burlington, VT) was used to compare the optical densities (405 nm) of each sample after the 30-min substrate reaction.

Laboratory test of aphid probing behavior. The green peach aphid (*Myzus persicae* (Sulzer), Homoptera: Aphididae) was used in laboratory experiments. Colonies were reared on radish (*Raphanus sativus* L. cv. Early Scarlet) in wooden-framed cages (35 \times 35 \times 56 cm high) under constant fluorescent illumination at 24 ± 2 C. Apterous specimens for experimentation were obtained by placing 10 large apterous aphids on a

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radish plant for 10–12 days and collecting their progeny.

Aphids starved 1–2 hr were placed on a detached healthy soybean leaflet floated on water in a petri dish and observed under a dissecting microscope. The number of probes, the duration of each probe, and probing time within a 10-min period were recorded for eight specimens of *M. persicae* on each isolate.

Field experiment with Clark isolines. A field experiment was conducted at the Vegetable Crops Farm of the University of Illinois at Urbana-Champaign in 1983 to compare aphid landing rates and SMV spread in plantings of four Clark isolines: Clark 63 (normal pubescence), L78-434 (SMV-resistant and normal pubescence), L63-2435 (curly pubescence), and L71-149 (sparse pubescence). Only the results from Clark 63 and SMV-resistant isolines are presented. The isolines were planted in plots 10 × 10 m arranged in a randomized complete block design with each isolate replicated five times. Rows were 0.75 m apart and plots were thinned to one plant per 10 cm along rows. After thinning, every 40th plant was mechanically inoculated with SMV-G5 at the primary leaf stage to produce a 2.5% initial level of infection. In the plots planted with the resistant isolate L78-434, susceptible Clark 63 plants were transplanted at every 40th position along rows and inoculated along with the other treatments. Twenty days after inoculation, disease incidence was recorded in the middle 3 m of the central four rows in each plot. Leaves from plants with questionable infections were assayed for SMV by ELISA.

Monitoring of aphid landing rates. A trap consisting of a horizontal mosaic green ceramic tile (Cambridge 815) in a sandwich box containing a 50% aqueous solution of ethylene glycol (12) was placed in the center of each plot to monitor aphid landings. The position of each trap was maintained at canopy level throughout the study. Aphids were collected daily between 11 July and 10 September 1983 and were stored in vials of 70% ethanol for later identification. The number of specimens of each aphid species trapped was recorded separately. Voucher specimens of each species have been deposited in the collection of the

Table 1. Transmission-related behavior of *Myzus persicae* on soybean cultivar Clark 63 and its SMV-resistant isolate L78-434 ($n = 8$)^a in the laboratory

Soybean isolate	Probe duration (sec)	Time spent not probing (sec)	Number of probes within 10 min
Clark 63	68.8 ^b	423.1	3.5
L78-434	65.2	411.1	3.8

^aTotal number of aphids observed for each soybean isolate.

^bNumbers in columns not significantly different at $P = 0.05$, paired t test.

Illinois Natural History Survey, Champaign.

Canopy cover data. Canopy cover measurements were taken in each plot at three growth stages by estimating the proportion of row and interrow space occupied by foliage. Estimates from five randomly selected locations in each experimental plot were averaged and expressed as percentages of canopy cover for each treatment.

RESULTS

Pubescence. The average number of hairs per square centimeter was 273 ± 10 for Clark 63 and 207 ± 10 for the resistant isolate L78-434. The average lengths of

hairs of Clark 63 and L78-434 were 0.87 and 0.95 mm, respectively.

Probing behavior. No significant differences were observed in number of probes, duration of probes, or time spent not probing by *M. persicae* for the two isolines, according to the paired t test (Table 1).

Disease incidence in the field. Striking differences existed in the disease incidence recorded in the center of each plot. The resistant isolate developed no virus infection during the season. Figure 1 illustrates the disease progress curves for the two isolines.

Canopy cover. Canopy cover measurements were taken on 3, 18, and 24 August

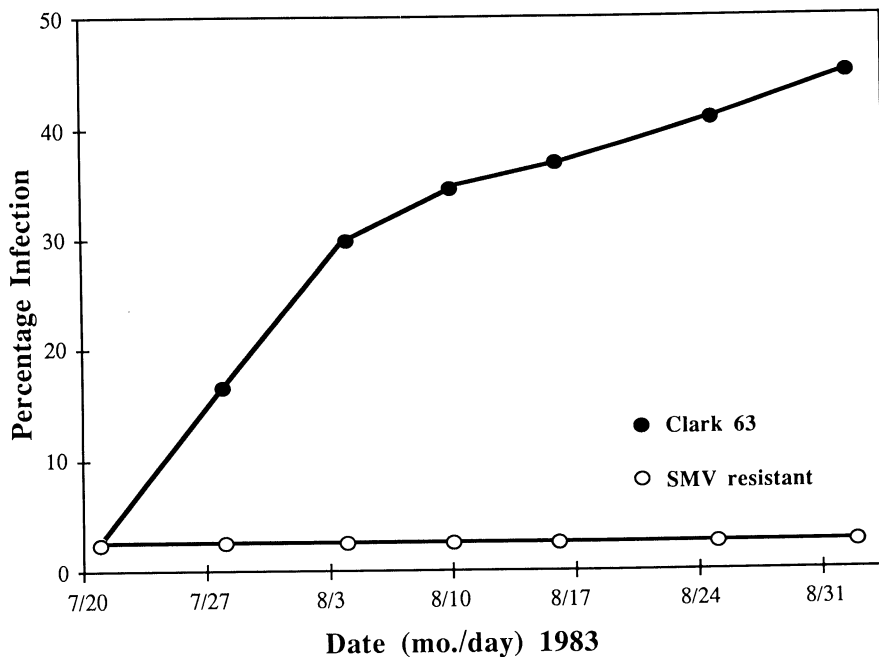


Fig. 1. Soybean mosaic virus disease progress curves on soybean cultivar Clark 63 (●) and its SMV-resistant isolate L78-434 (○) during a 1983 field experiment at Urbana, IL.

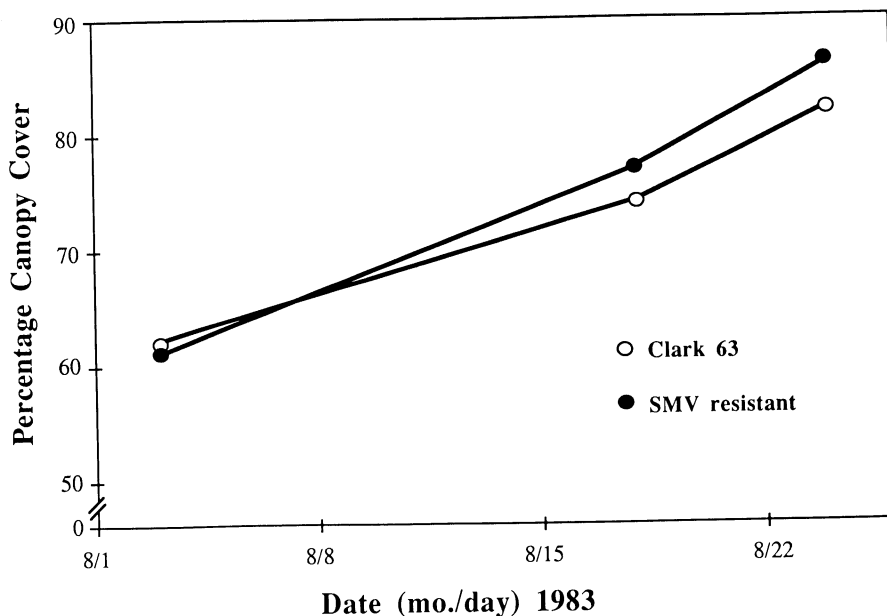


Fig. 2. Percent canopy cover of soybean cultivar Clark 63 (●) and its SMV-resistant isolate L78-434 (○) during the August 1983 growing season at Urbana, IL.

Table 2. Weekly aphid catch per horizontal mosaic green pan trap for plots of soybean cultivar Clark 63 and its SMV-resistant isoline L78-434 during the 1983 growing season, Urbana, IL

Soybean isoline	Mean number of specimens caught per trap per week									LSD
	11-17 Jul.	18-24 Jul.	25-31 Jul.	1-7 Aug.	8-14 Aug.	15-21 Aug.	22-28 Aug.	29 Aug.-4 Sept.	5-10 Sept.	
<i>Aphis</i> spp.										
Clark 63	3.8	2.4	1.2	0.4	2.6	0.2	0.0	0.0	0.0	NS ^a
L78-434	3.8	2.8	0.4	0.2	2.6	0.0	0.0	0.0	0.0	
<i>Macrosiphum</i> spp.										
Clark 63	14.2	6.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	NS
L78-434	10.6	2.8	0.4	0.0	0.0	0.0	0.0	0.0	0.0	
<i>Myzus</i> spp.										
Clark 63	0.8	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS
L78-434	1.4	0.0	0.0	0.4	0.0	0.2	0.0	0.0	0.0	
<i>Rhopalosiphum</i> spp.										
Clark 63	0.8	1.4	6.2	32.0	17.2	1.2	0.0	1.4	0.0	NS
L78-434	0.6	1.4	5.2	45.8	28.0	2.8	0.6	0.8	0.0	
All other species										
Clark 63	17.4	21.4	5.0	1.0	0.8	0.2	0.0	0.0	0.0	NS
L78-434	21.2	19.6	9.0	2.4	0.0	0.0	0.0	0.0	0.0	
All aphids ^b										
Clark 63	46.2	34.4	12.4	37.2	36.6	7.0	1.8	3.0	1.2	NS
L78-434	55.4	29.4	17.0	51.6	43.6	7.6	2.2	2.4	1.6	

^aNot significant at $P = 0.05$, LSD test.

^bUnidentified aphids also included.

1983. Both isolines maintained similar canopy covers except during the last measurement, when the canopy cover in the resistant isoline plots (86% canopy cover) was significantly greater than that of the cultivar Clark 63 plots (82% canopy cover) ($P = 0.05$, LSD test) (Fig. 2).

Landing rates of aphids. Daily counts of major genera of aphids known to be responsible for field transmission of SMV in central Illinois (11) were grouped on a weekly basis and analyzed. The following genera were analyzed independently: *Aphis*, *Macrosiphum*, *Rhopalosiphum*, *Myzus*, and all other genera together. No significant differences ($P = 0.05$) in mean weekly landing rates were observed within any aphid group on the two isolines, Clark 63 and L78-434 (Table 2).

DISCUSSION

SMV is very efficiently transmitted by *M. persicae* after a 30- to 60-sec inoculation probe on a susceptible plant (16). Atiri et al (1) recorded probes of shorter duration by *Aphis craccivora* Koch, a colonizing vector, on aphid-resistant cowpea cultivars than on aphid-susceptible ones. In our study, equal numbers of probes and probes of the same duration were recorded on the SMV-resistant isoline and the SMV-susceptible Clark 63. These data indicate that at least for this experiment, the SMV-resistant genotype did not interfere with the probing activity of *M. persicae*.

About 23 of the aphid species known to transmit SMV alight on soybean crops during any given year in central Illinois

(11). The landing rate of aphids depends in part upon canopy cover and color (10, 15). Both landing rate and probing activity of aphids have been positively correlated with field spread of SMV (17). In this study, Clark 63 and the SMV-resistant isoline maintained the same canopy cover throughout the experiment except at the last canopy cover measurement in late August (Fig. 2), at soybean growth stage R2 (9). Important genera of aphids that transmit SMV landed at the same rate on both isolines. This indicates that the resistant genotype does not influence the landing rates of aphids.

Reductions in the spread of pea mosaic and pea stunt viruses on Dollar red clover caused by resistance to the pea aphid, *Acyrtosiphon pisum* (Harris), have been reported (18). *A. pisum* colonizes red clover. In our study, increasing incidence of SMV on Clark 63 and the absence of the virus disease on its SMV-resistant isoline were not due to the differential reactions of vectors to these isolines, because aphids behaved similarly on both. No aphid species commonly colonizes soybean North America.

Our data strongly suggest that the resistant isoline L78-434 in no way alters aphid landing or probing activity from that of SMV-susceptible Clark 63. This further suggests that the resistant quality of this isoline is a result of the interaction of the virus and the isoline and does not involve the vector.

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