Susceptibility and Resistance of Soybeans to Peanut Stripe Virus

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ABSTRACT

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One hundred twenty-one soybean genotypes from the International Soybean Program collection were evaluated for their reactions to three symptom variants (isolates) of peanut stripe virus (PStV): stripe, blotch, and mild mottle. Symptoms on soybeans ranged from systemic necrosis, mosaic, and mild mottle to no reaction. About 35% of the genotypes are resistant to all isolates. Enzyme-linked immunosorbent assay (ELISA) readings were positive from susceptible genotypes showing symptoms but not from resistant genotypes without symptoms. PStV was transmitted efficiently (16%) from peanut to soybean by the aphid *Myzus persicae* but inefficiently (1%) by *Aphis craccivora*. None of the three virus isolates were transmitted in the 15,000 soybean seeds from infected plants harvested from all susceptible cultivars. Infective particles could be recovered from seed coats of immature seeds by bioassay on *Chenopodium amaranticolor*. Although low ELISA values were obtained from the seed coats of mature seed, infective particles could not be recovered by bioassay. Neither infective virus nor serologically detectable PStV was recovered from cotyledons or embryo axes of mature seeds.

In 1983, Xu et al (13) reported a virus in China causing a mild mottle in peanuts (*Arachis hypogaea* L.) that naturally infected nearby soybeans (*Glycine max* (L.) Merr.). Greenhouse trials showed a yield reduction of 53% in soybeans. Demski et al (6) reported a new virus in peanuts in the United States in 1984 and named it peanut stripe virus (PStV). The virus was isolated from peanut seed received from China (5). It is serologically related to the virus causing peanut mild mottle in China. Both viruses have very similar host ranges (7,14).

Peanut mottle virus (PMV), another potyvirus that infects peanuts, naturally infects soybeans (1,2,6). Field studies and virus disease surveys have demonstrated a relationship between peanut-growing areas of Georgia and the occurrence of PMV in soybean (4,10). A similar pattern may occur with PStV where infected peanuts are the primary source of virus for soybeans.

Soybeans are a major agricultural crop in the Western Hemisphere. Soybean germ plasm is frequently exchanged internationally, and it is not known if PStV is seedborne in soybeans. The susceptibility of soybean cultivars to inoculation with PStV prompted the present study.

The purposes of this work were to identify resistance to PStV in soybean germ plasm and to characterize the types of susceptible reactions. Tests were conducted to determine if PStV is seedborne in soybeans and to determine the efficiency of its aphid transmission from peanuts to soybeans.

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MATERIALS AND METHODS

Virus isolates. Three symptom variants (isolates) of PStV were used (5). The first isolate, stripe, was characterized by dark green stripes, discontinuous banding along lateral veins of young leaves, and oak-leaf patterns on older peanut leaves. The second isolate, blotch, was characterized by large dark green spots not associated with the veins on peanut leaflets (7). Both the stripe and the blotch isolates were obtained from naturally infected peanuts in Georgia. The third isolate, serologically indistinguishable from PStV, called virus producing mild mottle (VPMM), was obtained from O. W. Barnett (13). The three isolates can be distinguished by symptoms induced in peanuts, but all are considered to be PStV. All isolates were maintained in white lupine (*Lupinus albus* L.) in the greenhouse.

Reactions of soybean genotypes. The evaluations were performed using 121 soybean entries from the International Soybean Program. Seeds were planted in soil in 25-cm-diameter pots in a greenhouse. Each of the three PStV isolates was separately inoculated, mechanically, to unfoliate leaves of 15 plants of each of the 121 soybean accessions 7–9 days after planting. Plants were evaluated by visual observation 1 wk later for local reactions and after 2–3 wk for systemic reaction. Mechanical inoculations from each soybean accession with blotch-, stripe-, or VPMM-infected soybean plants were made to peanut (cultivar Argentine) to observe the original symptoms of each PStV isolate expressed in peanuts. Additionally, serological assays were made on plants from each soybean accession.

Resistance as used in this paper is intended to imply a high degree of resistance that may be close to immunity because symptoms were not expressed, virus could not be mechanically recovered, and virus could not be detected by enzyme-linked immunosorbent assay (ELISA) 3 wk after the soybean plants were mechanically inoculated with PStV.

ELISA. Antiserum to the stripe isolate of PStV was prepared by injecting rabbits intramuscularly with preparations of purified virus (7). The direct double-antibody sandwich (DAS) ELISA procedure used was the one described by Clark and Adams (3) and modified by Lister (11). The γ-globulin fractions were precipitated with sodium sulfate. A portion of the γ-globulin fraction was conjugated to alkaline phosphatase type VII-S (Sigma) with 0.06% glutaraldehyde (12). The remainder of the γ-globulin fraction was used for coating polystyrene plates. The sample extraction buffer contained 0.25 M potassium phosphate and 0.1 M ethylenediaminetetraacetic acid (EDTA), pH 7.5.

Three virus controls, three healthy controls, and three blank wells were randomly distributed in each plate. Test sample triturate (200 μl) was added to each of two wells. All ELISA reactions were assessed by reading absorbance at 410 nm in a Dynatech ELISA reader. Absorbance values for blank wells were subtracted from absorbance values from plate wells containing healthy controls giving absorbance values of healthy controls. Samples judged positive for PStV had absorbance values at least twice those of the healthy controls. All PStV isolates were readily detected using the antisera to the stripe isolate (5,7).

Aphid transmission. Tests for aphid transmission of the blotch isolate were done in the laboratory with *Aphis craccivora* Koch and *Myzus persicae* (Sulz.). These species were selected because they are commonly trapped in both peanut and soybean fields and were reported vectors of VPMM (13). *A. craccivora* colonies were maintained on cowpeas (*Vigna unguiculata* (L.) Walp subsp. *unguiculata* 'California Blackeye No. 5') and *M. persicae* were maintained on pepper (*Capsicum annuum* L.

'Yolo Wonder'). Nonviruliferous aphids were starved in glass vials for 2–3 hr, then 100 aphids of each specie were individually placed on a virus-infected peanut leaflet for a 2-min acquisition access. These aphids were then individually transferred to a healthy soybean plant for a 24-hr inoculation access. Thirty aphids of each specie were similarly placed on infected soybeans, then individually transferred to a healthy soybean plant. All plants were visually examined and tested by ELISA 3 wk after aphid inoculation.

Seed transmission. Immature (full size but green) and mature soybean seeds were collected from infected susceptible accessions (Tulumayo-2, Coc Chun, and PR139-10). One hundred mature and 100 immature seeds were harvested from each accession. Seeds were separated into seed coats, embryo axes, and cotyledons, then assayed by DAS-ELISA, using the procedure for peanut seed assay (8), and by local lesion assay on *C. amaranticolor*. To determine if the desiccation of seeds had any effect on infective virus particles, an additional 100 green seed coats were dried in a 37 C oven overnight, then tested by DAS-ELISA and bioassayed on *C. amaranticolor*. From all the infected plants, a total of 15,000 mature seeds were harvested, and 120 seeds were planted in each of 125 germination trays. Seedlings were observed for symptoms until the fifth true leaf developed, then assayed by DAS-ELISA (sample = one top expanded leaflet from each of five plants were pooled).

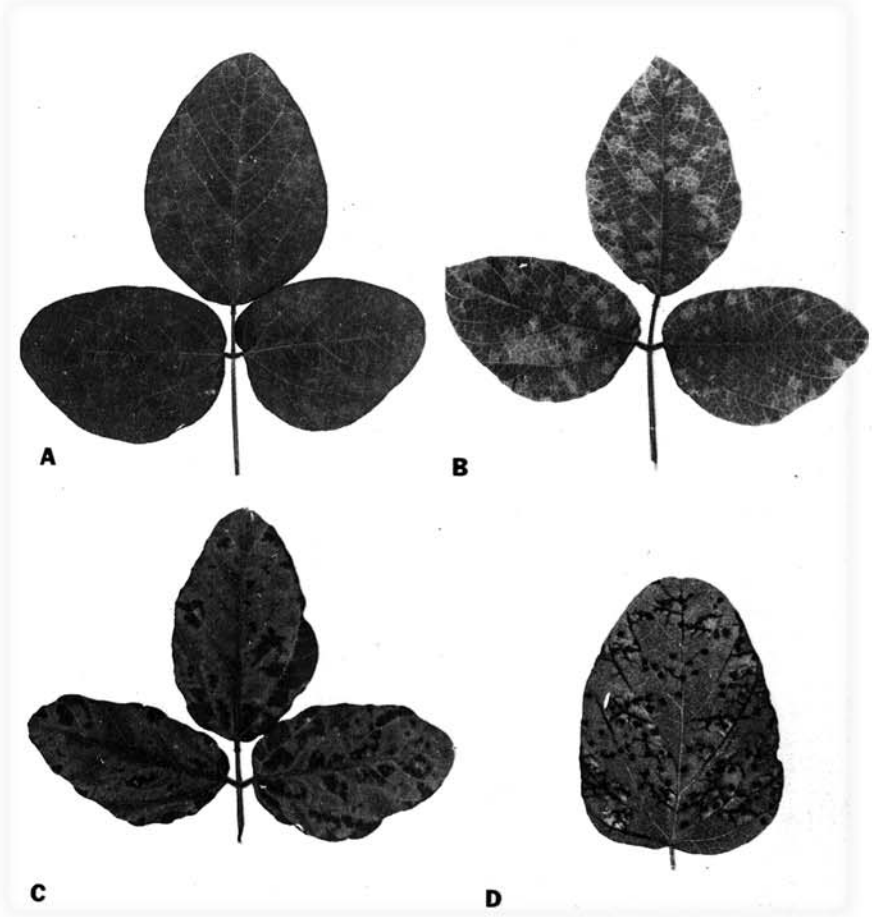

Fig. 1. Reactions of different soybean lines to inoculation with the blotch isolate of peanut stripe virus. **(A)** Davis (resistant), **(B)** Laurence (mild mottle), **(C)** IAC-6 (systemic mosaic), and **(D)** PR141-24 (necrotic lesions).

Table 1. Examples of symptom expression in soybean genotypes 15 days after inoculation with peanut stripe virus isolates stripe, blotch, and virus producing mild mottle

Type of reaction	Cultivars
Necrotic local lesions followed by systemic necrosis	PR139-10, PR141-24, Tulumayo-2
Chlorotic local lesions followed by systemic mottle	Coc Chun, MTD-22W, PR21-43-1-B-2
Systemic mosaic	BS54, Elgin, IAC-6, Jupiter, PR13-34-4-B-3
Systemic mild mottle in the first trifoliolate leaves[a]	Lawrence, Tamayulas, Will

[a] Virus was recovered from the primary leaves but not from the secondary growth.

RESULTS

Reactions of soybean genotypes. Based on three criteria, symptom development in soybeans, characteristic symptom production after mechanical inoculation from the soybeans to peanuts, and positive ELISA readings, 77 of the 121 soybean entries artificially inoculated with PStV were found susceptible (having met all three criteria) to at least one of the three PStV isolates. The following entries were susceptible: AGS 8, Almonozar, Amsoy, Bard, Bossier 19, Braxton, BS54, BSR201, Cayeme, CN 210, CN 290, Clark 63, Coc Chun, Con Khuong, Crawford, DH4, DII-61-66, Durock, Duocrop, EGA y-91-7, Elgin, F81-7636, F82-7156, F82-7824, G2261, H-80-20555, IAC-6, Imp. Pelic Blt-6, IPB 212-81, Jupiter, Jupiter R, Kabanyolo-1, LO 4035-1, Lawrence, LK soy 21-6, Maple Amber, Maple Arrow, Maple Presto, McCall, M-73-244, M-98, Mead, MTD-22W, MTD-63, Oscland, OC 78-503, Ocepar 2-IAPO, Pixie, Platte, PR13-34-4-B-3, PR139-10, PR140-2, PR141-24, PR15-100-4-B-8, PR21-43-1-B-2, PR30-34-4-x-2, PR30-38-3-x-3, Rinconada, Rinconada 44, Rocio, Rosales-S-80, S76-2109, SJ2, Tamayulas 80, TGX 330-04E, TGX 711-01D, Tulumayo-2, Udo Magaly, UFV-1, Wells-2, Will, Williams 82, Wright, 576 2109, 78-W110, 79-W330, and 80-B-4007

Susceptible reactions differed dramatically among cultivars (Fig. 1). Leaves of PR141-24, Tulumayo-2, and PR139-10 developed necrotic local lesions that eventually developed into systemic necrosis, whereas PR21-43-1-B-2, Coc Chun, and MTD-22W developed only mild mottle. These reactions were separated into four categories (Table 1). However, the type of symptom expression in a given soybean accession was the same regardless of the PStV isolate. The only difference between the isolates of PStV infecting soybean appeared to be virulence; the blotch isolate consistently infected more plants per entry, and four accessions were susceptible only to the blotch isolate (Mead, Platte, UFV-1, and Williams 82) by all three criteria. No differences in soybean susceptibility were found between stripe and mild mottle isolates. Cultivars PR141-24, Jupiter R, and Tulumayo-2 showed discrete necrotic local lesions against all three PStV isolates, indicating their usefulness as assay hosts.

The resistant reaction was observed on 44 cultivars. These cultivars did not develop symptoms from any of the three isolates, and the virus could not be detected by ELISA or when inoculations were made to the indicator plant *C. amaranticolor*. Parallel inoculations to *C. amaranticolor*, using sap from infected soybean, induced >10 lesions per leaf. Among soybean cultivars currently recommended for Georgia, only Davis was identified as resistant.

Other resistant entries were: AGS 147, Amcor, Beeson, Birch, Clay, Chico, Corsoy 79, Cumberland, Cutler, Dawson, Desoto, Douglas, Duiker, D75-9207, D75-10-194, Epps, F75-9207, Harcor, Hardin, Harper, Hobbit, HLS, IAC-73-1385, ICA-L-129, IPB 163-91, Keller, Lakota, LeSoy 273, LS 77-952, MACS 75, Mayo 80, Nebsoy, OC 7934, Pella, Simpson, SJ 5, Sparks, Sprite, Union, Vickery, Weber, Woodworth, and Yagui 80.

Aphid transmission. *M. persicae* was more efficient than *A. craccivora* in transmitting PStV from peanuts to soybeans; 16 of 100 plants were infected by *M. persicae*, whereas only one of 110 plants was infected by *A. craccivora*. No transmission from PStV-infected soybean to healthy soybean was observed when 30 individual *M. persicae* and 30 individual *A. craccivora* were used.

Seed transmission. The 77 susceptible infected soybean cultivars were grown to maturity, and seeds were harvested. The virus was not detected by ELISA or by bioassay in seedlings raised from seed that developed from the infected plants (15,000 seeds). Infective virus was recovered from 11 of 20 seed coats of immature seeds (from five selected lines) and from six of 25 seed coats of artificially dried seeds by bioassay to *C. amaranticolor*, on which characteristic chlorotic local lesions were observed. Neither infective virus nor serologically detectable virus particles were recovered from the embryos of immature or dried seeds. PStV could be detected in 56 of 200 seeds serologically but not by infectivity tests in the testa of dried seeds.

DISCUSSION

Recent (1985 and 1986) field surveys (J. W. Demski and D. L. Pinnow, *unpublished*) indicate that although intensive efforts have been made, PStV has not been eliminated from the peanut germ plasm in the United States. One primary source of PStV is seed transmission in peanut. Peanuts often need 135 days to mature, and infected peanut plants are an available virus source for this 4.5 mo. Thus, there is a possibility that it can be disseminated to fields of soybeans from infected peanuts throughout the growing season. Other primary sources have not been identified. Natural infection of soybean with PMV occurs in Georgia and other areas of the world where peanut and soybean are grown near each other (1,2,10). A similar situation could occur with PStV.

The type of symptom expression in susceptible soybeans was generally the same for each of the three PStV isolates and appears to be a function of the soybean accession. If one isolate induced local lesions on a particular accession, the other isolates also induced local lesions on this accession. However, the percentage of soybean plants infected appears to be a function of the PStV isolate. The blotch isolate consistently infected a higher percentage of the soybean plants in each susceptible accession than either stripe or VPMM.

Most soybean germ plasm tested was susceptible to PStV. Symptoms varied from severe systemic necrosis in the lines from Puerto Rico (PR141-24 and PR139) to mild mottle (Elgin and Coc Chun). It is interesting to note that Braxton, Duocrop, Ransom, and Wright, four of the 1986 recommended soybean cultivars for Georgia, are susceptible to all three isolates.

Resistance to mechanical inoculation was expressed by lack of virus infection and/or replication because inoculation with all three PStV isolates did not induce symptoms and the virus could not be detected by bioassay or ELISA. If one or more isolates caused infection in an accession, the accession was termed susceptible. Although the three virus isolates are related serologically, the blotch isolate could successfully infect the cultivars Mead, Platte, UFV-1, and Williams 82, whereas stripe and VPMM did not.

Because PStV is seed-transmitted in peanuts, there was a possibility that it is also seed-transmitted in soybeans. The studies with 15,000 seedlings from a total of 77 soybean accessions, however, provide strong evidence that PStV is not seed-transmitted in soybeans. PStV thus differs from soybean mosaic virus (SMV), a serologically related potyvirus, which is seed-transmitted in soybeans (11).

Infective particles of all three PStV isolates studied can be recovered from seed coats of immature soybean seeds. As the seeds mature, there is still a positive serological reaction from seed coats, but the particles are not infective, as evidenced by lack of lesions on bioassay hosts. The fact that the virus is infective when isolated from artificially dried, immature seed coats provides an argument against desiccation as a physical inactivation process. Irwin and Goodman (9) proposed that inhibitors or specific chemicals may inactivate SMV as the seed matures. Our data do not disagree with this proposal. The results with PStV demonstrate that as the soybean seed matures, the virus in the seed coats loses its infective properties.

A source of inoculum for peanut fields and possibly soybean fields appears to be infected peanut seed. If PStV becomes established in commercial peanuts, then susceptible soybeans should not be planted adjacent to peanuts. From 200 randomly selected peanut plants that were 100 m from a PStV source in peanuts, only one plant was infected by PStV as determined by ELISA (J. W. Demski, *unpublished*). Other susceptible hosts such as soybean probably would also be helped by space between a source and the host. Cognizance of crop spacings between peanuts and soybeans should be beneficial.

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