Production of Peanut Seed Free of Peanut Stripe and Peanut Mottle Viruses in Florida

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ABSTRACT

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Peanut (*Arachis hypogaea* L.) seed free of both peanut stripe (PStV) and peanut mottle (PMoV) potyviruses were harvested in 1986–1990 from virus-indexed, greenhouse-grown plants for field propagation. Neither virus was detected in field plots sown in 1987 and 1989 with virus-indexed, greenhouse-grown seed and isolated by at least 5 km from any commercial or experimental plantings. PMoV, but not PStV, was detected in a similar field plot sown in 1988 and located about 1.5 km from a 128-ha commercial field. PStV was detected in 247 of 497 plants in an experimental breeder's field sown in 1988–1991 with seed infected with this virus but was detected in only one of 1,431 plants in a similar field located 13 km away and sown with virus-indexed, greenhouse-grown seed. PStV was not detected in Sunrunner peanut plants derived from seed that were virus-indexed in 1985, sown in a greenhouse in 1986, and field-grown from 1987 to 1991.

Additional keywords: enzyme-linked immunosorbent assay (ELISA), groundnut, sodium dodecyl sulfate (SDS) immunodiffusion serology

Peanut stripe disease is caused by peanut stripe virus (PStV), a potyvirus that is seedborne in peanut (*Arachis hypogaea* L.). Because its potential economic impact was unknown, the discovery of this virus in the United States in 1982 caused national concern. PStV is believed to have been introduced via contaminated peanut seed imported from the Orient. Efforts to eliminate PStV resulted in reduced domestic and international exchange of peanut germ plasm, delays in the release of new cultivars, and the destruction of some breeding material in experimental plantings (6,26).

Although PStV has been detected in experimental peanut plantings in several southeastern states, it does not appear to be in commercial plantings in the United States. In contrast, peanut mottle virus (PMoV), which is also seedborne in peanut, has been in the United States longer than PStV and is widespread in both commercial and experimental peanut plantings (6,7,14).

The primary source of inoculum for both viruses is infected peanut seed. Secondary transmission of both viruses is by aphids in a styletborne manner (4,6). Estimates of yield loss vary (6,15,17), but neither virus is currently considered a threat to U.S. peanut production. Both viruses have been reported to cause yield loss in other legume crops, such as soybean (*Glycine max* (L.) Merr.), when the other crop is grown near plantings of infected peanut (4,5,28).

PStV was first identified in Florida in 1983 in experimental peanut breeding plots (J. W. Demski, *unpublished*). Surveys in Florida during 1984 and 1985 confirmed findings in other states that PStV incidence was much higher in experimental than in commercial plantings (20).

Most commercial peanut seed in Florida is derived from certified stock from the Florida Foundation Seed Producers, Inc. (Greenwood, FL). Peanut cultivars developed by the Institute of Food and Agricultural Sciences of the University of Florida and released through the Florida Foundation Seed Producers at the time of this study included Florigiant, Florunner, Southern Runner, and Sunrunner, which make up more than half of total U.S. peanut production. To reduce the prospect of PStV spreading into commercial plantings through this seed chain, the University of Florida in 1986 established control guidelines similar to those developed in Georgia for this virus (6). Under these guidelines, breeder seed contaminated with PStV could be sown only at two sites in Florida, herein referred to as the Dozier and Green Acres sites, in Jackson and Alachua counties, respectively. Also, new breeder seed of the peanut cultivar Sunrunner (19) was withheld from release because all known seed stocks of its three component lines were infected with PStV.

We report on the development of virus-free peanut seed from stocks contaminated with PStV and PMoV, rates of reinfection by PStV and PMoV in various field plantings in Florida sown with virus-free seed, and the release in 1989 of PStV-free Sunrunner peanut planting stocks for commercial seed production. Portions of this work have been published previously (29).

MATERIALS AND METHODS

Serological tests. PStV and PMoV antisera and reference antigens used in this investigation were provided by D. E. Purcifull (20). All original seed were individually indexed for PStV and PMoV by indirect enzyme-linked immunosorbent assay (ELISA) at Prosser, WA. Cotyledon tissue was sliced from the distal portion of each seed with individual razor blades, triturated 1:10 in carbonate buffer (pH 9.6) (3), and incubated for 2 hr at 25 C in duplicate wells. Antisera diluted 1:1,000 were incubated for 4 hr at 25 C; goat antirabbit antibodies conjugated with alkaline phosphatase enzyme (1:2,000 dilution) were incubated for 4 hr at 37 C. All ELISA-negative seed were returned to Gainesville, FL, for germination.

Leaf samples from greenhouse or field-grown plants were tested in immunodiffusion tests as described by Purcifull and Batchelor (21). The diffusion medium consisted of 0.8% Noble agar, 0.5% sodium dodecyl sulfate (SDS), and 1% sodium azide. Leaf samples tested by this method were routinely expressed with a sap extractor (Model 1, Ravenel Specialties, Seneca, SC) and diluted 1:1 (w/v) in water.

Field samples consisted of quadrifoliolate leaves. Each field was sampled twice during the growing season, before and after anthesis. The initial samples were taken in June or July and the later samples in August or September. Leaves with and without evident foliar mosaic and/or mottle symptoms were collected in routine surveys. Foliar symptoms were usually most evident on plants before anthesis. Both viruses induce relatively mild symptoms that are masked as plants

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mature under field conditions.

Greenhouse propagation of virus-indexed peanuts. Seed that indexed negative for PStV and PMoV by indirect ELISA were dusted with *Rhizobium* sp. (cowpea miscellany) and sown individually in 11.4-L pots. Plants were grown to maturity in greenhouses. The soil consisted of Metromix 300 medium (W. R. Grace & Co., Fogelsville, PA) supplemented with 8 g of 3-9-18 NPK granular fertilizer and 8 g of gypsum per pot. Forty-five days after planting, an additional 8 g of gypsum was added to each pot. Seedlings were individually inspected before anthesis for virus symptoms, and each was indexed for PStV and PMoV by SDS immunodiffusion serology. Initial seed used in this study were from parents that had been exposed to both PStV and PMoV. These seed were from breeding lines and the commercial cultivars Florigiant, Florunner, Southern Runner, and Sunrunner.

RESULTS

PStV was detected in one of the 2,360 plants grown in greenhouses between 1986 and 1990 from seed assessed as virus-free by indirect ELISA. Four seedlings infected with PMoV were also detected. Foliar mosaic symptoms were evident in all five of the infected plants before anthesis, and the identities of the respective viruses were confirmed in SDS immunodiffusion tests. Mosaic symptoms were not detected in any of the other seedlings, nor was either virus detected in any of them by SDS immunodiffusion. The infected plants were removed, and the remaining plants were retained for seed. The number of seed harvested from each pot varied but averaged 20–50 for most genotypes.

The incidence of PStV was high each year between 1988 and 1991 at the two sites designated in the guidelines for breeders' tests and sown with seed potentially infected with PStV. PStV was detected in 247 of 497 and 480 of 525 plants sampled at the Dozier (about 4 ha) and Green Acres (about 5.5 ha) sites, respectively, during the 4-yr period. Incidence ranged from 39% in 1989 to 69% in 1990 at the Dozier site and from 78% in 1988 to 96% in 1989 at the Green Acres site. In contrast, PStV was detected in only one of 1,431 plants sampled between 1988 and 1991 at another breeder's nursery (about 9 ha) in Jackson County about 13 km from the Dozier site. This plot (AREC site) was adjacent to peanut plantings maintained by the Florida Foundation Seed Producers (about 60 ha) and was sown with residual seed harvested before 1979 or with virus-indexed, greenhouse-grown seed. The PStV-infected plant at the AREC site was detected during the 1990 growing season. Incidence of PMoV during the 1988–1991 growing seasons was similar at all three sites (74% of 386 plants, 61% of 497 plants, and 76% of 1,431 plants sampled at the Green Acres, Dozier, and AREC sites, respectively).

Plots at various locations in north-central Florida sown with virus-indexed, greenhouse-grown seed were surveyed for PStV and PMoV. Neither virus was detected in 61 samples taken from a 0.5-ha plot (Sanford site) in Seminole County in 1987 or in 184 samples from a similar plot in Marion County in 1989. Both fields were at least 5 km away from any commercial peanut plantings or breeders' tests. PMoV, but not PStV, was detected (in 23 of 98 samples) in a 0.5-ha plot in Marion County sown in 1988 and located about 1.5 km from a 128-ha commercial peanut field.

PStV was detected in several experimental plots (less than 0.5 ha) at a site on the University of Florida campus (UF campus site) about 18 km from the Green Acres site. In 1989, PStV was detected in 24 of 54 samples from a plot sown inadvertently with seed harvested at the Green Acres site. In 1990, PStV was detected in two of 32 samples derived from seed from the U.S. Department of Agriculture ARS Southern Regional Plant Introduction Station; this virus was not detected, however, in 126 samples from an adjacent plot sown that year with virus-indexed, greenhouse-grown seed. Seed harvested from this adjacent plot were sown at the UF campus site in 1991. PStV was detected in 22 of 151 samples taken from that plot.

Sunrunner seed free of PStV were obtained. PStV was detected in five of 150 Sunrunner seeds indexed in 1985 by indirect ELISA but was not detected in any of the 70 greenhouse-grown plants sown the following year with virus-free seed. PStV was not detected in the field-grown plants at the Sanford site in 1987 or at the AREC site in 1988 (68 samples indexed) and 1989 (228 samples indexed). About 910 kg of in-shell peanuts were harvested from these plants in 1989 and provided to the Florida Foundation Seed Producers for commercial release the following year.

DISCUSSION

The results of this study demonstrate that PStV can be eliminated from infected peanut seed stocks and that fields in Florida can remain free of this virus for several growing seasons provided they are not grown close to sources of inoculum. Nevertheless, this virus probably will eventually become as widespread as PMoV in the United States. We detected one PStV-infected plant at the AREC site. In an independent study at the same location, D. E. Purcifull (*unpublished*) detected PStV in four of 288 samples in 1990. The source of PStV inoculum for these plants was not ascertained, but because winged aphid vectors are readily transported aerially (22), it is conceivable that the virus originated from the Dozier site, the nearest known source of inoculum.

Controlling PStV and PMoV would necessitate modifying the current peanut seed certification standards to resemble those currently in use for peas (*Pisum sativum* L.) (12) and lettuce (*Lactuca sativa* L.) (11), which have similar seedborne viruses. The results of this study suggest that such measures could be successful if applied to peanuts in Florida, but it is doubtful whether there are sufficient economic incentives at this time to implement them. Control of pea seedborne mosaic and lettuce mosaic viruses in their respective hosts is imperative because these viruses induce substantial crop losses and because the market value of virus-free seed is correspondingly high. Neither incentive applies to peanuts, however. Accordingly, Georgia rescinded its PStV control guidelines in 1987, and Florida did so in 1992.

The current situation for PStV and PMoV notwithstanding, international exchange of peanut germ plasm continues to pose a threat to the U.S. peanut seed chain. For example, peanut clump virus, which occurs in West Africa and India, apparently has not yet been introduced into the United States (10,23). This seedborne virus has a much wider host range than either PStV or PMoV, and the fungus *Polymyxa graminis* Ledingham, which has been implicated as a vector in Africa (24), already occurs in northern Florida, where it is a vector of soilborne wheat mosaic (13). Also, a severe, necrotic strain of PStV, not known to occur in the United States, was recently discovered in Taiwan (2). Vetten et al (25) reported several strains of PStV that are seedborne in soybean. Although the risks of introducing these and other foreign seedborne viruses can be reduced by serological testing of cotyledonary tissue in peanut germ plasm, infected seeds may still escape detection, as shown in this and other studies (1,9,27). Moreover, peanut viruses could be introduced from abroad in other species, such as the vegetatively propagated *A. pintoi* Krapovickas & Gregory (18). Likewise, a strain of PMoV was recently isolated in Florida from seed of *Voandzeia subterranea* (L.) Thouars imported from West Africa (16).

The position taken by the FAO/IBPGR (10) in its guidelines for the safe movement of legume germ plasm is that each new introduction should be grown under containment or otherwise isolated as far as possible from commercial planting stock until its health status can be assured. As shown in this and other studies for PStV and PMoV, locating fields even relatively short distances away from a source of inoculum can substantially reduce the risks of virus infection (4,8,15).

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LITERATURE CITED

1. Adams, D. B., and Kuhn, C. W. 1977. Seed transmission of peanut mottle virus in peanuts. Phytopathology 67:1126-1129.
2. Chang, C. A., Purcifull, D. E., and Zettler, F. W. 1990. Comparison of two strains of peanut stripe virus in Taiwan. Plant Dis. 74:593-596.
3. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
4. Demski, J. W. 1975. Source and spread of peanut mottle virus in soybean and peanut. Phytopathology 65:917-920.
5. Demski, J. W., and Kuhn, C. W. 1975. Resistant and susceptible reaction of soybeans to peanut mottle virus. Phytopathology 65:95-99.
6. Demski, J. W., and Lovell, G. R. 1985. Peanut stripe virus and the distribution of peanut seed. Plant Dis. 69:734-738.
7. Demski, J. W., Smith, D. H., and Kuhn, C. W. 1975. Incidence and distribution of peanut mottle virus in peanut in the United States. Peanut Sci. 2:91-93.
8. Demski, J. W., and Warwick, D. 1986. Peanut stripe virus in *Arachis hypogaea* L. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 18:77.
9. Demski, J. W., and Warwick, D. 1986. Testing peanut seeds for peanut stripe virus. Peanut Sci. 13:38-40.
10. Frison, E. A., Bos, L., Hamilton, R. I., Malthur, S. B., and Taylor, J. D., eds. 1990. FAO/IBPGR Technical Guidelines for the Safe Movement of Legume Germplasm. Food and Agricultural Organization of the United Nations and International Board for Plant Genetic Resources, Rome.
11. Grogan, R. G. 1980. Control of lettuce mosaic with virus free seed. Plant Dis. 64:446-449.
12. Khetarpal, R. K., and Maury, Y. 1987. Pea seed-borne mosaic virus: A review. Agronomie (Paris) 7:215-224.
13. Kucharek, T. A. 1990. Epidemics of diseases in agronomic crops in North Florida, 1970–1989. Proc. Soil Crop Sci. Soc. Fla. 49:187-192.
14. Kuhn, C. W. 1965. Symptomatology, host range, and effect on yield of a seed-transmitted peanut virus. Phytopathology 55:880-884.
15. Kuhn, C. W., and Demski, J. W. 1975. The relationship of peanut mottle virus to peanut production. Univ. Ga. Agric. Exp. Stn. Res. Rep. 213.
16. Li, R. H., Zettler, F. W., Elliott, M. S., Petersen, M. A., Still, P. E., Baker, C. A., and Mink, G. I. 1991. A strain of peanut mottle virus seed-borne in bambarra groundnut. Plant Dis. 75:130-133.
17. Lynch, R. E., Demski, J. W., Branch, W. D., Holbrook, C. C., and Morgan, L. W. 1988. Influence of peanut stripe virus on growth, yield, and quality of Florunner peanut. Peanut Sci. 15:47-52.
18. Morales, F. J., Castaño, M., Velasco, A. C., Arroyave, J., and Zettler, F. W. 1991. Natural infection of tropical forage legume species of *Arachis* and *Stylosanthes* by potyviruses related to peanut mottle virus. Plant Dis. 75:1090-1093.
19. Norden, A. J., Gorbet, D. W., and Knauft, D. A. 1983. Sunrunner, a runner market-type peanut. Univ. Fla. Inst. Food Agric. Sci. Circ. S-303.
20. Purcifull, D. E., Baker, C. A., Hiebert, E., Zettler, F. W., and Gorbet, D. W. 1986. Use of immunodiffusion tests in surveys of peanut plantings in Florida for presence of peanut stripe virus (PStV) and peanut mottle virus (PMoV). (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 18:77.
21. Purcifull, D. E., and Batchelor, D. L. 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Fla. Agric. Exp. Stn. Bull. (Tech.) 788.
22. Taylor, L. R. 1986. The distribution of virus disease and the migrant vector aphid. Pages 35-57 in: Plant Virus Epidemics. G. D. McLean, R. G. Garrett, and W. G. Ruesink, eds. Academic Press, Orlando.
23. Thouvenel, J.-C., and Fauquet, C. 1981. Peanut clump virus. No. 235 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, England.
24. Thouvenel, J.-C., and Fauquet, C. 1981. Further properties of peanut clump virus and studies on its natural transmission. Ann. Appl. Biol. 97:99-107.
25. Vetten, H. J., Green, S. K., and Lesemann, D.-E. 1992. Characterization of peanut stripe virus isolates from soybean in Taiwan. Phytopathol. Z. 135:107-124.
26. Wynne, J. C. 1986. Effect of peanut stripe virus on peanut breeding. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 18:78.
27. Xu, Z., Chen, K., Zhang, Z., and Chen, J. 1991. Seed transmission of peanut stripe virus in peanut. Plant Dis. 75:723-726.
28. Xu, Z., Yu, Z., Liu, J., and Barnett, O. W. 1983. A virus causing peanut mild mottle in Hubei Province, China. Plant Dis. 67:1029-1032.
29. Zettler, F. W., Elliott, M. S., Purcifull, D. E., and Mink, G. I. 1990. Production of peanut seed free of peanut mottle and peanut stripe viruses in Florida. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 22:40.