Peanut Mottle Virus Symptoms in Peanuts Inoculated with Different *Rhizobium* Strains

S. WONGKAEW, Research Assistant, and J. F. PETERSON, Associate Professor, Department of Plant Science, Macdonald Campus of McGill University, 21111 Lakeshore Road, Ste. Anne de Bellevue, Quebec H9X 1C0

ABSTRACT

Wongkaew, S., and Peterson, J. F. 1983. Peanut mottle virus symptoms in peanuts inoculated with different *Rhizobium* strains. Plant Disease 67:601-603.

Peanut plants (*Arachis hypogaea*) inoculated with a mild strain of peanut mottle virus and harboring an effective *Rhizobium* expressed typical mild symptoms. However, similarly inoculated plants harboring an ineffective *Rhizobium* showed more severe symptoms, resembling descriptions of those caused by a necrotic strain of the virus.

Peanut mottle virus (PMV) causes a disease of peanuts in most areas where the crop is grown. In Africa and Australia, there is no evidence of the occurrence of different strains of the virus (2). In the United States, however, a severe strain has been recognized (8). Paguio and Kuhn (5) have differentiated, mainly by symptom expression in peanuts, five strains that are serologically indistinguishable: M-1 and M-2 (mild mottle), N (necrotic), S (severe mosaic), and CLP (chlorotic line pattern). Field surveys have indicated that mild strains are more widespread than severe ones (5,8).

During an investigation of the effects of PMV infection on peanut nodulation, it was noted that symptom severity apparently varied with whether the plants had been inoculated with an effective (C_2H_2 reduction) or ineffective strain of *Rhizobium*. Therefore, the experiments were repeated to verify these results and to check the possibility that the virus inoculum source used might have become contaminated by other strains.

MATERIALS AND METHODS

Washed, rhizobium-free seeds of Early Prolific peanut (*Arachis hypogaea* L.) were pregerminated in autoclaved, washed sand for 4 days. Germinating seeds with hypocotyls 4–5 mm long were then harvested and inoculated with rhizobia.

Two strains of slow-growing *Rhizobium* sp., representing effective and ineffective microsymbionts, were used for peanut inoculation. The effective one, designated 8A54, was selected from four other strains (generously provided by J. C. Burton, Nitragin Co., Inc., Milwaukee, WI) because it showed the highest nitrogenase (C_2H_2) specific activity and stimulated the maximum production of leghemoglobin in the nodules (11). The ineffective strain, ATCC 10317, was purchased from the American Type Culture Collection, Rockville, MD. Both strains were isolated from single colonies cultured on yeast-extract mannitol agar (9,10). Three-day-old liquid cultures, grown in yeast-extract mannitol liquid medium without calcium carbonate, were used as seed inoculants after appropriate dilution with culture medium. The inoculant was poured onto the seeds and mixed thoroughly; seeds were air dried for 10 min before replanting. The dosage was approximately 10^7 cells per seed.

Two rhizobium-inoculated seeds were sown in each of 96 clay pots (15 cm diam.) containing an autoclaved soil mixture (sand:loam, 1:1), pH 6.2, with a total nitrogen content of about 0.02%. Plants were kept under controlled conditions of 10,000 lux light intensity for 12 hr/day, 28/25 C day/night temperature, and 75–85% relative humidity. One hundred milliliters of a quarter-strength, nitrogen-free Long Ashton solution (3) was added to each pot twice, 2 wk apart. Sterilized distilled water was used daily to irrigate the plants for the first 2 wk after planting, after which tap water was used.

A mild strain of PMV designated PMV 348 TC 2 (kindly supplied by S. A. Tolin) was used as a source of virus inoculum. The virus was reisolated from a single lesion formed on bean (*Phaseolus vulgaris* L. cv. Topcrop), after which it was transferred to pea (*Pisum sativum* L. cv. Alaska) and Early Prolific peanuts for routine culture maintenance under greenhouse conditions. Crude sap of PMV-infected pea leaves harvested 10 days after inoculation was diluted 1:5 (wt/vol) with chilled 0.05 M phosphate buffer, pH 7.5, containing 0.1% sodium sulfite and 1% Celite (4). The sap was then manually rubbed onto the lowest compound leaf of 17-day-old peanuts in 48 pots. Residue was washed off with distilled water immediately after inoculation. The remaining control plants were similarly rubbed with a blank inoculum prepared from healthy pea leaves.

RESULTS

Relative to plants with effective *Rhizobium*, virus-free peanuts inoculated with the ineffective *Rhizobium* were slightly stunted, with paler leaves and less stem branching. They produced numerous flowers, but only a few developed to pegging stage, and these pegs bore no seeds.

Symptoms typical of those caused by mild strains of PMV (5) appeared on all 96 PMV-inoculated plants with either the effective or ineffective rhizobial strain 9–10 days after virus inoculation. In plants with the effective rhizobial strain, symptoms first appeared on newly formed leaves as a severe mottling (Fig. 1). However, the mottling subsequently disappeared as leaves matured, and only pale green veinbanding was observed.

In PMV-infected plants with the ineffective rhizobial strain, the initial mottling progressed; by 20 days after virus inoculation, leaves showed interveinal chlorosis and small necrotic areas (Fig. 2), somewhat resembling the symptoms caused by a necrotic strain of the virus, PMV-N (5). Stipules also showed necrosis (Fig. 3), and the necrosis became more pronounced as the plants aged.

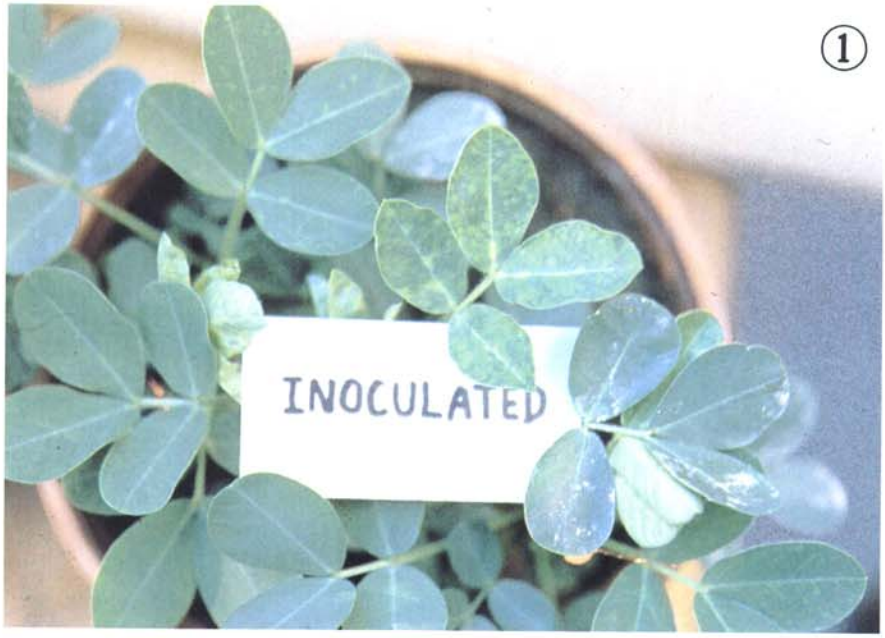
The work described was repeated in two confirmatory experiments, using 20 plants per treatment. Similar results were obtained; the more severe necrotic symptoms were consistently shown by plants harboring the ineffective rhizobial strain.

DISCUSSION

The virus used in this study had been reisolated from a single lesion, and no

Accepted for publication 23 November 1982.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.



indication of contaminating strains or heterogeneity appeared in peas. The consistency of obtaining these symptom differences when the same virus inoculum was used suggests that contamination by a necrotic strain of virus in only the plants with ineffective *Rhizobium* is unlikely. Also, stipule necrosis was not reported as a symptom caused by a Georgia PMV-N strain (5). The necrotic symptoms shown by plants with ineffective *Rhizobium* developed more slowly than would have been expected of a necrotic strain of virus. Because such symptoms were not shown by plants with effective *Rhizobium*, it is suggested that they resulted from an interaction of host, virus, and ineffective *Rhizobium*. Differences in virus symptom expression have also been reported between uninoculated field beans (*Lalab purpureus*) and those with an effective rhizobial strain infected by dolichos enation virus (6).

It is well established that various strains of *Rhizobium* are often present in field soils, and some studies (1,7) have indicated that peanuts nodulate readily with rhizobial strains in various cross-inoculation groups. Thus it is probable that plants in one field can be nodulated by various rhizobial strains. If, as our results indicate, the symptoms caused by a given virus strain can vary with the rhizobial strain present, the observation of symptoms of varying severity in a leguminous crop may reflect not only the presence of diversity of virus strains, but also a diversity of rhizobial strains. The potential specific influence of different rhizobial strains and their standardization, when possible, should be considered in studies concerning virus symptoms in leguminous crops.

LITERATURE CITED

1. Allen, O. N., and Allen, E. K. 1940. Response of the peanut plant to inoculation with rhizobia, with special reference to morphological development of the nodules. Bot. Gaz. 102:121-142.
2. Bock, K. R., and Kuhn, C. W. 1975. Peanut mottle virus. Descriptions of plant viruses. No. 141. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
3. Dart, P. J. 1977. Infection and development of leguminous nodules. Pages 367-472 in: A Treatise on Dinitrogen Fixation. R. W. F. Hardy and W. S. Silver, eds. John Wiley, New York. 675 pp.
4. Kuhn, C. W. 1965. Symptomatology, host range and effect on yield of a seed-transmitted peanut virus. Phytopathology 55:880-884.
5. Paguio, O. R., and Kuhn, C. W. 1973. Strains of

Figs. 1–3. Symptoms induced by peanut mottle virus infection on Early Prolific peanut **(1)** inoculated with the effective *Rhizobium* 8A54 (20 days after virus infection) and **(2)** inoculated with the ineffective *Rhizobium* ATCC 10317 (20 days after virus infection). **(3)** Side view of a peanut mottle virus-infected Early Prolific peanut inoculated with the ineffective *Rhizobium* ATCC 10317. Note the necrosis on stipules (20 days after virus infection).

peanut mottle virus. Phytopathology 63:976-980.
6. Rajgopalan, N., and Raju, P. N. 1972. The influence of infection by dolichos enation mosaic virus on nodulation and nitrogen fixation by field bean (*Dolichos lalab* L.). Phytopathol. Z. 73:285-309.
7. Saric, Z. 1963. Specificity of strains of the nodule bacteria *Rhizobium* sp. isolated from peanuts in some parts of Yugoslavia. J. Sci. Agric. Res. (Beograd) 16:60-67.
8. Sun, M. K. C., and Hebert, T. T. 1972. Purification and properties of a severe strain of peanut mottle virus. Phytopathology 62:832-839.
9. Vincent, J. M. 1970. The cultivation, isolation, and maintenance of Rhizobia. Pages 3-4 in: A Manual for the Practical Study of Root-Nodule Bacteria. J. M. Vincent. IBP Handbook 15. Blackwell Scientific Publications, Oxford. 164 pp.
10. Wangnai, S. 1975. Media for culture of Rhizobia. Page 97 in: Nitrogen Fixation: *Rhizobium*-legumes. Kasetsart University Press, Bangkok, Thailand. 139 pp.
11. Wongkaew, S. 1981. Effects of peanut mottle virus infection upon nodulation of peanut (*Arachis hypogaea* L.). Ph.D. thesis. McGill University, Montreal. 231 pp.