Effect of peanut mottle virus on reaction of peanut cv. Tamnut 74 to *Cercospora arachidicola*

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ABSTRACT

Two-week old seedlings of Tamnut 74 peanut were inoculated with peanut mottle virus (PMV) or left nontreated, and four weeks later were sprayed with a conidial suspension of *Cercospora arachidicola* (2 x 10^4 conidia/mL) prior to placement in a polyethylene enclosure in a growth chamber (22-30 C, 100% RH). After three weeks, number of lesions/leaflet, leaflet area (mm^2), necrotic area (mm^2)/leaflet, percent necrotic area, conidial density (conidia/mm^2) of necrotic area), and conidia/leaflet, was determined on four leaflets for each of 12 PMV-infected and 12 virus-free plants. Conidia/leaflet (one important parameter in evaluating reaction of peanut genotypes to *C. arachidicola*), lesions/leaflet, area of lesion, and leaflet area was reduced on PMV-infected plants compared to virus-free plants in each of 4 experiments. Similarly, necrotic area/leaflet were reduced in 3 of 4 experiments. Conidia/mm^2 of necrotic area was reduced in 2 of 4 experiments. PMV was not detected in conidia of *C. arachidicola* from PMV infected plants by inoculation to a PMV indicator host, serology, electron microscopy or electrophoresis.

Key Words: Peanut mottle virus interaction, *Arachis hypogaea*, reaction evaluations.

Cercospora arachidicola Hori, the early leaf spot pathogen, and peanut mottle virus (PMV) both occur worldwide and cause economically important diseases of peanut (*Arachis hypogea* L.) (6,13). All cultivars of peanut are susceptible in varying degree to *C. arachidicola* (1). No immunity to PMV has been found in cultivated peanut (8), but resistance to PMV has been found in wild peanut of the Rhizomatosae and Arachis sections (4,11). Hence, double infection by these pathogens is likely in some geographic areas.

Virus infection may render a plant more susceptible to a fungal pathogen (2,16,17,18), less susceptible to a fungal pathogen (3,7,9), or have no effect (12). Reduction of susceptibility to cucumber scab (*Cladosporium cucumerinum*) by cucumber masaic virus was noted in several varieties of cucumber (7). Similarly, virus-infected squash plants survived longer than virus-free when inoculated with *Fusarium solani* f. sp. *cucurbitae* race 1 (3). In contrast, *Helminthosporium maydis* Race O produced greater number of lesions and larger lesions on corn seedlings infected with maize dwarf mosaic virus (MDMV) than on virus-free seedlings (16). Also, sporulation of *H. maydis* Race O began sooner and was more abundant in lesions on MDMV-infected corn leaves (17). Similarly, corn seedlings infected with MDMV were more susceptible to root rots caused by *Gibberella zeae* and *H. pedicellatum* than were virus-free ones (18).

Number of conidia produced per leaflet has been proposed as an important selection criterion (6) for evaluating germplasm for resistance to early leaf spot. Research is needed to determine the relationship of susceptibility to *C. arachidicola* and infection with PMV; therefore, the purpose of this study was to determine the effect of PMV on expression of disease (number of lesions/leaflet, area of lesion, necrotic area/leaflet, leaflet area, conidial density and conidia/leaflet) on the leaf spot susceptible peanut cv. Tamnut 74 infected by *C. arachidicola*.

Materials and Methods

Three seeds of peanut cv. Tamnut 74 were planted in 16-cm diameter plastic pots containing a mixture of soil, sand and finely shredded peat (2:2:1, v/v/v) in a greenhouse maintained at 28 ± 2 C during the day and 22 ± 2 C at night. Two weeks after planting, the three seedlings in each pot were either left nontreated or mechanically inoculated with an isolate of PMV originally from a wild peanut, maintained in *Pisum sativum* L. cv. Little Marvel (13). Inoculations with PMV were made with small four-ply gauze pads saturated with inoculum [infected pea leaves ground in 0.01 M phosphate buffer, pH 8.0 with 0.001 M Dithioerythritol (2 mL/ g tissue)] and rubbed lightly on peanut leaves previously dusted with 225-μm corundum. Inoculated plants were then covered for 6-12 h with damp paper.

Four weeks later, four pots with 3 PMV-infected plants and four pots with 3 virus-free Tamnut 74 plants were inoculated with a single-spore isolate of *C. arachidicola* previously obtained from infected peanut grown in Stillwater, OK. Conidia were prepared for inoculum as described by Smith (15), except that leaves of Tamnut 74 were used to prepare the medium, and conidia were suspended (2 x 10^4 conidia/mL) in an emulsion of Amway (Amway Corp., Ada, MI 49301) all-purpose adjuvant (two drops/100 mL of H_2O). Both surfaces of leaflets were misted with a conidial suspension using a DeVilbiss No. 152 atomizer (The DeVilbiss Company, Somerset, PA 15501). Two pots with 3 PMV-infected Tamnut 74 plants and two pots with 3 virus-free Tamnut 74 plants were placed in each of two fabricated clear polyethylene moisture chambers (0.61 m x 1.22 m x 0.38 m). The chambers with plants were placed in a growth chamber maintained at 28 ± 1 C during the day and 22 ± 1 C at night with a 14-h photoperiod and irradiation of 68μE/m^2/sec. Relative humidity, recorded with a hygrothermograph, was maintained at approximately 100% by wetting burlap bags placed at the bottom of each polyethylene chamber.

Three weeks after inoculation with *C. arachidicola*, number of lesions/leaflet, leaflet area, necrotic area/leaflet, conidial density, (conidia/mm^2 of necrotic area), and conidia/leaflet were determined on 4 leaflets for each of 12 PMV-infected and 12 virus-free Tamnut 74 plants in each experiment. For conidial production, infected leaflets were incubated in petri-dish moist chambers for 96 h under continuous light (800 lux), provided by 40W Cool-White Econ-o-watt fluorescent tubes (Westinghouse Electric Corp., Dallas, TX 75247), at 25 ± 1 C. Lesions were examined under a dissecting microscope (50 X) for sporulation. Conidia were washed from surfaces of each four leaflets with 2 mL of distilled water containing Amway all-purpose adjuvant (two drops/100 mL of H_2O), and numbers of conidia in suspensions were determined with a hemacytometer. Area of leaflets was measured with a Li-Cor model 3100 area meter (Lambda Instruments Corporation, Lincoln, NE 68504), and lesions on leaflets were excised and their surface areas were determined. Sporulation of *C. arachidicola* was calculated as number of conidia produced on an infected leaflet under the incubation conditions stated above. Data were analyzed following standard proce-

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dures for analysis of variance and the least significant difference was computed.

Conidia of *C. arachidicola* collected from PMV-infected plants were examined as potential carriers of PMV. Conidia were collected from PMV-infected or virus-free Tamnut 74 plants either by rinsing leaves with a minimal amount of the phosphate buffer used in PMV inoculations or by suction. Conidia collected by rinsing were concentrated by a centrifugation at 10,000 g for 10 min at 10 C. The 5 to 6 mg of conidia from either PMV-infected or virus-free plants were each ground in 0.80 mL of phosphate buffer in a mortar and pestle and centrifuged at 10,000 g for 10 min at 10C. The supernatant was then examined for the presence of PMV by four methods. A 400 µL sample was applied with a glass spatula over 4 expanded primary leaves of *Phaseolus vulgaris* cv. Top Crop (a local necrotic lesion indicator host for PMV) previously dusted with 225-µm corundum. A 200 µL sample was used in capillary ring-interfacial tests as previously reported (13). A 50 µL sample was used for electrophoretic determination of the presence of PMV coat protein as previously reported (14). A fourth aliquot was examined by electron microscopy for the presence of PMV particles by uranyl acetate negative staining as previously reported (13).

Results and Discussion

Number of conidia of *C. arachidicola* produced per leaflet, number of lesions per leaflet, area of lesion and leaflet area were significantly different on PMV-infected and virus-free Tamnut 74 plants (Table 1). In all four experiments, fewer conidia per leaflet were produced on the PMV-infected than on virus-free Tamnut 74 plants. Similarly, the number of lesions per leaflet produced by *C. arachidicola* was significantly lower on PMV-infected than virus-free Tamnut 74 plants in all four experiments as was the leaflet area (Table 1). In three of four experiments there were significant reduction in necrotic area (mm^2)/leaflet on PMV-infected than on virus-free Tamnut 74 plants. The conidial density of *C. arachidicola* was significantly lower on PMV-infected than virus-free Tamnut 74 plants in two of four experiments (Table 1).

Table 1. The effect of peanut mottle virus (PMV) infection in peanut cultivar Tamnut 74 on its reaction to infection by *Cercospora arachidicola*

		Disease Reaction Parameter					
Expt.	Treatment	No. Lesions/ Leaflet	Area (mm^2) of Lesion	Necrotic Area (mm^2)/ Leaflet	Leaflet Area (mm^2)	Conidial density[b]	Conidia/ Leaflet
1	VF[a]	48.7	4.5	221	736	2,233	485,416
	VI	34.1	6.3	216	582	615	132,840
	$LSD_{0.01}$	14.2	1.3	N.S.	88	1,037	148,780
2	VF	15.3	4.2	64	639	165	10,708
	VI	11.0	3.5	39	548	142	5,729
	$LSD_{0.01}$	3.9	1.1	23	59	N.S.	4,770
3	VF	25.2	6.5	164	797	1,464	260,417
	VI	13.2	5.5	73	491	1,286	116,063
	$LSD_{0.05}$	5.3	1.4	52	51	N.S.	35,600
4	VF	39.5	2.5	100	641	1,892	197,000
	VI	28.1	1.8	51	477	995	55,000
	$LSD_{0.05}$	9.5	0.5	26	118	578	8,362

[a] VF = Virus-free
VI = PMV infected

[b] Conidia/mm^2 of necrotic area

It has been shown that urediospores of *Puccinia graminis-tritici* produced on brome mosaic virus (BMV)-infected wheat transmit BMV (19), but results of all our tests for the presence of PMV or PMV coat protein in conidia of *C. arachidicola* obtained from PMV-infected and virus-free Tamnut 74 plants were negative. No necrotic local lesions were produced on Top Crop beans nor were particles seen in specimens prepared for electron microscopy. There was no difference in the serological reaction of material collected from conidia of *C. arachidicola* from PMV-infected and virus-free Tamnut 74. Nor was there any difference in the electrophoretic pattern of the two conidial samples. Conidia of *C. arachidicola* do not seem to be a carrier of PMV as urediospores of *P. graminis-tritici* are of BMV.

Although the antagonistic interaction that may occur between a virus and a fungus has been documented (5), the influence on evaluating disease reaction has not been documented. The number of conidia of *C. arachidicola* produced per leaflet is an important parameter in evaluating peanut germplasm reaction to *C. arachidicola* (6). Because PMV infection of peanut germplasm may alter reaction of *C. arachicola,* especially the number of conidia of *C. arachidicola* produced per infected leaflet, it is important that the presence of the virus be considered in areas where PMV is endemic when germplasm are being evaluated for leaf spot resistance. A hazardous situation could arise where germplasm is selected for resistance to leaf spot in the presence of PMV, and new selections are distributed for evaluation to *C. arachidicola* resistance in PMV-free areas.

Many evaluations for resistance to *C. arachidicola* have been made on peanut germplasm under greenhouse and field conditions (1,6,10) without regard to the presence or absence of PMV. The data presented in this paper clearly indicate that infection of Tamnut 74 with PMV altered its reaction to *C. arachidicola.* High incidence of PMV-infected peanut in field tests could cause errors in selecting promising lines with resistance to *C. arachidicola.* Therefore, it is recommended to evaluate PMV-free and PMV-infected peanut germplasm for reaction to *C. arachidicola* to obtain information on the effects of PMV on the outcome of resistant evaluations. This may be of particular importance when utilizing the conidia of *C. arachidicola* produced per leaflet as the principal selection criterion.

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