Influence of Host Genotype on Uredospore Production and Germinability in *Puccinia arachidis*

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ABSTRACT

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Uredospore production by *Puccinia arachidis* was studied on inoculated detached leaves of one susceptible and five resistant genotypes of peanut (*Arachis hypogaea*). Significantly fewer uredospores were produced per unit leaf area and per unit pustule area on the resistant than on the susceptible genotypes. Germinability tests carried out on uredospores from three susceptible, one moderately resistant, and 15 resistant genotypes showed that uredospores from resistant genotypes had significantly lower germinability than those from the moderately resistant and the susceptible genotypes. The significance of uredospore production and germinability in relation to resistance is discussed.

Additional key words: components of disease resistance, groundnut, slow rusting.

Rust disease caused in peanut (*Arachis hypogaea* L.) by *Puccinia arachidis* Speg. has recently attracted much attention because of its rapid spread to almost all major peanut growing countries (1,5,28). Efforts to find genetic resistance have been successful and several sources of resistance have been identified (1–4,5–9,23–25,27). Cook (3) suggested that rust resistance in some peanut cultivars was mainly physiologic, resulting in necrotic lesions or poorly sporulating uredosori. Subrahmanyam et al (27) and Nevill (15), working with genotypes of both the cultivated peanut and wild *Arachis* species, showed that uredospores germinated on leaf surfaces and the fungus entered through stomata irrespective of whether a genotype was immune, resistant, or susceptible to rust. However, in immune species of *Arachis* the fungus died shortly after entering the substomatal cavity. Differences in resistance were associated with differences in rate and extent of mycelial development within the cavity and within leaf tissues. Subrahmanyam et al (26), investigating rust on 30 peanut genotypes, found that incubation period, infection frequency, pustule diameter, and rupturing of pustules were important components of resistance, and were significantly correlated with one another and with mean field rust scores recorded over several seasons. No immunity has been found in the cultivated peanut and the resistance described is of the slow rusting type found in cereals. Although data are available on pustule development, little is known of uredospore production and viability in resistant and susceptible peanut genotypes.

In three preliminary experiments, in vitro germinability of uredospores from a rust-susceptible peanut genotype (TMV 2) was significantly higher than germinability of those from a rust-resistant genotype (NC Ac 17090). In the present investigation, peanut genotypes representing a wide range of rust resistance were used to quantify uredospore production and germinability following monocyclic infection.

MATERIALS AND METHODS

Uredospore production. Seeds of five peanut genotypes resistant to rust (EC 76446 (292), NC Ac 17090, PI 405132, PI 407454, and PI 393643) and one susceptible to rust (TMV 2) were sown in a mixture of sandy red soil (alfisol) and farmyard manure (4:1, v/v) in 15-cm-diameter plastic pots in the glasshouse. Four seeds were sown in each pot, and the seedlings were later thinned to two per pot. Three pots were used for each genotype. Air temperature in the glasshouse ranged from 25–30 C during the plant growth period. At 50 days after sowing, the middle leaf on the main stem of each plant was excised through the pulvinus and its area was measured by tracing its outline and measuring the area with a leaf area meter (Hayashi Denkoh Co. Ltd., Tokyo, Japan). The detached leaves from different genotypes were arranged with their petioles buried in sterilized river sand in plastic trays (56 × 25 × 5 cm) in randomized blocks with three replications of each genotype, one replicate treatment comprising two leaves. The sand in the trays was moistened with a nutrient medium (10), the trays were covered with thin plastic sheets, and then incubated before inoculation for 24 hr in a Percival plant growth chamber (Percival Refrigeration and Mfg. Co., Boone, IA 50036 at 25 C with 12-hr photoperiod (4,000 lux).

Uredospore inoculum was produced on rooted detached leaves of the susceptible peanut genotype TMV 2 in a growth chamber. Uredospores were harvested with a Cyclone spore collector (ERI Instrument Shop, Iowa State University, Ames) and suspended in sterile distilled water containing Tween-80 (10 drops/1,000 ml). The suspension was adjusted to $\sim 5 \times 10^4$ spores per milliliter. The leaves were inoculated with the uredospore suspension sprayed on with a plastic atomizer. Leaves were then incubated in the dark for 12 hr at 25 C and subsequently with a 12-hr photoperiod (4,000 lux) until the end of the experiment.

At 13 days after inoculation, and on every second day thereafter, uredospores were collected with a Cyclone collector from each set of leaves at between 0830 and 0930 hours into 5-ml glass vials. Following spore collection, the numbers of ruptured and unruptured pustules were recorded for each leaf. Known volumes of water containing Tween-80 (10 drops/100 ml) were added to vials which were then shaken for 1 min on a Vortex-Genie mixer. Using a hemacytometer, six separate drops of suspension were examined under the microscope to determine the numbers of spores present. At 31 days after inoculation, the diameters of five randomly selected ruptured pustules from each leaf were measured with an ocular micrometer and their areas were estimated assuming that the pustules were circular.

From these data and for each treatment the total numbers of pustules and ruptured pustules per square centimeter of leaf area were calculated for each 2-day period. The numbers of uredospores produced for each 2-day period per unit leaf area and per ruptured

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pustule also were calculated. The quantity of uredospores produced during the experimental period (13–35 days after inoculation) was also calculated and expressed as number of uredospores per square millimeter of pustule surface.

Uredospore germination. Nineteen peanut genotypes providing a wide range of resistance to rust were selected for this study on the basis of their field reaction to *P. arachidis*. The methods of preparing the experimental material, inoculation, incubation, and design of the experiment are similar to those described above for the uredospore production trial except that the leaves were collected from 45-day-old plants and there were three leaves per replication.

Starting 8 days after inoculation, leaves were examined daily under the stereomicroscope (×20) and collection of uredospores commenced for each genotype when the pustules first ruptured. At 0900 hours uredospores were collected from the ruptured pustules and discarded. Forty-eight hours later uredospores were collected from the same pustules and used for germination tests on the same day. The same procedure was used three more times at 5-day intervals.

For germination tests, uredospores were brushed over the surface of 2% water agar in 9-cm-diameter petri dishes and five dishes were prepared for each replicate treatment. The dishes were incubated in the dark for ~3 hr at 25 C and then exposed to formaldehyde vapor to prevent further germination and germ tube development. A stereomicroscope (×70) was used to examine 100 single uredospores per petri dish for germination. Clumps of spores were ignored although there was no evidence of autoinhibition.

RESULTS

In the rust-susceptible TMV 2, the pustules appeared early, were numerous, and all had ruptured by 19 days after inoculation (Fig. 1). On the resistant genotypes the pustules appeared later, were fewer in number, and only a few of them had ruptured within the observation period (Fig. 1). Uredospore production started earlier and was significantly greater in the rust-susceptible TMV 2 than in the resistant genotypes (Fig. 2). In PI 405132, uredospore production started earlier than in the other resistant genotypes; however, the numbers of uredospores produced were significantly lower than in the susceptible TMV 2 (Fig. 2).

Pustules in TMV 2 were larger and produced more uredospores per unit pustule area than did those on the resistant genotypes (Table 1), but there was no apparent difference in uredospore morphology (Fig. 3). There was a positive correlation between pustule diameter and spores per pustule ($r = 0.908$) and spores per square millimeter of pustule surface ($r = 0.874$). Spores produced per pustule and spores produced per square millimeter of pustule area were also positively correlated ($r = 0.984$).

The results of germination tests on uredospores collected from 19 peanut genotypes, listed in order of decreasing resistance to rust as evidenced by their mean field rust scores, are presented in Table 2. In general, uredospores from the resistant genotypes (mean field rust scores between 2.2 and 3.0) had significantly lower percentage germination than those from moderately resistant (mean rust score 4.2) or susceptible (mean rust score 9.0) genotypes. Uredospores from the resistant genotypes PI 405132, PI 259747, PI 341879, and PI 393646 had germination percentages almost similar to those of the moderately resistant and susceptible genotypes at the first sampling, but germination percentage was lower in later samples. There was no definite pattern in germination percentage over the sampling periods. Some genotypes showed a slight increase in germination percentages with time, while others showed a decrease.

TABLE 1. Uredospore production by *Puccinia arachidis* on six peanut genotypes

Genotype	Mean pustule area (mm^2)[x]	Spores/ pustule[y]	Spores/mm^2 pustule area
TMV 2	1.2 a[z]	1,015 a	855 a
EC 76446 (292)	0.4 c	22 b	61 b
NC Ac 17090	0.4 c	50 b	121 b
PI 405132	0.7 b	84 b	127 b
PI 407454	0.3 c	47 b	139 b
PI 393643	0.4 c	48 b	121 b

[x] Estimated at 31 days after inoculation.
[y] Mean uredospore production during 13 to 35 days after inoculation.
[z] Values within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.01$.

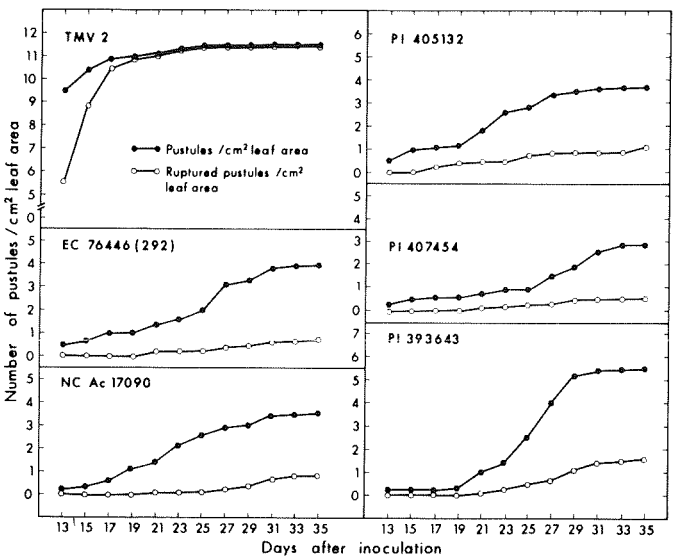

Fig. 1. Total number of pustules and of ruptured pustules of *Puccinia arachidis* per unit leaf area for six peanut genotypes.

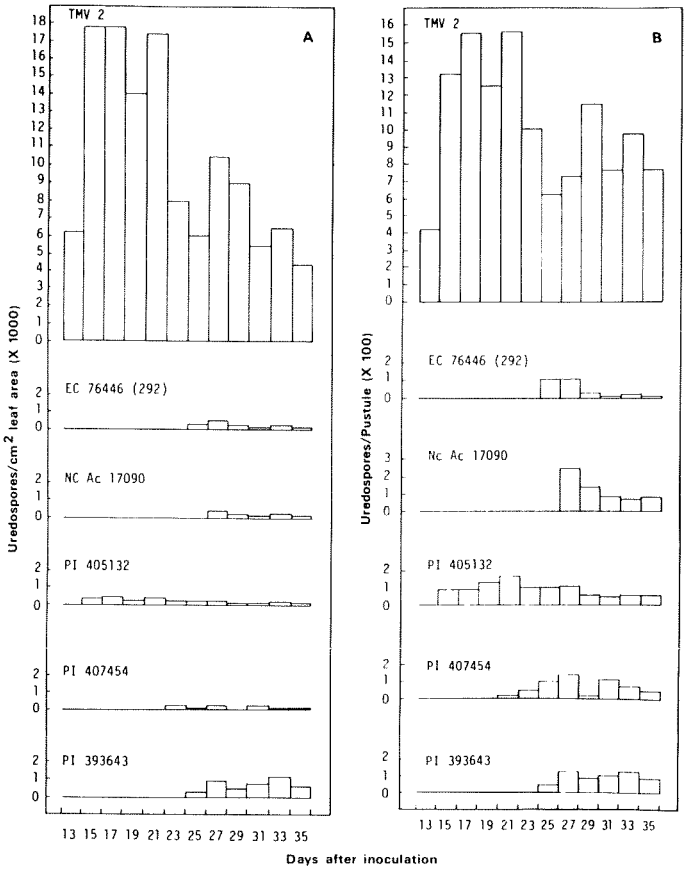

Fig. 2. Production of uredospores of *Puccinia arachidis* **A**, per unit leaf area and **B**, per pustule, for six peanut genotypes.

The genotype PI 315608 had the lowest mean uredospore germination percentage. There was a highly significant ($P = 0.01$) positive correlation between field rust scores and percent germination of uredospores sampled at first rupturing of pustules ($r = 0.6095$), 5 days later ($r = 0.7350$), 10 days later ($r = 0.8082$), and 15 days later ($r = 0.7770$) and also with mean uredospore germination percentage ($r = 0.8186$).

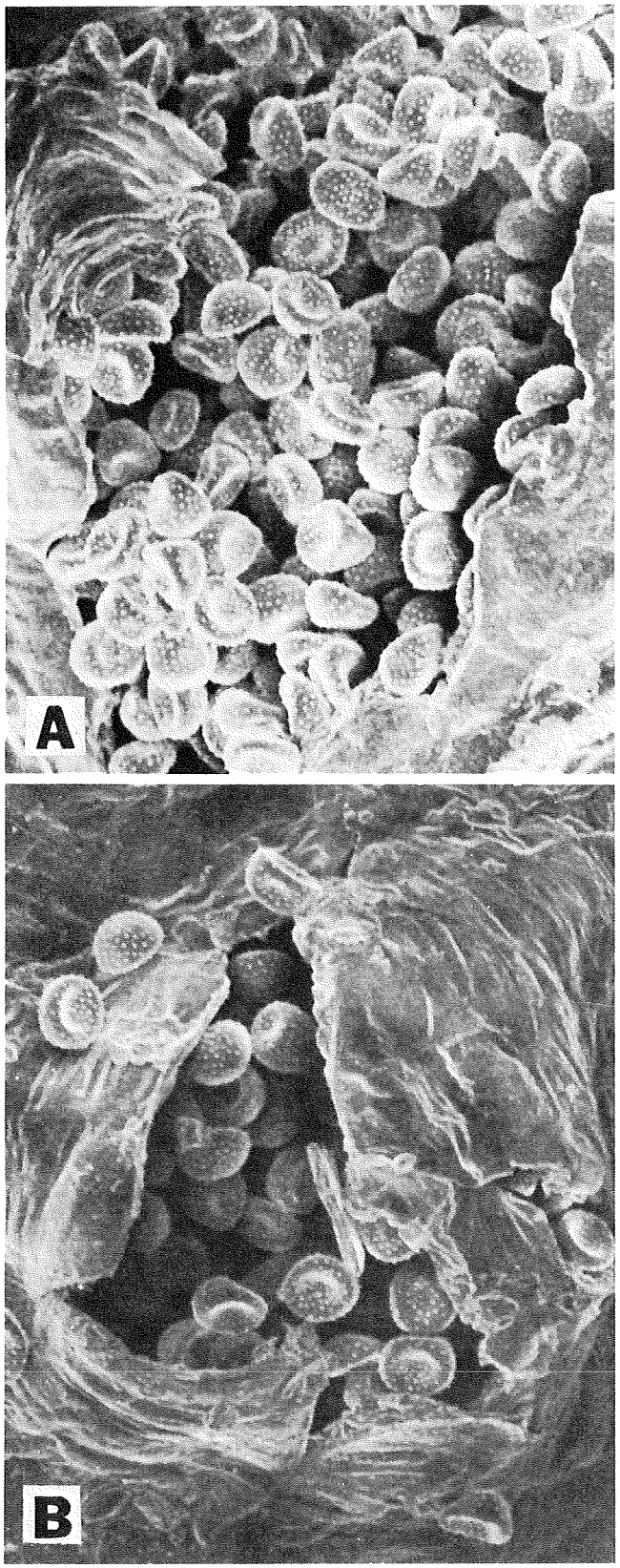

Fig. 3. Scanning electron photomicrographs (×400) of pustules of *Puccinia arachidis* on **A,** the susceptible genotype TMV 2 and **B,** on the resistant genotype NC Ac 17090.

DISCUSSION

Prolonged incubation and/or latent period, low infection frequency, reduced lesion size, reduced sporulation, and short infectious period are important components of disease resistance reducing the rate of epidemic buildup (18). This kind of reaction to disease is characteristic of "horizontal resistance" (29,30) and is similar to the "slow rusting" or "partial resistance" reported by several investigators of cereal rusts (11–14,16–22). Mackenzie (12) described slow rusting as a reduced rate of epidemic acceleration. Resistant genotypes subjected to the same pathogen populations under the same environmental conditions should have lower apparent infection rates (r) than susceptible genotypes. Resistance to rust in peanut genotypes has been associated with prolonged incubation period, reduced infection frequency, reduced lesion size, and inhibition of pustule rupturing (26). The present investigation has confirmed the importance of these resistance factors and provided further information on the production of uredospores in resistant and susceptible genotypes.

The production of uredospores per square centimeter of leaf area is significantly lower in resistant than in susceptible genotypes and dependent upon rupture of pustules to release uredospores, on the number and size of the ruptured pustules, and on the productivity of pustules. Estimation of uredospore production per ruptured pustule and per square millimeter of pustule area also indicated that uredospore production is significantly reduced in resistant genotypes. The latter figure gives a useful measure of uredospore productivity for comparison of susceptible and resistant genotypes. There were no apparent differences in morphology of uredospores produced on susceptible and resistant genotypes, but the uredospores produced on resistant genotypes showed significantly lower percentages of germination than those from moderately resistant and susceptible genotypes. In the present investigation,

TABLE 2. Germinability of uredospores of *Puccinia arachidis* collected from 19 peanut genotypes

Genotype	Field rust score[a]	Percentage germination of uredospores sampled at: First rupturing of pustules	5 days later	10 days later	15 days later	Mean uredospore germination (%)[b]
NC Ac 17090	2.2	29.8	32.5	40.3	46.3	37.2
PI 414332	2.4	34.6	37.4	38.0	38.7	37.2
PI 341879	2.4	54.5	54.9	45.7	41.3	49.1
PI 393646	2.4	54.5	49.4	50.6	45.0	49.9
PI 405132	2.4	59.5	53.4	37.8	41.5	48.1
PI 390593	2.6	33.9	41.2	60.2	32.7	42.0
PI 414331	2.8	30.9	21.6	47.7	45.3	36.4
PI 407454	2.8	31.9	38.5	50.4	49.6	42.6
EC 76446 (292)	2.8	45.4	64.0	44.3	38.7	48.1
PI 315608	3.0	23.0	16.2	19.3	33.3	23.0
PI 393527-B	3.0	30.1	33.0	51.7	56.4	42.8
PI 314817	3.0	35.8	45.7	51.6	39.4	43.1
PI 393643	3.0	47.1	38.3	44.7	43.3	43.3
PI 381622	3.0	50.2	35.7	38.1	33.1	39.3
PI 259747	3.0	57.9	50.7	44.5	49.4	50.6
NC Ac 17127	4.2	59.3	71.9	82.4	76.9	72.6
Robut 33-1	9.0	69.2	73.9	82.5	67.3	73.2
TMV 2	9.0	59.3	77.9	85.1	78.0	75.1
J 11	9.0	65.7	83.8	82.7	68.7	75.2
WDLSD[c] ($P = 0.05$)	0.48	16.50	12.89	14.10	18.15	11.49

[a] Mean rust scores recorded at ICRISAT Center over the years 1979–1982 using a 9-point disease scale on which 1 = no disease, and 9 = more than 50% of foliage destroyed by the disease.
[b] Analyzed after eliminating the sampling time differences.
[c] Waller and Duncan's Bayesian least significant differences.

uredospores from a susceptible genotype were used to inoculate the test material. We do not know if the same results would have been obtained if uredospores from a resistant genotype had been used for the inoculation.

Reduced uredospore production on resistant genotypes of several cereal crops has been reported (18); however, the quality of uredospores produced on resistant and susceptible genotypes has seldom been reported. The reduced germinability of uredospores produced on resistant peanut genotypes observed in the present investigation may have a significant role in reducing the epidemic buildup. For instance, uredospores formed on a genotype like NC Ac 17090 would give rise to only half as many infections as uredospores produced on a genotype like TMV 2. However, the importance of specific components of disease resistance are difficult to assess precisely in terms of overall field resistance as they interact with one another and are cumulative over the course of the epidemic (20,21).

At ICRISAT Center (18°N; 78°E) climatic conditions in the rainy season when the main peanut crop is grown are very favorable for development of rust disease. All commercial cultivars are highly susceptible and epidemics occur each year. In rust-resistance screening trials, high inoculum potential is assured by growing "infector rows" of rust-susceptible genotypes together with the test entries (25). However, this is likely to mask the contribution to host resistance to rust conferred by the reduced uredospore production and germinability found in resistant genotypes. To obtain a true estimate of rust resistance it would be necessary to measure epidemic buildup on resistant and susceptible genotypes grown in isolation.

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