Histological Responses of Peanut Germplasm Resistant and Susceptible to *Cylindrocladium crotalariae* in Relationship to Inoculum Density

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ABSTRACT

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Taproots of nine peanut entries including susceptible (Florigiant and NC 2) and resistant (NC 3033, NC 3033 × NC 2, NC 3033 × Florigiant, Florigiant × NC 3033, Argentine, Argentine × Florigiant, and Florigiant × Argentine) germplasm inoculated in greenhouse tests with one, two or four microsclerotia (ms) of *Cylindrocladium crotalariae* showed no qualitative histological differences in the basic formation of original or additional taproot periderms or in the suberization of periderm. Florigiant sustained more breachments of its original periderm and protected fewer of its periderm breachments with additional periderm than did the other entries. Additional phellogens were initiated in all parts of the peanut taproot where viable parenchymatic cells existed. In infested field plots with an inoculum density <2.9 ms/g of soil, the nine entries gave responses similar to those obtained in greenhouse tests. Primary branch roots in all entries showed potential for limited secondary growth including additional periderm formation when infected by *C. crotalariae*. The sites of emergence of branch roots from the taproot cortex were favorable entry points for the fungus. All entries initiated phellogens in infected nodules. The Poisson distribution was used to describe the theoretical probability of the occurrence of microsclerotia on the taproot surface, assuming mean densities of ms commonly occurring in North Carolina peanut fields. Resistant NC 3033 remained highly resistant with four ms per square millimeter of taproot, whereas susceptible Florigiant was moderately to severely diseased with one ms per square millimeter of taproot.

Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea* L.) was reported first in 1965 in Georgia (2), and it presently threatens peanut production throughout the southeastern United States (5,13). The ineffectiveness of agricultural chemicals in disease control (1,14) stimulated a breeding program to identify and evaluate CBR-resistant genotypes (9,18). Although all peanut cultivars are susceptible, the Spanish cultivars show more resistance than either the Valencia or Virginia cultivars (18). Also, considerable genotype resistance differences exist within each cultivar group (18). Argentine (a small-seeded cultivar) and NC 3033 (a small-seeded Virginia-type) possess moderate to high resistance and were selected as donor parents in the breeding program for the Virginia-North Carolina region (6,8,18). Resistance in peanut to CBR was inoculum-density dependent, with Argentine and NC 3033 surviving at microsclerotia (ms) densities as high as 100 ms per gram of soil (9). Florigiant, a commercial cultivar planted to 75% of the peanut acreage in North Carolina, had severe symptoms at densities of 0.5 ms per gram of soil (9).

Johnston and Beute (8), in their histological studies of susceptible Florigiant hypocotyls, suggested a possible correlation between a plant's ability to quickly "wall off" lesions by additional periderm formation and the capacity to survive infection that was observed in an occasional Florigiant plant. Florigiant fibrous roots were reported to lack the ability to initiate phellogen (14) and were rapidly invaded and destroyed by the fungus. The ability to rapidly form additional periderm was suggested to be a beneficial trait for consideration in breeding for disease resistance.

In our present histological studies, an attempt was made to utilize more realistic inoculation techniques and statistical analyses in an examination of the possible relationship of additional periderm formation to CBR resistance, the role of fibrous roots in disease development, and the relationship of inoculum density to the host's histological defense response.

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MATERIALS AND METHODS

Inoculum production. The isolate (C-64) of *Cylindrocladium crotalariae* (Loos) Bell and Sobers that we used was obtained from an infected peanut plant in eastern North Carolina (12). The isolate was maintained on acidified potato-dextrose agar (PDA) and was periodically inoculated into and isolated from plants of Florigiant to maintain a high level of virulence. To obtain microsclerotia, the fungus was grown on PDA in darkness for 3–4 wk, after which the cultures were comminuted in a Waring Blendor for 2 min and passed through nested sieves with 246 and 240 μm openings (60 and 100 mesh, respectively). Microsclerotia in the 240-μm sieve were separated from mycelial fragments by passing a forceful stream of water through the sieve for 1 min. Microsclerotia were then rinsed into a small beaker containing a few milliliters of water.

Plant material and culture. Hadley (6) studied the mechanism of inheritance of CBR resistance in peanut and determined the following mean disease rating (6) for his parental lines on a scale of 0 to 5: Argentine, 2.21; NC 3033, 2.13; NC 2, 3.50; and Florigiant, 4.28. For his F_2 progeny, the ratings were Argentine × Florigiant, 3.01; Florigiant × Argentine, 3.09; NC 3033 × NC 2, 2.46; NC 3033 × Florigiant, 2.88; and Florigiant × NC 3033, 3.23. Cultivars Florigiant and NC 2 and seed from single-plant selections of Hadley's (6) resistant material made in F_5 or later generations were used in these histological tests. Seeds were dusted lightly with a 30% botran-30% captan protectant (Upjohn Company, Kalamazoo, MI 49001) and germinated in moist vermiculite. Seedlings were removed approximately 5 days after germination, dipped in an inoculum suspension of *Rhizobium* ("Nitragin"; Nitragin Co., Inc., Milwaukee, WI 53209), and transplanted two per box into hinged polyethylene boxes (25 × 16.5 × 4 cm, Tri-State Plastics, Henderson, KY 42420) containing washed vermiculite previously amended with one teaspoon of osmocote (19-6-12, N-P-K; Sierra Chemical Co., 1001 Yosemite Dr., Milpitas, CA 95304) per 1,512

cm^3. Two 2.5-cm-diameter holes were drilled at the upper and lower ends for watering and plant emergence, respectively. Boxes were wrapped in aluminum foil and tilted in shallow trays for subirrigation. Plants were allowed to grow for 10 days in the boxes before inoculation. Temperatures within the boxes averaged 25 C.

Selection of inoculum densities. An attempt was made to evaluate tissue responses to inoculum densities similar to those found in naturally infested fields. Consideration was given to a range of population densities of 0.5 to 100 ms/g of soil, all of which were reported at some time in field samplings (9). Assuming a random distribution of separate propagules in soil, the Poisson model was selected to describe the theoretical distribution of propagules along the taproot surface. Given the formula $P(x=k)=(e^{-\lambda}\lambda^x)/x!$, in which λ = the mean number of ms occupying a cubic millimeter of soil for a specific number of ms per gram of soil, x = the frequency class (0,1,2,3,4, . . . ms), and $e = 2.718$, then the probability of k number of ms in any one cubic millimeter of soil (bulk density = 1.25 grams per cubic centimeter) at some point along the taproot is $(e^{-\lambda}\lambda^x)/x!$. Calculated from the formula for the surface area of a cone without the base, S.A. $= \pi r \sqrt{(x^2+h^2)}$, the surface area (S.A.) of a conical segment of taproot of radius 4 mm and length 102 mm is 1,277 mm^2. At a population density of 0.5 ms/g soil, which may cause severe disease in plants of cultivar Florigiant, calculations $([e^{-\lambda}\lambda^1]/1 \times 1{,}277)$ indicate that 0.7976 mm^3 of soil in contact with the root surface are occupied with one ms. Only 0.000249 mm^3 of soil in contact with the root surface contains two ms at 0.5 ms/g soil. NC 3033 remains resistant up to densities of 50 ms/g soil (9). Calculations indicate that at 50 ms/g soil, 74.96 of the 1,277 mm^3 soil in contact with the root surface are occupied with one ms. However, 2.34 mm^3 of soil in contact with the root surface are occupied with two ms at 50 ms/g of soil. Only 0.00078 mm^3 of soil in contact with the root surface contains four ms at 50 ms/g of soil. The current opinion is that population levels of microsclerotia in peanut fields are stabilizing at lower levels than previously anticipated, with numbers of ms per gram of soil being more frequently below 20 than above (M. K. Beute, *personal communication*). It would be extremely rare for k to be greater than four ms together at one point on the taproot at population levels commonly encountered. This model resulted in the selection of the inoculum densities of one, two, or four ms per square millimeter of taproot.

Inoculation technique. Microsclerotia were harvested as previously described (11) and suspended in water. The suspension was stirred and drops were pipetted onto filter paper as needed. Individual ms of uniform size were picked up from the filter paper with a fine needle and placed on roots. The small propagules adhered well to the moist root surfaces. Inoculation sites were marked by placing colored pins in the vermiculite adjacent to the roots.

Histological techniques. Taproot and fibrous root samples were killed and fixed in formalin-2-propanol-propionic acid (7), infiltrated and imbedded with Paraplast (Brunswick Company, St. Louis, MO 63130), sectioned on a rotary microtome at 12 μm and stained (3) with Triarch's quadruple stain (George H. Conant, Triarch, Inc., Ripon, WI 54971). Histochemical tests included Sudan IV for suberin and phloroglucinol and orcinol for wound gum (7). In all histological tests, 1-cm-long taproot tissue samples were taken at the point of inoculation and sectioned completely. Ribbons of sections were taken uniformly through the mounting blocks; approximately equal numbers of sections were obtained for each treatment.

Periderm formation in susceptible and resistant peanut germplasm. To determine the importance of additional periderm formation in plants of susceptible cultivar Florigiant compared to that in plants of NC 3033, the most resistant available germplasm, plants were inoculated with either one, two, or four ms by placing inoculum on surface of the taproot 3 cm below the collar. Each treatment was replicated 16 times in a completely randomized design and the experiment was repeated once. Uninoculated plants of each cultivar were included. Cultivars were observed after 5 wk for necrotic fibrous roots and collar necrosis. A 1-cm-long segment of taproot at the point of inoculation was taken for histological processing.

Host defense responses of nine peanut germplasms. A completely randomized design was used in each test. Lines were separated into two tests of 10 plants per inoculum density per plant line. The extremes of susceptibility and resistance (cultivars Florigiant and NC 3033, respectively) were included in each test as references. Plants were inoculated with one, two, or four ms as previously described, or (as controls) were not inoculated. Test I included Florigiant, NC 3033, NC 3033 × Florigiant, Florigiant × NC 3033, NC 2, and NC 3033 × NC 2. Test II consisted of Florigiant, NC 3033, Argentine, Florigiant × Argentine, and Argentine × Florigiant. Taproot samples were taken for histological evaluation and for isolation of the fungus after a 5-wk incubation period.

Field studies. To evaluate greenhouse findings under field conditions, the nine lines were placed in a randomized complete block design with two replications in a Cylindrocladium-infested field in Martin County, North Carolina. Plots were sampled in late May for initial ms population levels by using the elutriation method of Phipps (10). Taproot segments were taken randomly (six per plot) on 27 October and fixed for histological observation.

Role of fibrous roots in disease development. Seedlings of Florigiant and NC 3033 were transplanted to plastic boxes and grown for 10 days to allow development of a fibrous root system. Several primary or secondary branch roots on each plant were inoculated with two ms placed on the root 3 cm from the point of emergence from the taproot. Each treatment was replicated four times in a completely randomized design, and uninoculated plants were included. Fibrous roots were sampled 3 wk after inoculation and fixed for histological observation. This experiment was repeated once.

Calculations and data analysis. Histological parameters measured were number of breachments of the original periderm, number of additional periderms formed, number of breachments of the additional periderms, walling-off of additional periderm breachments, number of occluded vessel elements, and number of periderm breachments occurring at the bases of emerging fibrous roots. Periderms exhibiting obvious necrotic cellular changes as well as those actually invaded by hyphae were scored as breached periderms. Certain calculations were performed upon the data to give better indications of differences in host response:

$$\text{Overall protection of lesions} = \frac{\text{(Number of protected breachments of original periderm)}}{\text{(Number of breachments of the original periderm).}} \times 100$$

$$\text{Contribution of branch roots} = \frac{\text{(Number of breachments of original periderm at branch root bases)}}{\text{(Number of breachments of the original periderm).}} \times 100$$

Analysis of variance was performed to determine significance of cultivar main effects. When significant F-test results were obtained, a Duncan's multiple range test was also performed. The information derived from the branch root pathogenesis test was strictly qualitative; therefore, a statistical analysis was not performed.

RESULTS

General taproot pathogenesis. In the peanut taproot, phellogen is typically initiated in the pericycle about 10 days after germination (19), resulting in the production of a few layers of suberized phellem (Sudan IV-positive). All lines observed in these tests produced a suberized original periderm. Concomitantly, taproot cortical cells had begun to collapse and slough, leaving the periderm as the protective dermal tissue. Branch roots were initiated in the pericycle at 4–5 days and appear in four ranks opposite the primary

xylem arms. These roots grew through the taproot, creating crevices and gaps in both cortex and later periderm (19). Ms of *C. crotalariae* germinated and produced numerous slender hyphae, which grew over the taproot surface and penetrated intercellularly. Infection cushions were rarely observed. Sites of penetration observed included bases of emerging branch roots, cortex, phellem or phellogen, and nodules.

Johnson and Beute (8) reported a strong tissue reaction to some toxic substance(s) in advance of fungal hyphae. In the present studies, sensitive cells became prenecrotic (enlarged nuclei and

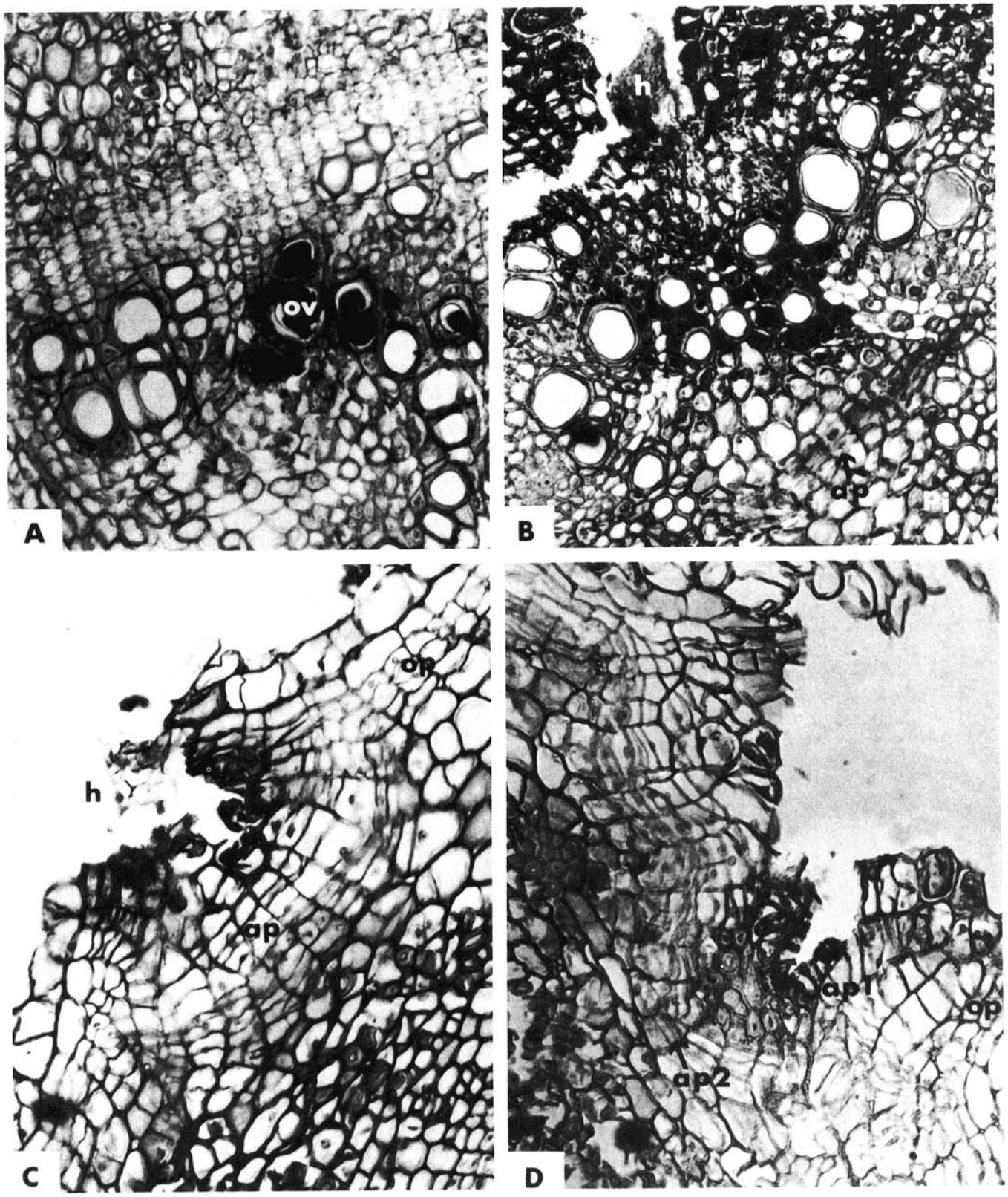

Fig. 1. Taproot pathogenesis and additional periderm formation in taproot tissues of *Arachis hypogaea* infected with *Cylindrocladium crotalariae*: **A**, necrotic xylem parenchyma and occluded vessels (ov) surrounded by healthy tissue in Florigiant taproot (×100); **B**, formation of additional periderm in the pith of NC 3033 taproot (×100); **C**, successful walling-off of lesion by additional periderm in NC 3033 × NC 2 taproot (×100); **D**, successive formation of additional periderms in NC 3033 taproot (×100). Pith (pi), original periderm (op), additional periderm (ap), first, second, and third additional periderms (Ap1, Ap2), fungal hyphae (h).

increased staining intensity) and eventually necrotic as evidenced by abnormal staining and cytology. Invasion of prenecrotic or necrotic tissue appeared to be the habit of this fungus. Vascular parenchyma cells were particularly sensitive to the toxic substance(s). They became necrotic and produced amorphous (phloroglucinol- and orcinol-positive) materials that accumulated in and occluded adjacent vessel elements. It is suspected that the toxic substance(s) may move for some distance in the xylem as evidenced by groups of necrotic vascular parenchyma cells and occluded vessels occurring at least 0.5 cm above and below the lesion site (Fig. 1A). At any single point along a Florigiant taproot as many as one-fourth of the total conducting vessels were observed to be completely occluded in some cases.

In all lines tested, as the phellogen cells of the original periderm became prenecrotic, cell divisions were observed in parenchyma cells below the enlarging lesion. Cells involved in resumption of meristematic activity were phelloderm cells, phloem and xylem parenchyma, pith parenchyma, and nodular cortical cells. Phellogen was observed in phloem and xylem tissues and nodules of all lines. Phellogen was observed in the pith of all lines except Florigiant. Initiation of phellogen in the pith occurred when pith parenchyma cells adjacent to necrotic vascular parenchyma were stimulated to dedifferentiate and become meristematic (Fig. 1B). Histochemical tests with Sudan IV stain indicated that all lines produced suberized phellem as components of additional periderms. In some cases, additional periderm appeared to effectively restrain the fungus (Fig. 1C), while in others, successive breachments of additional periderms resulted in a series of unsuccessful barriers below the expanding lesion (Fig. 1D). Lesions expanded inward as well as laterally, with phellogen constantly being initiated on the lesion borders. Phellem layers were few or absent altogether with rapid expansion of necrosis. This was especially true in Florigiant taproots, suggesting that the total defense system of the plant simply could not keep pace with the fungus. A nonexpanding or slowly expanding lesion was usually indicated by the presence of several layers of well-formed phellem cells. Sections were observed in which whole quadrants of the taproot had been sloughed as a result of expanding necrosis and walling-off of the lesion (Fig. 2A).

Role of branch roots in disease development. At harvest, taproots occasionally showed no observable necrosis except at the bases of emerged branch roots. In cases of severe lesion development where the fungus had invaded the stele, the entry point could be traced to the base of an emerged fibrous root. Results showed that 45–97% of all original periderm breachments were associated with fungal entry at these sites (Tables 1–4, Fig. 2).

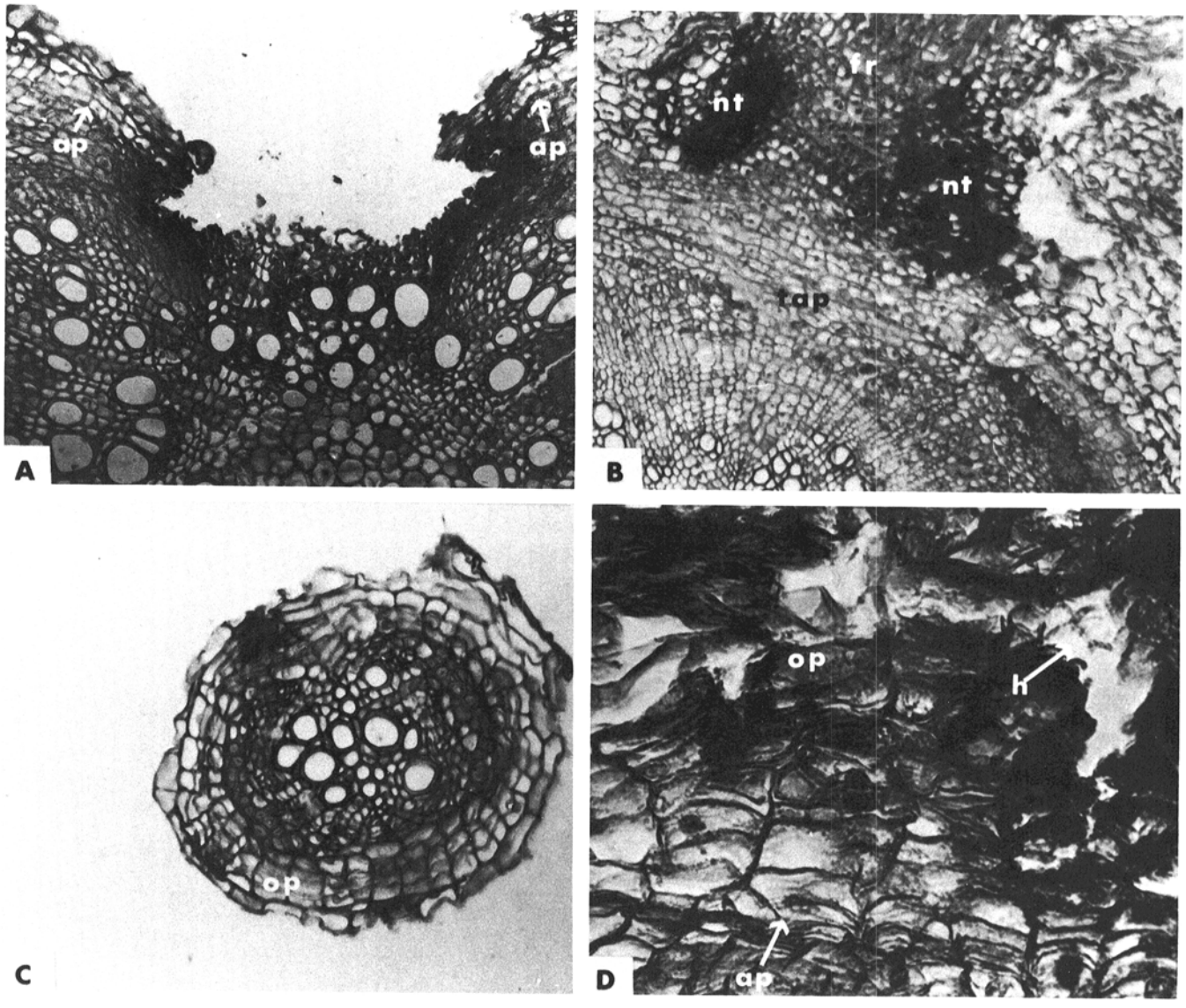

Fig. 2. Taproot pathogenesis and the role of fibrous roots in disease development of *Arachis hypogaea* infected with *Cylindrocladium crotalariae*: **A**, taproot lesion expanding inward and laterally with additional periderm forming on the borders (×63); **B**, entry of the fungus into the taproot at the base of an emerging fibrous root (×63); **C**, primary branch root showing periderm formation (×63); **D**, additional periderm walling-off lesion on primary branch root (×100). Fibrous root (fr), original periderm (op), additional periderm (ap), taproot (tap), necrotic tissue (nt), fungal hyphae (h).

Florigiant showed significantly fewer ($P = 0.01$) total breachments through bases of branch roots than NC 3033 at inoculum densities of two and four ms (Table 1). Florigiant also had fewer breachments ($P = 0.05$) associated with fibrous roots than all lines with four ms ($P = 0.05$) (Tables 2 and 3). When tested in the field, Florigiant had fewer taproot lesions associated with branch root exit points than all other lines ($P = 0.05$) (Table 4).

Although previous studies (8) indicated an absence of periderm in peanut primary branch roots, these roots were found to behave similarly to the taproot. Primary branch roots were ~ 4–5 wk old when harvested 3 wk after inoculation. Many showed signs of secondary growth—initiation of a vascular cambium with production of secondary xylem and phloem, initiation of a phellogen with production of suberized phellem and phelloderm, and sloughing of the cortex (Fig. 2C). Both Florigiant and NC 3033 branch roots produced periderm. Breachments of the periderm of a branch root resulted in a similar host response, initiation of phellogen, and formation of an additional periderm (Fig. 2D). Secondary branch roots did not form a periderm. These roots were in a primary state of growth with an intact epidermis and cortex.

Vascular parenchyma necrosis and vessel occlusions commonly extended from an infected branch root stele into an uninfected taproot stele. These tissue reactions in the otherwise healthy taproot stele frequently extended at least several millimeters above and below the point of branch root emergence.

Periderm formation in susceptible and resistant peanut germplasms. Susceptible Florigiant regularly formed additional periderms in response to breachment of its original periderm barrier by *C. crotalariae*. Florigiant sustained more breachments of the original periderm at all inoculum densities than did NC 3033 ($P = 0.01$), and the percentage of all breachments of the original periderm, which were protected at the time of harvest, was also greater in NC 3033 at all inoculum densities ($P = 0.01$) (Table 1). Florigiant protected only 72.6, 73.4, and 69.1% of all breachments with one, two, and four ms, respectively, whereas NC 3033 protected 100, 91.3, and 95.8% of all breachments with one, two, and four ms, respectively (Table 1).

Host defense responses of the nine peanut germplasms. In Test I

TABLE 1. Histological responses of susceptible (cultivar Florigiant) and resistant (cultivar NC 3003) peanut taproots inoculated with one, two, or four microsclerotia (ms) of *Cylindorcladium crotalariae* and harvested after 5 wk[w]

Entry	Number of ms	Periderm breachment[x]	Percent protection[y]	Contribution branch roots[z]
Florigiant	1	13.63 a	72.68 l	58.75 o
NC 3033	1	8.00 b	100.00 m	63.00 o
Florigiant	2	19.56 a	73.43 l	46.37 o
NC 3033	2	9.75 b	91.25 m	72.00 p
Florigiant	4	19.63 a	69.13 l	45.25 o
NC 3033	4	0.25 b	95.75 m	71.15 p

[w] Numbers in columns followed by different letters are significantly different, $P = 0.05$, according to Duncan's multiple range test.
[x] Each treatment represents 16 1-cm-long root segments.
[y] Percent of original periderms that had additional associated periderm formation, subsequent to breachment.
[z] Percent of periderm breachments occurring through bases of branch roots.

TABLE 2. Test I. Histological responses of taproots of cultivar Florigiant and five other peanut germplasms inoculated with one, two, or four microsclerotia of *Cylindrocladium crotalariae* and harvested after 5 wk[v]

Entry	Number of ms	Periderm breachment[w]	Percent protection[x]	Contribution branch roots[y]
Flor[z]	1	9.1 a	85.2 NS	73.2 NS
NC 3033	1	4.2 bc	95.0	90.6
NC 2	1	2.0 c	93.6	95.0
NC 3033 × NC 2	1	4.9 bc	97.4	82.4
NC 3033 × Flor	1	7.2 ab	95.8	95.0
Flor × NC 3033	1	5.0 abc	93.1	87.2
Flor	2	14.9 a	84.3 j	73.4 m
NC 3033	2	6.8 b	93.5 jk	85.8 mn
NC 2	2	5.2 b	88.9 jk	82.2 mn
NC 3033 × NC 2	2	4.1 b	96.9 k	81.0 mn
NC 3033 × Flor	2	8.6 b	99.2 k	94.8 n
Flor × NC 3033	2	8.9 b	95.4 k	77.8 mn
Flor	4	12.4 a	64.0 n	59.3 q
NC 3033	4	9.4 ab	94.1 o	83.5 r
NC 2	4	3.8 c	91.3 o	88.0 r
NC 3033 × NC	4	7.1 bc	93.9 o	79.3 r
NC 3033 × Flor	4	10.9 a	96.7 o	78.5 r
Flor × NC 3033	4	9.3 ab	91.5 o	70.1 r

[v] Numbers in columns within a ms level followed by different letter are significantly different, $P = 0.05$, according to Duncan's multiple range test. NS = values in the group not significantly different.
[w] Each treatment represents 16 1-cm-long root segments.
[x] Percent of original periderms that had developed additional periderms, subsequent to breachment.
[y] Total periderm breachments through bases of branch roots. Each treatment represents 16 1-cm-long root segments.
[z] Flor = Florigiant.

TABLE 3. Test II. Histological responses of taproots of cultivar Florigiant and four other peanut germplasms inoculated with one, two, or four microsclerotia of *Cylindrocladium crotalariae* and harvested after 5 wk[v]

Entry	Number of ms	Periderm breachment[w]	Percent protection[x]	Contribution branch roots[y]
Flor[z]	1	10.5 NS	82.2 d	72.0 NS
NC 3033	1	7.8	97.7 e	87.8
Arg	1	9.3	99.4 e	83.2
Flor × Arg	1	7.6	96.3 e	77.5
Flor	2	17.4 a	87.2 h	57.6 q
NC 3033	2	11.0 b	99.3 o	82.7 rs
Arg	2	8.9 b	100.0 o	88.9 qrs
Flor × Arg	2	9.1 b	96.7 o	73.7 qrs
Arg × Flor	2	14.2 ab	93.9 h	67.5 qs
Flor	4	20.5 a	73.8 g	53.3 j
NC 3033	4	10.3 b	95.6 h	82.2 k
Arg	4	9.7 b	98.9 h	74.8 k
Flor × Arg	4	13.8 b	92.0 h	69.4 k
Arg × Flor	4	14.8 b	94.5 h	78.3 k

[v] Numbers in columns within a ms level followed by different letter are significantly different, $P = 0.05$, according to Duncan's multiple range test. NS = values in group not significantly different.
[w] Each treatment represents 16 1-cm-long root segments.
[x] Percent of original periderms that had developed additional periderms, subsequent to breachment.
[y] Total periderm breachments through bases of branch roots. Each treatment represents 16 1-cm-long root segments.
[z] Flor = Florigiant, Arg = Argentine.

TABLE 4. Histological responses of 1-cm-long taproot segments of nine peanut entries grown in a field infested with *Cylindrocladium crotalariae*[v]

Entry[w]	Periderm breachment[x]	Percent protection[y]	Contribution branch roots[z]
Florigiant	28.75 a	69.42 p	43.33 r
NC 3033	16.08 cd	93.50 q	79.08 tu
NC 3033 × Flor	15.08 cd	93.25 q	75.08 u
Flor × NC 3033	18.50 bc	88.25 q	62.58 v
Argentine × Flor	11.00 e	93.17 q	85.50 s
Flor × Argentine	13.42 de	91.25 q	76.50 tu
Argentine	13.58 de	94.67 q	84.00 st
NC 2	19.12 b	87.50 q	63.92 v
NC 3033 × NC 2	13.50 de	93.50 q	81.42 stu

[v] Numbers in columns within a ms level followed by different letter are significantly different, $P = 0.05$, according to Duncan's multiple range test.
[w] Flor = Florigiant.
[x] Each treatment represents 16 1-cm-long root segments.
[y] Percent of original periderms which had associated additional periderms, subsequent to breachment.
[z] Total periderm breachments through bases of branch roots. Each treatment represents 16 1-cm-long segments.

of the experiments designed to compare the various germplasms (Table 2), there were differences ($P = 0.05$) in the number of breachments of the original periderm at all inoculum densities. Florigiant showed more breachments with one ms than NC 3033, NC 2, and NC 3033 × NC 2. With two ms, Florigiant differed from all other lines in the number of breachments. Florigiant also had more breachments than NC 2 and NC 3033 × NC 2 with four ms. Florigiant showed more occluded vessels at four ms ($P = 0.05$) than all other lines. Differences in overall protection of original periderm breachments at harvest occurred with an inoculum density of two ms between Florigiant and NC 3033 × NC 2, NC 3033 × Florigiant, and Florigiant × NC 3033 ($P = 0.05$), and with four ms between Florigiant and all other lines ($P = 0.05$).

In Test II of the experiment (Table 3), susceptible Florigiant had more breachments of the original periderm than NC 3033, Argentine, and Florigiant × Argentine ($P = 0.05$) with two ms, and more than all lines ($P = 0.05$) with four ms. Florigiant showed significantly fewer protected original periderm breachments at harvest than did all lines with one ms ($P = 0.05$), less than NC 3033, Argentine, and Florigiant × Argentine with two ms ($P = 0.05$), and less than all other lines with four ms ($P = 0.05$).

In all experiments, Florigiant showed a greater percentage of plants with obvious fibrous root necrosis and collar rot at all inoculum densities than either NC 3033 or the other lines.

Three dead Florigiant plants were recorded in the experiment testing Florigiant and NC 3033 (two with two ms, and one with four ms). No dead plants were observed in any other experiments. In all experiments, the Sudan IV test for suberin revealed neither qualitative nor quantitative differences among the nine lines.

Field testing of the nine peanut lines. This test tended to support greenhouse findings. Initial population densities of ms in the plots were low, ranging from 0.0 to 2.95 ms/g of soil. Florigiant showed significantly more breachments of the original periderm than all other lines (Table 4). Overall, Florigiant protected fewer of its taproot lesions with effective periderm and had significantly more occluded vessels than all other lines ($P = 0.05$).

DISCUSSION

Except for the periderm parameters, taproot histopathology appeared similar in all peanut lines tested. The apparent sensitivity of xylem parenchyma to toxic substance(s) resulted in the accumulation of wound gum in adjacent vessels of taproots at least 0.5 cm above and below the lesion in some cases. At any one point along the taproot, no more than approximately one-fourth of the conduction vessels were occluded, even in susceptible Florigiant (greenhouse tests). However, if such occlusions occurred at numerous points along the taproot, this could result in reduced water flow through the main conducting channels and be partly responsible for the wilting seen in early stages of the disease. The ability of peanut taproot parenchyma to initiate phellogen in all parts of the root enables even susceptible Florigiant to retard or delay ingress of toxic substances and the fungus. The frequent initiation of phellogen in the pith parenchyma of resistant lines enables these to essentially slough a whole infected quadrant of the root and thus exclude the fungus. Initiation of phellogen in the pith was never observed in Florigiant, perhaps because the rate of spread of necrosis and invasion by the fungus was too rapid, and potential meristematic cells were debilitated before they could respond.

Branch or fibrous roots are important in CBR development from two standpoints: Their own defense capabilities and the contribution they make to taproot infections. Both susceptible and resistant primary branch roots (4–5 wk old) appear to have the potential for limited secondary growth including initiation of a phellogen with production of suberized phellem layers. Presumably, similar host defense mechanisms operate in both taproot and fibrous roots, and the role of original and additional periderms in defense could be similar. Secondary branch roots (4–5 wk old) were never observed to have secondary growth or periderm. Having the capacity for periderm formation, primary branch roots may limit or retard invasion of the fungus into the stele, maintaining functional vascular transport for extended periods of time. Additional periderm formation in infected primary branch roots may also prevent or delay spread of the fungus into the taproot.

The breaks in the original periderm cylinder of the taproot, which occur when a fibrous root pushes its way to the outside, are favorable sites for entry of *Cylindrocladium* into the taproot. The fungus penetrates at sites around the bases of the emerged root and into the branch root itself, which is extremely vulnerable until a periderm forms. Toxic substance(s) appear to be translocated from an infected fibrous root through its vessels to the taproot in advance of actual fungal invasion, resulting in considerable necrosis of adjacent vascular parenchyma and gum accumulation in fibrous root and taproot vessels.

Although Florigiant produces additional periderms in response to injury of its naturally occurring phellogen cylinder, the efficiency with which it responds and the apparent effectiveness of these additional barriers were generally lower than in the other lines tested. Overall, Florigiant confined fewer lesions in the presence of suberized barriers at harvest than did the other lines. Florigiant tended to sustain more breachments of its original taproot periderm than the other lines, suggesting the possibility of inherent biochemical differences in some aspect of the periderm. Florigiant generally had a smaller percentage of its taproot lesions associated with entry at the bases of fibrous roots than the other lines. In taproots of resistant entries, a high percentage of successful infections occurred via breaks in the original periderm barrier caused by emerging fibrous roots, whereas in susceptible taproots the barrier itself may be weaker, allowing easier direct breachment of the periderm. Hypothetical periderm-related differences (15,16) between susceptible and resistant germplasms could be subtle biochemical differences in suberin component(s) resulting in a weaker structure and more readily degraded product in susceptible lines (17), more sensitive mechanism for the detection of tissue injury with earlier initiation of phellogen in resistant lines, more metabolically active suberin biosynthetic pathways resulting in the laying down of suberin lamellae more rapidly in resistant germplasm, or decreased sensitivity of phellem and phellogen to toxic substance(s) in resistant germplasms. No obvious differences in the total suberin content of cork cell walls were detected among the nine lines using the Sudan IV test.

Although it is possible that periderm barriers may form and function only after some other resistance mechanism has already inhibited or killed the fungus, the potential importance of periderm lies in its routine formation as an inherent response to injury (4), its presumed ability to delay spread of the disease, its apparent relationship, however direct, to resistance based upon the histological parameters measured, and its visibility as a marker of potential resistance.

From these experiments a ranking of resistant lines based upon histological response was not possible. Either the resistant lines responded similarly with respect to periderm production, the tests were not sensitive enough to detect differences, or other mechanisms were involved that determine the differences in the level of resistance of germplasms.

A somewhat unexpected finding was the seemingly resistant response of susceptible NC 2 as determined by the histological parameters measured. Hadley (6) rated NC 2 as susceptible, but intermediate in response to *Cylindrocladium* (root rot index significantly different from that of the resistant lines and Florigiant). In his F_1 analysis, general combining abilities (GCA) were −0.1313, NC 2; −0.1238, Argentine; and −0.2263, NC 3033. In the F_2 analysis, NC 2 showed the most negative GCA (−0.2450), suggesting that in hybrid combination the resistance factors in NC 2 were highly expressed (6). NC 3033 × NC 2, one of the most promising lines in the CBR breeding program at present, gave consistently more resistant histological responses than did NC 3033.

In these greenhouse histological tests, intermediate NC 2 performed as well as the resistant lines based upon single point inoculations and according to the parameters measured. It consistently sustained fewer breachments of the original periderm

and protected a greater percentage of taproot periderm breachments with additional periderm than Florigiant. In the one field test of low initial inoculum density, NC 2 performed better than Florigiant, but not as well as NC 3033, suggesting an intermediate type of response. It is possible that under natural field conditions there are other biotic and/or abiotic interactions influencing the tissue response of NC 2 to *Cylindrocladium*. Further field and greenhouse testing would be required to clarify the situation concerning NC 2.

Phipps and Beute (9) reported that root rot reached a maximum in NC 3033 at an inoculum density of 50 ms/g of soil, whereas Florigiant showed severe disease symptoms at 0.5 ms/g of soil. They also reported that NC 3033 and Argentine were capable of surviving densities as high as 100 ms/g of soil. In the present study, Florigiant generally sustained more breachments of the original periderm and protected fewer breachments overall than NC 3033 at all inoculum densities tested (one, two, and four ms per square millimeter of taproot). The percentage of all lesions walled off decreased rapidly in Florigiant (range of 87 to 64% depending upon inoculum density) as inoculum densities increased from one to two to four ms, whereas this parameter remained high (>90%) in the other lines. The Poisson probabilities of finding k number of ms per square millimeter of taproot indicated that with 0.5 ms/g of soil, an inoculum level at which Florigiant is severely diseased, the probability of k = one ms at some point on a 102-mm conical taproot segment is 0.7976. The probability of k = four is only 0.00078 at 50 ms per gram of soil, 0.01145 at 100 ms/g of soil, 0.3835 at 250 ms per gram of soil, and one at 500 ms/g of soil. It appears from the histological data that the "breaking point" in the inoculum density-dependent resistance of NC 3033 is greater than four ms per square millimeter of taproot. In greenhouse tests with 4 ms, NC 3033 protected 94.1–95.7% of its taproot periderm breachments; whereas Florigiant protected 64.0–73.8% (Tables 1–3). Assuming that microsclerotia in soil occur as separate, randomly distributed propagules and that the Poisson model is appropriate, a density of approximately 500 ms per gram of soil would be required to assure the occurrence of four ms together somewhere on the 102-mm-long taproot piece. The extreme rarity of densities giving k = four in naturally infested soils may account for the high degree of density-dependent resistance of NC 3033, whereas Florigiant tissue may become severely diseased—protecting considerably less than 90% of taproot lesions and sustaining fibrous root and collar necrosis at k = one (which concurs with a probability of 0.7976 in a soil with 0.5 ms/g of soil).

The Poisson distribution for the occurrence of rare events assumes that the occurrence of one event does not influence the occurrence of another event (ie, that microsclerotia occur as separate independent particles in the three-dimensional soil system). In actuality, these propagules are produced in necrotic peanut roots and are only dispersed following mechanical and biological processes resulting from cultural practices and natural tissue degradation. After one season or cycle of susceptible peanuts in an infested field, numerous microsclerotia may be clumped together in small pieces of organic debris (Harris and Black, *unpublished*). The probability of having greater than four microsclerotia together at some point on the taproot would then be greater than that predicted by the Poisson model for a given number of ms per gram of soil. This line of reasoning lends support to the practice of crop rotation in an attempt to minimize CBR damage to successive peanut crops in infested fields. It further suggests that when inoculum density-dependent resistant lines are released, some disease may be expected depending upon the initial inoculum density and degree of dispersion of propagules in the soil. Crop rotation and cultural practices that promote degradation of organic debris and dispersal of propagules should reduce disease severity in the resistant lines.

These studies suggest the need for better information related to the distribution of *C. crotalariae* propagules over time and space in the field. Such information should result in improvements in field sampling techniques and interpretations of soil samples for predictive purposes and facilitate decisions concerning the use of density-dependent resistant lines in the future.

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