Differences in Plant and Pod Reaction of Peanut Lines to Infection by **Diplodia gossypina**[1]

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ABSTRACT

In greenhouse inoculation tests we screened peanut (**Arachis hypogaea**) cultivars Florigiant and Florispan Runner and breeding lines Tifton 8, F393-9-5-1-2-3, F420-100 and F334A-B-14 for resistance to **Diplodia gossypina**, using a low and a high level of inoculum. Thirty days after inoculation at the low inoculum level, all of the F393-9-5-1-2-3 and 50% of the Florigiant plants were infected. Thirty days after inoculation at the high inoculum level, all plants of F393-9-5-1-2-3, F420-100, Florigiant, and Tifton 8 were infected. Sixty days after inoculation, 50% of the Florispan Runner plants were infected. Ninety days after inoculation, 60% and 35% of Florispan Runner and F334A-B-14 plants, respectively, were infected. Pods of F393-9-5-1-2-3, F420-100, Tifton 8, and Florigiant were severely rotted 30 days after inoculation. During this time, only 3% of the Florispan Runner pods were rotted, whereas pods of F334A-B-14 remained free of rot. **D. gossypina** was isolated from all seed from nonrotted pods of F393-9-5-1-2-3, F420-100, Florigiant, and Tifton 8 after inoculation. Only 10% of Florispan Runner seed yielded isolates of this fungus. **D. gossypina** was not isolated from F334A-B-14 seed.

Additional index words: Diplodia collar rot, **Arachis hypogaea**, resistance. soil-borne fungi.

Diplodia collar rot of peanut (*Arachis hypogaea* L.), caused by *Diplodia gossypina* Cooke, found throughout the world wherever peanuts are grown (4, 7), occurs sporadically in Southeastern United States. Peanut yields were reduced by 1% in 1965 (10). Mortality rates of up to 25 and 50% in isolated fields have been reported (6, 8). *D. gossypina* occurs in the soil mainly as a saprophyte, but it can be pathogenic and attack many different plant types, including such major crops as cotton (*Gossypium hirsutum L.*) and soybean (Glycine *max* (L.) Merr.). Crop rotation is thought to reduce disease severity in areas having a history of diplodia collar rot. Extremely high soil temperatures favor development of this disease (1, 2). Boyle (1) and McGuire and Cooper (9) noted that peanut branches were rarely infected unless plants had been predisposed to heat injury.

Although evidence of plant infection by seed-borne inoculum of *D. gossypina* is lacking, this fungus is a part of the seed mycoflora of the peanut. Garren and Higgins (3) showed that *D. gossypina* was isolated consistently from seed with concealed damage. Although *D. gossypina* was not isolated from dried seed of Virginia-grown peanuts, over 70% of dried seed of Puerto Rican-grown peanuts were infested with this fungus (5).

The objectives for this study were to determine (a) the relative susceptibility of Southeast-developed peanut cultivars and breeding lines to infection by *D. gossypina* in the greenhouse and (b) the relationship between seed-borne inoculum of *D. gossypina* and disease severity.

Materials and Methods

Peanut cultivars and breeding lines screened for resistance to diplodia collar rot, pod rot, and pod colonization by **D. gossypina** in the greenhouse were Florigiant, Tifton 8, Florispan Runner, F393-9-5-1-2-3, F420-100 and F334A-B-14. All of these, except Tifton 8, have certain common ancestors in their pedigrees. Plants of each cultivar or breeding line were grown on greenhouse benches in 20-cm clay pots (one plant per pot) containing a sterilized mixture of one part sand, one part soil and one part peat (v/v). Although the greenhouse was shaded and ventilated, air temperatures often exceeded 38°C.

The isolate of **Diplodia** used in this experiment was obtained from infected greenhouse-grown peanut plants. Cultures grown for 14 days on potato dextrose agar were macerated for 3 sec in water (100 ml distilled water per Petri dish containing agar and the fungus). Two rates of inoculum, 100 and 200 ml of fungal suspension per pot, were used in the screening studies. The low inoculum rate (100 ml per pot) was used in the mycoflora and seed transmission studies. Plants were about 8 weeks old when inoculated. Inoculum was evenly distributed over the surface of the soil and immediately covered with a layer (6 mm) of sterilized soil mixture. Plants were watered, and the soil was kept moist for several days after inoculation. In each experiment, pots (10 pots/treatment) were randomly arranged on greenhouse benches. Each experiment was repeated three times, except for the mycoflora sampling experiment, which was repeated twice.

The percentages of plants showing visible collar rot symptoms were determined 30, 60, and 90 days after inoculation. Pod rot percentages and pod colonization were determined 30 days after inoculation. Pods removed from 30 plants (ca. 300 pods) of each cultivar or breeding line were washed and inspected for pod rot. To determine the isolation frequency of **D. gossypina** and other fungi, we plated 100 shell pieces (ca. 1 cm^2) and 100 seed that had been surface-disinfested (3 min in 0.5% NaOCl) of each cultivar or breeding line on potato dextrose agar in Petri dishes. After incubation for 8 days at 25 C, fungi growing from the shell pieces and seed were identified.

In the seed transmission study, 25 seed from pods collected from inoculated plants of each cultivar or breeding line were planted as described previously and observed for collar rot symptoms. Seed were not treated with a protectant. Plants were considered infected when they showed above ground symptoms of diplodia collar rot.

Results

Wilting of a single lateral branch was one of the early symptoms of diplodia collar rot noted on peanut plants after inoculation with *D. gossypina* in the greenhouse. Lateral branch infection sites usually began at soil contact points. The lesions

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had a light-brown center surrounded by a dark-brown margin. With time, the lesions elongated along the logitudinal axis of the branch. The branch died when it was completely girdled. The fungus sometimes moved from infected branches into the main branch and tap root, thereby killing the plant. Sometimes, several branches of a plant became infected, but the rest of the plant remained free of infection. Pegs (gynophores) sometimes were infected by the fungus. Infected plants were characterized by erumpent, black pycnidia embedded in the surface of the necrotic stem tissue.

Cultivar and breeding line susceptibility to *D. gossypina* in the greenhouse is given in Table 1. Breeding line F393-9-5-1-2-3 was extremely susceptible to *D. gossypina*. Florigiant was highly susceptible at the low inoculum rate, with 50% of the plants becoming infected 30 days after inoculation. All Florigiant plants were infected at the high inoculum rate 30 days after inoculation. Tifton 8 and F420-100 were susceptible to *D. gossypina* only at the high inoculum rate. Florispan Runner and particularly F334A-B-14 appeared to be most resistant to *D. gossypina*. Although 50% of Florispan Runner plants became infected by 60 days after inoculation at the high inoculum rate, breeding line F334A-B-14 remained free of infection until 90 days after inoculation with the high rate.

Table 1. Infection of six peanut cultivars or breeding lines by Diplodia gossypina 30, 60 and 90 days after inoculation with two rates of inoculum.

Cultivar or breeding line	Plants infected by D. gossypina (%) [1]					
	Days after infection					
	Low inoculum rate			High inoculum rate		
	30	60	90	30	60	90
F393-9-5-1-2-3	100 [2]	100	100	100	100	100
Florigiant	50	50	75	100	100	100
Tifton 8	0	0	0	100	100	100
F420-100	0	0	0	100	100	100
Florispan Runner	0	0	0	0	50	60
F334A-B-14	0	0	0	0	0	35

[1] Plants were considered infected if any diseased tissue was present.

[2] Mean percentages of infected plants from three tests.

The incidence of pod rot in the cultivars and breeding lines is given in Table 2. Pods of F393-9-5-1-2-3 and Florigiant were extremely susceptible to *D. gossypina*. More than half of the pods of these cultivars were rotted 30 days after inoculation with the low inoculum rate. All pods were rotted in soils with the high inoculum rate. Although very few pods of Tifton 8 and F420-100 were rotted at the low inoculum rate, pod rot was severe at the high inoculum rate. No pods of Florispan Runner rotted within 30 days at the low inoculum rate, but a few pods rotted at the high inoculum rate. Pods of F334A-B-14 remained free of infection at both inoculum rates after 30 days.

Table 2. Incidence of pod rot caused by Diplodia gossypina in six peanut cultivars or breeding lines 30 days after inoculation with two rates of inoculum.

Cultivar or breeding line	Pod rot (%)	
	Low inoculum rate	High inoculum rate
F393-9-5-1-2-3	70 [1]	100
Florigiant	53	100
Tifton 8	5	80
F420-100	8	85
Florispan Runner	0	3
F334A-B-14	0	0

[1] Mean percentages of rotted pods from three tests.

However, slight pod rot was noted on some pods of F334A-B-14 after 60 days at the high inoculum rate.

Diplodia gossypina was isolated readily from shells of F393-9-5-1-2-3, Florigiant, Tifton 8, and F420-100 30 days after inoculation, even though no rot was visible (Table 3). The isolation frequency of this fungus from shell pieces of Florispan Runner and F334A-B-14 was much ower. Thirty days after inoculation, all seed from F393-9-5-1-2-3, Florigiant, Tifton 8, F420-100 and 10% of the seed of Florispan Runner were colonized by *D. gossypina*. *D. gossypina* was not isolated from any seed of F334A-B-14.

Table 3. The isolation frequency of fungi, including Diplodia gossypina, from shell pieces and seed of non-rotted pods from six peanut cultivars or breeding lines 30 days after inoculation with D. gossypina.

Cultivar or breeding line	Isolation frequency of fungi (%)			
	Shell		Seed	
	Total	Diplodia	Total	Diplodia
F393-9-5-1-2-3	100 [1]	55	100	100
Florigiant	90	50	100	100
Tifton 8	100	85	100	100
F420-100	95	70	100	100
Florispan Runner	30	15	15	10
F334A-B-14	25	10	5	0

[1] Percentage of total number of shell pieces or seed from which fungi were isolated after incubation for 12 days at 27 C.

Seed-borne *D. gossypina* inoculum could transmit the fungus into F393-9-5-1-2-3, Florigiant, Tifton 8, and F420-100 plants. Mortality of F393-9-5-1-2-3 plants that was caused by seed-borne inoculum of *D. gossypina* was over 25%. *D. gossypina* was not transmitted by seed of Florispan Runner or F334A-B-14.

Discussion

Of the peanut cultivars and breeding lines tested for susceptibility to *D. gossypina*, Florispan Runner and F334A-B-14 appeared to be the most

resistant. F334A-B-14 plants did not show symptoms of infection by *D. gossypina* until 90 days after inoculation, seed were not colonized by *D. gossypina* after inoculation, and seed obtained from pods grown in infested soil did not transmit the fungus.

Genes for resistance to *D. gossypina* appear to be inherited through one of the parents of Florida cross 334A. This cross, made in 1944, combined two complex breeding lines, Ga. 207-3 x F230-118-2-2. Ga. 207 was an infraspecific combination of Spanish 18-38 x Basse. F230-118-2-2 was an infraspecific combination between Dixie Giant and Small White Spanish. The Florispan Runner variety, developed by continuous selection in the F334A cross, was released in 1953. Breeding line F334A-B-14 derives from additional selection in the same hybrid material. F393-9-5-1-2-3, F420-100, and Florigiant each have Florispan Runner or Florispan lines in their pedigrees. These susceptible cultivars and breeding lines appear to lack the genetic factors for resistance. The unrelated Tifton 8 also has no natural resistance. Since F334A-B-14 is a highly productive breeding line, it may be valuable in the development of agronomically acceptable cultivars resistant to *D. gossypina.*

Earlier reports (1, 9) suggested that *D. gossypina* was a wound parasite and that only wounded or damaged plant tissue became infected. Boyle (1) noted that moribund plant tissue had to be present before *D. gossypina* would attack peanuts in Georgia. Although McGuire and Cooper (9) obtained some infection under field conditions by using infested cotton bolls and oat grains, they thought that *D. gossypina* entered peanut branches mainly through heat lesions. However, the fungus colonized surrounding undamaged cortical tissue extensively, once initial infection sites were established. The isolate of *D. gossypina* used in these studies was extremely pathogenic on susceptible cultivars and breeding lines. Predisposition of plants was not necessary for infection to occur.

Several reports (3, 5, 9) show that *D. gossypina* generally is a part of the seed mycoflora of the peanut. Garren and Higgins (3) isolated *D. gossypina* from seed with concealed damage. McGuire and Cooper (9) noted that *D. gossypina* constituted a part of a fungal mat present between seed cotyledons of inoculated field-grown peanuts. Garren and Porter (5) recently isolated *D. gossypina* at high frequencies from seed of peanuts grown in Puerto Rico. In our study, isolates of *D. gossypina* were obtained often from unblemished peanut seed of susceptible cultivars and breeding lines. Furthermore, the seed-borne fungus persisted and caused diplodia collar rot on progeny plants of susceptible cultivars including F393-9-5-1-2-3, Florigiant, Tifton 8, and F420-100.

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