Fungal Colonization of Peanut Fruit as Related to Southern Corn Rootworm Injury

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Cooperative investigations of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture and the Research Division, Virginia Polytechnic Institute and State University. Contribution No. 257, Department of Plant Pathology and Plant Physiology, Virginia Polytechnic Institute and State University, Blacksburg.

Accepted for publication 30 July 1973.

ABSTRACT

Field-grown peanut fruit injured by the feeding of southern corn rootworm larvae were more susceptible to fungal colonization than noninjured fruit. Seed from injured fruit were colonized by fungi at a much greater frequency than seed from noninjured fruit. However, colonization by the toxigenic species *Aspergillus flavus* was not affected. In greenhouse tests, pod breakdown, an important in-soil rot of peanut fruit caused by *Pythium myriotylum*, was greatly enhanced by the presence of rootworm larvae. Under high inoculum densities of *P. myriotylum* and abnormally high rootworm populations the incidence of pod breakdown was almost twice that observed when only the fungus was present. Rootworm population densities influenced the severity of pod breakdown; severity increased as rootworm populations increased. The data suggest that insect feeding sites could provide portals of entrance into the peanut fruit for many fungi, including *P. myriotylum*.

Phytopathology 64:249-251

Additional key words: Arachis hypogaea, Diabrotica undecimpunctata howardi.

Fruit of the peanut (*Arachis hypogaea* L.) develop to maturity in the upper 51 mm (2 inches) of the soil. This soil layer, continuously shaded by dense foliage and rich in nutrients, abounds in fungi, insects, mites, and nematodes. Fungi associated with peanut fruit received careful study after the discovery in 1960 (11) that one of these fungi, *Aspergillus flavus* [Link.] Fries, can produce metabolites that are harmful to mammals. Recent research (3, 9, 10, 12) has shown that fungi are readily isolated from peanut fruit as they develop in the soil. Fungi are especially common in overmature, damaged, and termite-injured fruit. Griffin (8) showed that unless the fruit surface is injured, spores of *A. flavus* germinate infrequently in the geocarposphere soil adhering to the fruit. Also, seed from fruit with microscopic openings in the shell are more prone to fungal colonization (13).

Pythium myriotylum Drechs., the major cause of a severe in-soil fruit rot (pod breakdown), has increased greatly in importance since 1960 (5). In some instances pod breakdown has become epidemic and yields have been greatly reduced. The severity or incidence of this disease seems to be increasing each year. However, certain available cultivars have a degree of resistance to this pathogen (15).

One of the most common insect pests of peanuts in the Virginia-North Carolina area is the southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) (4). All active immature stages feed on peanut fruit, but injury becomes serious only with the feeding of second- and third-stage larvae. In severely rootworm-infested fields about 35 to 40% of the fruit have rootworm injury. Feeding may involve only surface areas of the fruit or may completely penetrate it. Population densities usually are greatest in medium textured, poorly drained soils (7). Insecticides that provide satisfactory control are available and are used by about 85% of the growers. In addition, some peanut breeding stocks may offer some degree of resistance (2, 14).

This research was undertaken to determine the relationship of southern corn rootworm larvae injury on subsequent colonization of fruit of Virginia-type peanuts by fungi, especially *P. myriotylum*.

MATERIALS AND METHODS.—The presence of microorganisms in field-grown peanut fruit injured by the feeding of southern corn rootworm larvae and in similar noninjured fruit (checks) was determined. Two-seeded fruit nearing maturity were segregated into injured and noninjured lots. Injured fruit with only one rootworm penetration site were selected and used in this study. Seed from the end of the fruit nearest the penetration site also were selected. Shell pieces about 1 cm^2 (1,200 pieces containing one penetration site and 1,200 pieces not penetrated) and seed (1,200 from injured fruit and 1,200 from noninjured fruit) were disinfested in 0.5% NaOCl for 3 min before they were plated on Rose Bengal-streptomycin agar. Most of the microorganisms that grew on the medium, from either the shell piece or the seed, could be identified after incubation for 10 days at 25 C. Percentages of shell pieces and seed from which at least one microorganism grew were determined.

To determine the effects of penetration of peanut fruit by southern corn rootworm larvae on predisposing fruits to colonization by *P. myriotylum*, a greenhouse experiment was designed with the following treatments: (i) untreated or check, (ii) *P. myriotylum* alone, (iii) rootworm larvae alone, and (iv) *P. myriotylum* and rootworm larvae added simultaneously. Seed of the peanut cultivar 'Virginia Bunch 46-2' were planted in bushel baskets containing a pasteurized soil (soil maintained at 38 C for 2 hr) mixture consisting of two parts of Dragston type topsoil, one part vermiculite, and one part peat. After 12 wk of growth, plants were segregated into four treatment groups containing seven replications each.

P. myriotylum was grown in 300-ml Erlenmeyer flasks containing an autoclaved medium of 200 g washed sand, 6 g cornmeal, and 40 ml distilled water. Each flask was seeded with one No. 4 cork borer plug from the growing edge of a colony of *P. myriotylum* growing on cornmeal-dextrose agar in petri dishes. After inoculation, flasks were maintained at 23 C for 12 days. The contents of a single flask were evenly distributed and worked into the top 5-cm of soil in baskets containing the plants in treatments 2 and 4. At the same time baskets containing the plants in treatments 3 and 4 were infested with 50 laboratory reared, second-stage larvae of the southern corn rootworm. Approximately 20 days after infestation with *P. myriotylum* and rootworm larvae, plants were removed from baskets and percentages of pod breakdown were determined. This experiment was repeated three times.

In another study, *P. myriotylum* tolerant ('Florigiant') and susceptible (Virginia Bunch 46-2) peanut cultivars were exposed to rootwrom populations to determine whether the presence of the rootworm larva altered the susceptibility of the tolerant cultivar to fungal colonization. These two cultivars, grown in 25-cm diam clay pots containing sterilized soil, were inoculated with either the fungus alone or with the fungus plus 30 or 60 second-stage rootworm larvae. Inoculum of *P. myriotylum* was prepared as previously described. Also, soil was infested with *P. myriotylum* and rootworm larvae by using techniques previously described. Plants were removed from the pots 14 days after soil infestation and percentages of pod breakdown were determined. Each treatment was replicated eight times and the experiment was repeated twice.

RESULTS.—Table 1 shows the frequencies that microorganisms were isolated from shells and seed of peanut fruit injured in the field by the feeding of southern corn rootworm larvae and from similar noninjured fruit. The average incidence of rootworm injury in test plots during both years of this study was 21%. Microfloral counts were considerably greater in shell pieces and seed from injured fruit than from noninjured fruit. In fact, the isolation frequency of total microorganisms was 50% and 138% greater, respectively, from shell pieces and seed of injured fruit than from shell pieces and seed of similar noninjured fruit. Twice as many *Fusarium* spp. and *Trichoderma* spp. were isolated from shell pieces from injured fruit as from shell pieces from noninjured fruit. Isolation frequencies of *Penicillium* spp., *Aspergillus* spp., *Rhizopus* spp., *Chaetomium* spp., and *Alternaria* spp. did not differ in shell pieces.

Trends were similar in the seed mycoflora. *Fusarium* spp. and *Trichoderma* spp. were isolated more frequently from rootworm-injured than from noninjured fruit. The isolation frequency of *Aspergillus* spp. was not influenced by rootworm feeding.

Large two-seeded peanut fruit from plants inoculated with *P. myriotylum* and simultaneously infested with southern corn rootworm larvae had more pod breakdown than fruit from plants inoculated with the fungus alone (Table 2). When the averages of three tests were combined, pod breakdown was 85% greater in treatments containing both organisms than in treatments containing only *P. myriotylum*. In experiments B and C, the amount of pod breakdown in treatments containing both organisms was over twice that in treatments containing the fungus alone. *P. myriotylum* was isolated readily from discolored pod tissue surrounding the penetration site. Of fruit in pots infested with rootworm larvae, 87% were injured (Table 2).

Table 3 shows the effects of rootworm injury and cultivar reaction to pod breakdown caused by *P. myriotylum*. When plants were inoculated with *P. myriotylum* alone, pod breakdown was significantly greater on fruit of the Virginia Bunch 46-2 cultivar than on fruit of the Florigiant cultivar. The addition of rootworm larvae to plants inoculated with *P. myriotylum* increased the severity of pod breakdown in both cultivars. Although pod breakdown severity increased considerably with the addition of 30 larvae per plant, differences in cultivar susceptibility remained apparent. However, with

TABLE 1. Isolation of microorganisms from pieces of shell and seed of field-grown peanut fruit injured by the feeding of southern corn rootworm larvae and from similar noninjured fruit

	Isolation frequency (%)[a]			
	Shell		Seed	
Microorganisms	Non-injured	Injured	Non-injured	Injured
Total microorganisms	64 a	96 b	32 a	76 b
Penicillium spp.	10 a	12 a	15 a	16 a
Fusarium spp.	9 a	19 b	1 a	13 b
Trichoderma spp.	14 a	32 b	2 a	5 a
Chaetomium spp.	3 a	4 a	1 a	1 a
Alternaria spp.	2 a	2 a	1 a	2 a
Aspergillus spp.	1 a	1 a	3 a	2 a
Rhizopus spp.	3 a	1 a	0 a	0 a

[a]Data subjected to Duncan's Multiple Range Test and analyzed separately for shell and seed. Data in a horizontal row having the same letters are not significantly different at the 5% level.

TABLE 2. The influence of high populations of southern corn rootworm (50 second-stage larvae per basket) on predisposition of greenhouse-grown peanut fruit of the cultivar 'Virginia Bunch 46-2' to pod breakdown caused by *Pythium myriotylum*

	Root-worm injury[a] (%)	Pod breakdown in three experiments[b] (%)			
Treatments	Mean	A	B	C	Mean
Check	0	1 a	1 a	0 a	0
P. myriotylum alone	0	46 b	34 b	39 b	39
Rootworm larvae alone	87	5 a	7 a	2 a	5
P. myriotylum + rootworm larvae	95	67 c	96 c	81 c	72

[a]Figure given is the average number of peanut fruit injured by southern corn rootworm feeding in Experiments A, B, and C.

[b]Data subjected to Duncan's Multiple Range Test. Treatments having the same letters in vertical columns are not significantly different at the 5% level.

TABLE 3. Cultivar reaction to pod breakdown caused by *Pythium myriotylum* of peanut fruit grown in the greenhouse in relationship to two population levels of southern corn rootworm larvae

	Pod breakdown[a] (%)		Rootworm injury (%)	
Treatments	Flori-giant	Virginia Bunch 46-2	Flori-giant	Virginia Bunch 46-2
P. myriotylum alone	16 a	28 b	0	0
P. myriotylum + 30 larvae	36 a	48 a	29	33
P. myriotylum + 60 larvae	64 a	61 a	46	54

[a]Data were analyzed according to Duncan's Multiple Range Test. Cultivars having the same letters in horizontal columns are not significantly different at the 5% level.

the addition of 60 larvae per plant, the differential between cultivars and susceptibility of *P. myriotylum* was not apparent.

DISCUSSION.—The results indicate that under field conditions injury caused by the feeding of southern corn rootworm larvae can provide avenues of entrance into peanut fruit by the soil-borne fungi surrounding the in-soil fruit during its development (Table 1). Of particular significance is the fact that the isolation frequency of fungi from seed from rootworm-injured fruit was over twice that of seed from noninjured fruit. Although the isolation frequency of *Aspergillus* sp. was not influenced by rootworm feeding, other toxin-producing fungi such as *Fusarium* spp. (6) were more prevalent in seed from injured fruit.

Under greenhouse conditions, peanut pod breakdown caused by *P. myriotylum,* is greatly enhanced by fruit injury caused by the southern corn rootworm larvae (Table 2). After injury, the shell tissue surrounding the penetration site apparently undergoes certain physical and chemical changes that enhance colonization. *P. myriotylum* was isolated easily from injured tissue surrounding the penetration site. Whether this particular fungus enters the penetration site and then radiates into the shell proper or directly colonizes the injured tissue surrounding the penetration site is not known.

According to van Schaik et al. (15), Florigiant, a commonly grown peanut cultivar, is less susceptible to colonization by *P. myriotylum* than are other cultivars, including Virginia Bunch 46-2. However, both cultivars are highly vulnerable under field conditions. In this greenhouse study, development of pod breakdown in these two peanut cultivars was significant with high rootworm populations (30 larvae per pot). With very high rootworm populations (60 larvae per pot), fruit from the cultivar Florigiant were as susceptible to colonization by *P. myriotylum* as fruit from cultivar Virginia Bunch 46-2 (Table 3). Under field conditions where rootworm populations would be much lower, predisposition may not be as great as that observed in this test.

In fields where rootworm populations are very high, approximately 35 to 40% of the fruit are injured by their feeding. However, rootworms are effectively controlled insecticides and injury in problem fields can be reduced to <5%. Because the majority of the peanut growers use insecticides, the role of rootworms in the predisposition of fruit to attack by soil-borne fungi is probably slight, occurring only in isolated instances. However, new rootworm races that are resistant to these insecticides may develop; e.g., the development of rootworm resistance to aldrin prior to 1960 (1).

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