Incidence of *Verticillium nigrescens* in Soybeans

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ABSTRACT

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Verticillium nigrescens was isolated from soybean flowers and from pods at several stages of development at eight locations in Georgia during 1977–1981. The incidence in pods ranged from 0 to 36%, was highest 3–5 wk after initial pod set, and declined gradually toward maturity. The highest incidence of the fungus in soybean pods occurred in fields where cotton had been grown repeatedly. The fungus was readily isolated from soil and high soil moisture levels were related to increased infection. Although other weed species were examined, the pathogen was isolated only from *Solanum carolinense*. When surface-sterilized pods were plated, *V. nigrescens* frequently grew from the end of the pedicel and only rarely grew from other parts of the pod. The fungus never caused vascular discoloration or wilt symptoms, but its presence was associated with decreased numbers of pods on infected plants. In growth chambers, *V. nigrescens* significantly reduced numbers of pods per plant and the weight of seed in inoculated plants. In the field, benomyl application reduced pod infection by 50–90% and treated plants produced more pods than control plants.

Verticillium nigrescens Pethybr. was first described as a saprophyte, but was later found to be pathogenic on potato, tomato, antirrhinum, eggplant, chrysanthemum, hop, and salsify, especially if the soil was very damp (5,7,8). It has been isolated from several other crops, but was only weakly pathogenic on most of them (2,12,17). *V. nigrescens* is a mild pathogen of cotton (20,21) and has been associated with the boll rot complex (11,15). Isaac (8) considered *V. nigrescens* to be a soil inhabitor and that its parasitism on plants may be incidental to its saprophytic existence in soil. In several studies of the microflora of soybean pods and seeds (1,9,10,16) this fungus was not isolated but Miller and Roy (13) reported isolating *V. nigrescens* in low frequency from leaves, pods, and seeds.

During a survey of brown stem rot in 1969 (14) and on several subsequent occasions, *V. nigrescens* was isolated from the stems of soybean (*Glycine max* (L.) Merr.) plants. We disregarded this fungus as a pathogen since soybean plants inoculated with it never developed wilt or vascular discoloration like that caused by *V. dahliae* (18).

To detect the succession pattern of fungi on soybean pods and their effects on seed quality, fungi were isolated from pods at various developmental stages. *V. nigrescens* was detected at surprisingly high frequency at some locations, particularly during early pod development. Fungicide treatments and also soil moisture conditions were related to the frequency of isolation of this fungus.

The purpose of the research reported in this article was to measure the occurrence of *V. nigrescens* in field grown soybeans and to test its pathogenicity to plants of a selected soybean cultivar in tests under controlled conditions.

MATERIALS AND METHODS

Field studies. Eight field plot areas, representing three major soybean-growing regions were planted with either cultivar Ransom or Bragg as indicated in Table 1. Chlorothalonil, fentin hydroxide, or benomyl was applied to eight-row plots 10 m long at 560 g (a. i.)/ha in 187 L of water per hectare. Unsprayed plots of equal size served as controls. The plot sprayer used was equipped with three nozzles per row suspended from a side-mounted boom which permitted the sprayer to be driven outside the plot area. This essentially eliminated mechanical damage to the plants from the spray applications at the R3 and R5 (3) growth stages. A randomized block design with four replications was used in each test.

In 1979, at Experiment and Plains, GA, duplicate experiments were established to determine the effects of irrigation on infection of soybeans by *V. nigrescens*. At each location, the irrigated plots received a 2.5 cm irrigation each week that the total natural rainfall was <1.5 cm and the other plot received natural rainfall only. This resulted in seven irrigations at each location. At Midville in 1978, a separate location was planted with 18 soybean cultivars in four-row plots 5 m long. A randomized block design with three replications was used.

Isolation of *V. nigrescens*. At each location (Table 1, Fig. 1) pod samples were taken on two to five sampling dates between 1 and 8 wk after initial pod set. At each sampling date, 1 pod was randomly

TABLE 1. The incidence of *Verticillium nigrescens* in soybean pods at eight locations and the influence of soil moisture and benomyl

Location	Year	Cultivar	Rainfall plus irrigation (cm)[a]	Pods with *V. nigrescens* (%)[b] No fungicide	Benomyl[c]
Midville 1[d]	1977	Ransom	–	18	8[f]
Midville 2[d]	1978	Ransom	–	14	3[f]
Tifton 1	1977	Bragg	–	11	1[f]
Tifton 2	1978	Bragg	–	2	1
Williamson	1977	Ransom	51.7	9	1[f]
	1978	Ransom	30.8	1	0
	1979	Bragg	–	10	2[f]
Plains	1979	Bragg	55.4	10	6
	1979	Bragg	37.9	3	1
Experiment 1[e]	1979	Bragg	57.8	2	3
	1979	Bragg	40.3	0	1
Experiment 2[d]	1980	Bragg	40.4	18	4[f]
	1981	Bragg	58.3	36	16[f]

[a] Total during June, July, August, and September.
[b] Mean of four replicate plots. Fifty pods were assayed per plot at each sampling date. Data presented are from the sampling date when the highest incidence was detected in plots with no fungicide applied.
[c] Two applications, one at R3 and one at R5. Benomyl applied at the rate of 560 g of active ingredient per hectare in 187 L of water.
[d] Field with a long history of cotton production.
[e] No cotton has been planted at this location for at least 25 yr.
[f] Significantly lower than the control at $P = 0.05$ by Student's t-test.

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collected from 50 different plants in each plot. All pods were surface-sterilized for 3 min in 5.25% sodium hypochlorite and plated on potato-glucose agar (PGA). After 1 wk, and again after 2 wk, the fungi emerging from the pods were identified and enumerated. Data presented in Tables 1 and 2 are from the sampling date when the highest incidence was detected in control plots. In Fig. 1, data are presented for all sampling dates in all experiments where the incidence of *V. nigrescens* in pods exceeded 10%.

After harvest, seeds from each location were surface sterilized for 4 min in 0.525% sodium hypochlorite as described by Grybauskas et al (4). Fifty seeds from each plot were plated on PGA and the emergent fungi were identified and enumerated.

Distribution of the fungus in the pods on different parts of the soybean plant was determined at Williamson in 1978 by selecting a plant from each of five infested plots and cutting each plant into three sections of approximately equal size designated top, middle, and bottom. All pods from each section were treated as above.

At Williamson in 1979, soybean flowers and stem sections as well as stems, flowers, and seed pods of various weeds present in the field were plated to evaluate the presence of *V. nigrescens*. The procedure described above for isolating *V. nigrescens* from pods was used in these isolations.

To determine how rapidly *V. nigrescens* was moving systemically from the soil, into the roots, and to the aboveground plant organs, Ransom soybeans were planted in infested soil in a greenhouse. Each week, a 2 cm-long stem section from each internode of 10 plants was surface sterilized, aseptically split longitudinally and plated on PGA. This was continued until *V. nigrescens* was isolated.

In 1978, five soil samples from each location were plated on the following medium. One hundred grams (fresh weight) of 10-day-old soybean plants (stems and leaves) were blended for 1 min in distilled water, strained through cheesecloth, and autoclaved. Twenty grams of agar and distilled water were added to make a total volume of 1 L, and the medium was again autoclaved. Upon cooling to 45 C, PCNB (0.15 g), $ZnSO_4 \cdot 7H_2O$ (0.75 g), and rose bengal (0.05 g) were added. Five grams of soil was mixed with 10 ml of sterile water and 1 ml of the suspension was spread over the surface of the solidified agar in each of 10 petri dishes.

Pod and seed counts. Prior to harvest, pods were counted in the field on 10 randomly selected plants in each replicate plot. The plants were tagged and numbered so that the same plants could be counted later. At harvest, 10 plants from each replicate plot were removed from the field, the pods were counted, and the seeds were removed from the pods and counted.

Pathogenicity studies. Ransom soybeans were planted (one per pot) in 30-cm-diameter plastic pots in a growth chamber equipped with a combination of VHO cool-white fluorescent and incandescent lamps. A photoperiod of 16 hr light (500 μE) and 8 hr dark was maintained for 60 days, then changed to 12 hr light and 12 hr dark until harvest. Temperatures were maintained at 27 C and 18 C, respectively, during the light and dark periods. Three weeks after planting, 25 plants were inoculated by injecting the stems with a suspension of conidia of *V. nigrescens* (10^6/ml) in sterile water. Twenty-five plants, similarly injected with sterile water, served as controls. At maturity (135 days), each plant was cut, its pods were counted, and the seeds were counted, dried to 13% moisture, and weighed.

RESULTS

Species of *Phomopsis, Alternaria, Fusarium*, and other fungi emerged from random areas on surfaces of soybean pods. In contrast, *V. nigrescens* always emerged from vascular tissues, and, in most isolations, from the end of the pedicel. The incidence of *V. nigrescens* increased until the 3rd to 5th wk of pod formation and then decreased (Fig. 1). The fungus was rarely isolated from pods near maturity and was not isolated from seed. When pods were selectively sampled by position on the plant, 3% of the pods from the upper third of the plants were infected, compared to 9% of the pods from the middle third and 12% of the pods in the lower third.

The highest incidence of *V. nigrescens* recorded in this study was 36% of the pods in a field at Experiment (location 2) in 1981. In the same field in 1980 the soil moisture was lower and the highest incidence was 18% (Table 1). The influence of soil moisture was also evident at three other locations (Williamson, Plains, and Experiment 1). The percentage of pods with *V. nigrescens* was higher when soil moisture was higher at all locations (Table 1). The difference was significant ($P = 0.05$) at all locations except Experiment 1.

No cotton has been planted at the Experiment 1 location for at least 25 yr. In contrast, the three locations with the highest infestations (Experiment 2, Midville No. 1 and 2) all had long histories of cotton production. The cropping history of the other four locations could not be determined.

The fungus was isolated from soil at all locations. The fungus was first isolated from the aboveground parts of soybean seedlings grown in soil contaminated with *V. nigrescens* when the plants were 6 wk old. Isolation from weeds revealed that horse nettle, *Solanum carolinense* L., contained the fungus; and that morning glory, *Ipomea purpurea* (L.) Roth; sicklepod, *Cassia obtusifolia* L.; lamb's-quarters, *Chenopodium album* L.; kudzu, *Pueraria lobata* (Willd.) Ohwi; and pokeweed, *Phytolacca americana* L., did not.

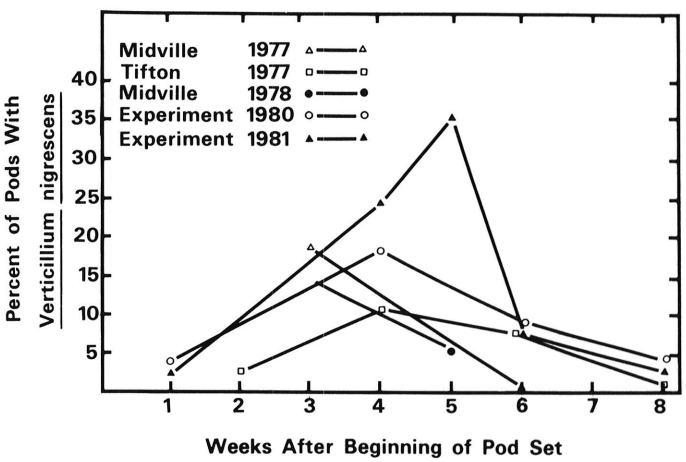

Fig. 1. Relationship of the stage of development of soybeans to the frequency of isolation of *Verticillium nigrescens* from pods. Each data point is the mean of four replicate plots. Fifty pods from each plot were assayed on each sampling date.

TABLE 2. Relation of *Verticillium nigrescens* to numbers of pods and seeds per plant and seed weight of soybeans

Location and treatment	*V. nigrescens* in pods[a] (%)	Pods per plant[b] (no.)	Seeds per plant[b] (no.)	Weight of 100 seeds[b] (g)
Midville				
Benomyl[c]	8[e]	55[e]	81[e]	16.6
Control	18	41	71	15.7
Tifton				
Benomyl[c]	1[e]	69[e]	125[e]	18.7
Control	11	57	86	17.7
Williamson				
Benomyl[c]	1[e]	67[e]	141[e]	20.4
Control	9	63	126	19.6
Growth chamber				
Inoculated[d]		24[e]	46[e]	9.0[e]
Control		28	49	10.0

[a] Mean of four replicate plots. Fifty pods were assayed per plot at each sampling date. Data presented are from the sampling date when the highest incidence was detected in plots with no fungicide applied.

[b] At the Midville, Tifton, and Williamson locations, 10 plants per plot were counted from each of four replicate plots. In the growth chamber, there were 25 replicate inoculated plants and 25 control plants.

[c] Benomyl applied at R3 and at R5 at the rate of 560 g of active ingredient per hectare in 187 L of water.

[d] Inoculated with *V. nigrescens* by injection of conidia into the stem.

[e] Significantly different ($P = 0.05$) from the control by Student's *t*-test.

Verticillium nigrescens was isolated from the pods of 16 of 18 cultivars in 1978 at a test location where the highest incidence found in pods was only 6%. The fungus was isolated from pods of the other two cultivars at another location in 1980.

Application of benomyl to plants growing in the field consistently reduced the recovery of *V. nigrescens* from the pods (Table 1). Applications of chlorothalonil or fentin hydroxide had no significant ($P = 0.05$) effect on numbers of *V. nigrescens* isolated from pods. The reduction by benomyl in the number of infected pods was accompanied by an increase in the number of pods per plant at maturity (Table 2). Pod counts on control plants at the Experiment 2 location in 1981 indicated no significant ($P = 0.05$) change in numbers of pods per plant among counts made at 5, 8, and 10 wk after the beginning of pod formation.

Inoculation with *V. nigrescens* under controlled conditions significantly reduced the number of pods produced, the number of seeds, and the size of the seeds (Table 2). The percentage of *V. nigrescens* in the pods of these plants was not determined because the removal of pods for plating might have significantly influenced the final pod and seed counts. In preliminary experiments, *V. nigrescens* was isolated from pods of plants inoculated by this procedure. Vascular discoloration or wilting were not observed either in inoculated plants or in naturally infected plants.

DISCUSSION

The presence of *V. nigrescens* in soybean pods appears to be associated with pod loss. In normal soybean plants most of the flowers never develop into mature pods, and 87% of the abscisson occurs before the pods are 2 cm long (19). Pod counts made well after this stage in 1981 indicated that most pod loss resulting from the activities of the fungus occurs earlier in the season when the natural abscission rate is high.

A decline of pod infection from 36% at 5 wk to 2.5% at 8 wk (Fig. 1) was obviously not a result of loss of infected pods since only a nonsignificant ($P = 0.05$) 3% decline in number of pods was recorded during the period. The increased presence of faster-growing fungi (*Alternaria* spp. and *Phomopsis* spp.) may contribute to the observed decline either by direct competition in the pods or by masking the presence of the slower-growing *V. nigrescens* on the culture plates. Miller and Roy (13) isolated *V. nigrescens* from soybean seed, but presented no data on the incidence of this fungus. During the course of this study, we cultured several thousand seeds, many from plots with a high level of pod infestation, and did not isolate this fungus. Apparently, the incidence of *V. nigrescens* in soybean seed is extremely low.

Although *V. albo-atrum* and *V. dahliae* are poor soil competitors (6), *V. nigrescens* seems to survive well in host-free soil. It is widely distributed in Georgia and has recently been observed in soybeans in Mississippi (13). *V. nigrescens* has been associated with cotton (11,15,21) and production of this crop seems to promote infestation of a field. *V. nigrescens* can also be a pathogen on potato, tomato, antirrhinum, eggplant, chrysanthemum, hops, and salsify (7,8), but except for potato, these crops are rarely grown in rotation with soybeans. We found *V. nigrescens* in only one weed and soil is probably the source of inoculum.

It appears that the fungus moves slowly and systemically upward through soybean plants since it took 6 wk for the fungus to reach the aboveground plant organs and the percentage of infected pods declined from the lower to the upper portion of the plant. Isaac (7) reported that plants growing in wet soil were most susceptible to *V. nigrescens*. This appears to be true for soybeans, since irrigated fields and experiments conducted in wetter years consistently had greater levels of infested pods. Isolation of *V. nigrescens* from each of 18 cultivars attempted, indicates that most soybean cultivars are susceptible to invasion.

Apparently, *V. nigrescens* can be a subtle pathogen of soybeans but produces none of the vascular discoloration and wilting symptoms typical of systemic fungi in soybeans. Rather, there is a loss of pods and a reduction in the number and size of seeds.

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